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Whole-Cell *Pseudomonas aeruginosa* Localized Surface Plasmon Resonance Aptasensor

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Abstract

The detection of whole-cell *Pseudomonas aeruginosa* presents an intriguing challenge with direct applications in health care and the prevention of nosocomial infection. To address this problem, a localized surface plasmon resonance (LSPR) based sensing platform was developed to detect whole-cell *Pseudomonas aeruginosa* strain PAO1 using a surface-confined aptamer as an affinity reagent. Nanosphere lithography (NSL) was used to fabricate a sensor surface containing a hexagonal array of Au nanotriangles. The sensor surface was subsequently modified with biotinylated polyethylene glycol (Bt-PEG) thiol/PEG thiol (1:3), neutravidin, and biotinylated aptamer in a sandwich format. The 1:3 (v/v) ratio of Bt-PEG thiol/PEG thiol was specifically chosen to maximize PAO1 binding while minimizing nonspecific adsorption and steric hindrance. In contrast to prior whole-cell LSPR work, the LSPR wavelength shift was shown to be linearly related to bacterial concentration over the range of $10\text{--}10^3$ cfu mL⁻¹. This LSPR sensing platform is rapid (~3 h for detection), sensitive (down to the level of a single bacterium), selective for detection of *Pseudomonas* strain PAO1 over other strains, and exhibits a clinically relevant dynamic range and excellent shelf life (~2 months) when stored at ambient conditions. This versatile LSPR sensing platform should be extendable to a wide range of supermolecular analytes, including both bacteria and viruses, by switching affinity reagents, and it has potential to be used in point-of-care and field-based applications.

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ASSOCIATED CONTENT

Supporting Information

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SEM images of tilted and dissected Au nanotriangle array, ratio of Bt-PEG thiol and PEG thiol selection, a confocal fluorescence image and an overlaid fluorescence and transmission image of fluorescently labeled aptamer bound PAO1, box plots showing the effects of surface derivatization as well as bacterial pull-down in both PBS and air, LSPR spectra of the sensor chip after surface modification, optical microscope images of PAO1 on Au thin film, inter- and intrabatch variations of sensor chips, bacterial growth curves, SEM image of *S. aureus* on modified sensor surface, and the results of various statistical comparisons (PDF)

The authors declare no competing financial interest.

Graphical abstract

Bacterial infections have posed a persistent health threat to humans, livestock, and crops throughout human history. Fortunately, the discovery of antibiotics in the mid-20th century caused fatality rates associated with bacterial infections to plunge. However, with the recent development of multidrug resistant bacteria,^{1,2} bacterial infections have again become an emerging health issue worldwide. Consequently, there is an urgent need to develop both efficient bacterial diagnostics as well as effective treatments.^{3–6} In this context, *Pseudomonas aeruginosa* has been listed as a critical pathogen by the World Health Organization in 2017.⁶

Current gold standard microbiology methods for bacterial detection have excellent specificity and sensitivity, allowing for bacterial strain identification and single-cell detection; however, these protocols require culturing bacteria in a strictly controlled laboratory environment and often take days for an accurate reading. Immunoassays, an alternative strategy, are rapid but suffer from relatively low sensitivity with a typical limit of detection (LOD) of 10^3 – 10^6 cfu mL⁻¹ (cfu = colony-forming unit).⁷ Other approaches like polymerase chain reaction (PCR) and its derivatives afford fast and sensitive detection but require extensive sample preparation to extract DNA from bacteria as well as expensive reagents and instrumentation.⁸ Because it is desirable to optimize detection speed, sensitivity, selectivity, and cost for bacterial diagnostics, research into bacterial detection continues to be of substantial interest.^{5,9–11}

Recent efforts have concentrated on developing new methods to detect whole-cell bacteria. Affinity reagents such as antibodies,^{7,12–14} siderophores,^{15,16} antimicrobial peptides,^{17,18} and aptamers^{19–23} have been utilized as recognition motifs due to their extraordinarily selective affinity toward target analytes. Aptamers, single-stranded oligonucleotides, utilize their unique combination of secondary and tertiary structures to bind small molecules, proteins, and even cellular targets with high affinity.^{24–26} As affinity reagents, aptamers are desirable, because they: (1) can be synthesized in large batches with minimal variability, (2) are amenable to surface modification, (3) are stable to temperature, pH, and buffer conditions, (4) are relatively inexpensive, and (5) support the construction of a database for each pathogen.^{24–26} The major drawbacks of aptamers are the tedious initial selection through the systematic evolution of ligands by exponential enrichment (SELEX)²⁴ as well as their susceptibility to nucleases in the sample medium.²⁷ Various aptamers have been selected against specific whole-cell pathogens, such as *Staphylococcus aureus*,²⁰ *Listeria monocytogenes*,²¹ *Lactobacillus acidophilus*,²² *Salmonella typhimurium*,^{23,28} *Salmonella enteritidis*,²⁸ *Mycobacterium tuberculosis*,²⁹ *Campylobacter jejuni*,³⁰ and *Escherichia coli*.³¹

some of which have been utilized for whole-cell bacterial detection.^{19–23} Currently, there are very few reports on whole-cell *Pseudomonas aeruginosa* specific aptamers, this in spite of the increasing multidrug resistance of *P. aeruginosa*. Wang et al. identified several specific aptamer sequences against inactivated whole-cell *P. aeruginosa* and demonstrated detection of *P. aeruginosa* using a fluorescein isothiocyanate (FITC) labeled aptamer with fluorescence microscopy.³² While this is a promising development, in general, label-free detection is desirable in an optimal sensing platform.

Localized surface plasmon resonance (LSPR) based nanosensors are label-free and are capable of sensitive, quantitative detection of target analytes.^{14,27,33–35} With the advent of nanosphere lithography (NSL), developed by Van Duyne and co-workers, periodic arrays of surface confined nanostructures can be easily fabricated with tunable geometry, composition, and spectral response.³⁶ The LSPR of metal nanoparticles (e.g., Au, Ag, Cu), which arises from the surface-confined collective oscillation of free electrons, results in selective absorption, scattering, and local electromagnetic field enhancement.³⁷ It is well established that the position of the LSPR extinction maximum (λ_{max}) is extremely sensitive to the material, size, shape, interparticle distance, and, most importantly, the surrounding environment, i.e., local refractive index, which is the key to LSPR sensing.³⁷ Changes in the local refractive index can originate from a change of solvent environment or from molecular adsorption. Thus, LSPR sensors are inherently nonselective. However, they may be rendered selective by carefully designed surface modifications, which typically contain affinity agents to capture analytes and nonspecific adsorption resistant molecules to repel interfering molecules from adsorbing onto the surface. LSPR nanosensors have been widely used for the detection of small molecules,³⁸ proteins,^{39,40} and disease biomarkers, e.g., amyloid- β derived diffusible ligands for Alzheimer's disease;¹⁴ however, they have rarely been applied for whole-cell bacterial detection.^{13,19} Recently, an LSPR sensor array functionalized with three specific aptamers was developed to detect *L. acidophilus*, *S. typhimurium*, and *P. aeruginosa* simultaneously. The detection of bacteria was observed through the increase in plasmon peak intensity.¹⁹ In addition, an LSPR sensor was developed to detect whole-cell *Salmonella* on a substrate containing gold nanoparticles, produced by oblique angle deposition. A plasmon peak shift was observed, indicating the binding of *Salmonella* onto surface-bound anti-*Salmonella* antibody; however, the magnitude of the wavelength shift was not correlated with bacterial concentration.¹³

Here, we demonstrate a sensitive, selective, and semi-quantitative LSPR sensing platform to detect whole-cell microorganisms. As a proof-of-concept, this sensing platform was used to detect *P. aeruginosa* strain PAO1, using a surface confined aptamer as an affinity reagent. In this approach, *Pseudomonas* bacterial cells are directly pulled down from solution onto a sensor surface by a surface-confined aptamer, which serves as the cell-recognition motif. The aptamer used in our study was previously selected against inactivated *P. aeruginosa* using whole-cell SELEX.³² The sensor surface was derivatized with a mixture of biotinylated polyethylene glycol (Bt-PEG) thiol and PEG thiol, the Bt-PEG thiol being used to immobilize aptamers via a Bt-neutravidin-Bt linkage (the tetrameric neutravidin enables noncovalent binding of up to four biotin molecules), while PEG thiol was used to minimize nonspecific adsorption. Importantly, here we show that with this strategy bacterial concentration in solution can be correlated to the LSPR wavelength shift. Overall, this LSPR

sensing platform is characterized by (a) rapid detection (~3 h), (b) extraordinary sensitivity, down to the level of a single bacterial cell, (c) a clinically relevant dynamic range (10 – 10^3 cfu mL⁻¹), (d) selectivity over detection of other strains of *Pseudomonas*, and (e) excellent shelf life (2 months) when stored in ambient conditions. The LSPR aptasensor represents an important advance in pathogen diagnostic platforms and, as such, supports wider implementation of pathogen-targeted, narrow-spectrum antibiotics, as opposed to traditional broad-spectrum antibiotics.

EXPERIMENTAL SECTION

Materials

Biotin PEG thiol (MW 1000 Da) was purchased from Nanocs. PEG thiol (MW 550 Da) was purchased from Creative PEGWorks. Neutravidin (NA) and tris(2-carboxyethyl)phosphine hydrochloride (TCEP) were purchased from Thermo Fisher Scientific. Polystyrene beads (0.6 μ m mean particle size), sulfuric acid (95.0–98.0%), hydrogen peroxide (30% w/w in H₂O), and ethyl alcohol (200 proof) were purchased from Sigma-Aldrich. Paraformaldehyde (16% w/v aq soln, methanol free), Dulbecco's Phosphate Buffered Saline (1 $\times$, without calcium and magnesium), and micro cover glass (No. 1, 18 mm $\times$ 18 mm) were purchased from VWR. Lysogeny broth and granulated agar were purchased from BD Difco.

Biotinylated aptamer (Bt-aptamer, 5' [Bt]-ATACCA-GCTTATTCAATTCCCCCGTTGCTTTCGCTTTTCCTT-TCGCTTTTGTTTCGTTTCGTCCCTGCTTCCTTTCTTG-AGATAGTAAGTGCAATCT3') and fluorescently labeled biotinylated aptamer (6FAM-aptamer-Bt, 5' [6-carboxyfluorescein-ATACCAGCTTATTCAATTCCCCCGTTGCTT-TCGCTTTTCCTTTTCGCTTTTGTTTCGTTTCGTCCCTG-CTTCCTTTCTTGAGATAGTAAGTGCAATCT-[Bt]3') were synthesized by Sigma-Aldrich. Deionized (DI) water used in all experiments was prepared using a Milli-Q Gradient water purification system with a resistivity of 18.2 M Ω -cm at 25 °C (Millipore).

Bacterial Cell Culture, Fixation, and Counting

P. aeruginosa strains PAO1 and PA14, *Escherichia coli* DH5 α , and *Staphylococcus aureus* RN4220 used in these experiments were obtained from J. Shrout's laboratory (University of Notre Dame). All bacteria were streaked from freezer stocks and grown on lysogeny broth (LB) agar plates in a 37 °C incubator for 20 h. Cell cultures were first grown in a glass tube containing 6 mL of LB media for 9 h in a 37 °C incubator with shaking set at 240 rpm. Nine-hour bacterial samples (30 μ L) were removed and serially diluted three times. All four serial dilutions were incubated at a 37 °C incubator for 14 h with shaking set at 240 rpm. All bacteria were harvested in their exponential growth phase. The cell culture was then washed twice using phosphate buffered saline (PBS) solution, and its optical density at 600 nm (OD₆₀₀) was adjusted to 0.80. A serial dilution was carried out to achieve various bacterial concentrations. To inactivate bacterial cells, 0.7 mL of bacterial solution was added into a microcentrifuge tube containing 0.7 mL of 6% paraformaldehyde solution, prepared by diluting 16% paraformaldehyde solution in PBS. As a control, 0.7 mL of PBS solution was added to a microcentrifuge tube containing 0.7 mL of 6% paraformaldehyde solution. Both tubes were heated at 62.5 °C water bath for 1 h to inactivate bacteria in solution. All samples

were cooled to room temperature prior to use. All bacterial cell densities were confirmed using standard microbiology plating and counting methods. Specifically, the OD₆₀₀ of bacterial solution was adjusted to 0.80 upon harvesting cell culture. Each bacterial solution was diluted 6 orders of magnitude in LB media, and 100 μ L of diluted bacterial solution was added onto a LB agar plate. The bacterial solution was spread evenly on a LB agar plate using a metal spreader, which was dipped in ethanol and flamed before and after each use. The bacteria containing LB agar plates were then incubated overnight at 37 °C, and the colonies were counted the next day. Bacterial cell density was obtained by averaging the counts from nine LB agar plates (three plates were prepared in one experiment, and the same experiment was repeated three times on different days).

Sensor Fabrication

LSPR sensor chips were fabricated using NSL.³⁶ Briefly, glass slides were soaked in piranha solution (*Caution: piranha, 3:1 sulfuric acid/hydrogen peroxide, is a strong oxidizer and should be used with extreme caution!*) overnight and rinsed with water. Polystyrene (PS) beads were gently spread as a monolayer at an air–water interface. The PS bead monolayer was transferred by first immersing a clean glass slide through the monolayer at an oblique angle, then translating the slide laterally to transfer PS beads onto the glass surface. The PS bead coated glass slides were dried at 60 °C prior to electron beam evaporation (UNIVEX 450B, Oerlikon) of Cr (1 nm) and Au (50 nm). The slides were then sonicated in chloroform to remove the PS beads, rinsed with water, dried with N₂ gas, and stored under N₂. Each Au nanotriangle patterned glass slide (76.2 mm $\times$ 25.4 mm) was diced into six pieces (10.16 mm $\times$ 15.24 mm) using a dicing saw (Disco DAD3240) equipped with a diamond blade (Thermocarbon).

Sensor Surface Modification

A 1:3 (v:v) mixture of 1 mM Bt-PEG thiol:1 mM PEG thiol was self-assembled onto the sensor surface with mild shaking for 16 h. Then, 0.2 mL of neutravidin solution (1 mg/mL) was added with shaking for 1 h. Bt-aptamer solution (0.2 mL per tube) was heated in a 95 °C water bath for 30 min, cooled to room temperature, and then incubated with the neutravidin-modified sensor chips for 1 h with mild shaking under controlled humidity conditions. Sensor chips were gently rinsed with water and dried with N₂ after each step.

Bacterial Detection

In a typical experiment, one chip was exposed to 0.2 mL of control solution, and five chips were exposed to 0.2 mL of inactivated bacterial solution under controlled humidity conditions. Control and bacterial samples were incubated at 37 °C for 1 h. Each chip was then washed three times in PBS solution for 5 min per wash, followed by water wash for 30 s. All sensor chips were dried with N₂ gas setup for 5 min prior to characterization.

Characterization

LSPR spectra were acquired on a UV–visible/NIR spectrometer (Jasco V-670) equipped with a 60 mm integrating sphere (Jasco ISN-723). Each spectrum was averaged over three spectral accumulations collected from 400–1400 nm at 0.5 nm interval, and all extinction

spectra were normalized. The probe beam size was ca. 8 mm × 9 mm. For LSPR measurements in liquid, ~50 µL of PBS solution was added onto the sensor surface, and a coverslip was added to confine the liquid prior to measurement. Scanning electron microscope images of Au nanotriangle arrays with and without bacteria were collected using a field-emission scanning electron microscope (FEI Magellan 400) at 5.00 kV. Prior to imaging, a thin layer of iridium (2.5 nm) was sputtered on the substrate to avoid surface charging.

RESULTS AND DISCUSSION

Sensor Fabrication and Sensor Surface Modification

A schematic illustration of a Au nanotriangle array, its layer-by-layer surface construction, and the bacterial detection scheme are shown in Figure 1a. NSL was used to fabricate Au nanotriangle arrays on glass, as shown in Figure 1b. Au nanotriangles with in-plane width of 210.5 ± 9.1 nm, out-of-plane height of 47.6 ± 0.4 nm, interparticle distance of 121.3 ± 7.7 nm, and a truncated tetrahedral shape were obtained. SEM images of tilted and dissected Au nanotriangle arrays are shown in Supporting Information, Figure S1(a),(b), respectively.

Au nanotriangle arrays were modified by self-assembly of (1:3) Bt-PEG thiol:PEG thiol directly onto the Au surface of the nanotriangles. Tetrameric neutravidin (NA) was then linked to the surface bound biotin through its strong noncovalent interaction ($K_a \sim 10^{13} \text{ M}^{-1}$).³⁹ PEG thiol was specifically chosen to repel nonspecifically adsorbed molecules and bacteria.⁴¹ The molecular weight difference between Bt-PEG thiol (MW 1000 Da) and PEG thiol (MW 500 Da) results in different chain lengths, facilitating the longer chain Bt-PEG thiol in its function to capture NA. The 1:3 (v:v) ratio (Bt-PEG thiol:PEG thiol) was selected, after careful optimization experiments, viz. Supporting Information, Figure S2, to maximize PAO1 binding onto the surface while minimizing steric hindrance between affinity reagents.

Subsequently, biotinylated aptamer (Bt-aptamer) was introduced to the neutravidin-modified sensor surface in order to provide a capture agent for *P. aeruginosa*. This aptamer sequence was previously selected against fixed *P. aeruginosa* ($K_d = 17.27 \pm 5.00$ nM) using whole-cell SELEX.³² The sensor surface was then exposed to inactivated bacteria, which were pulled down from solution by the surface-immobilized aptamers. The maximum amount of neutravidin and aptamer immobilized on the sensor surface was estimated to be $8.5 \times 10^{12} \text{ cm}^{-2}$ and $1.7 \times 10^{13} \text{ cm}^{-2}$, respectively. A representative SEM image of a captured *P. aeruginosa* cell is shown in Figure 1c. The binding of aptamer and *P. aeruginosa* cells was verified using confocal fluorescence microscopy. A fluorescence image and an overlaid image of fluorescence and transmission of 6FAM-labeled aptamer bound to *P. aeruginosa* are shown in Supporting Information, Figure S3(a),(b), respectively.

Characterization of LSPR Measurements in Buffer and Air

UV–visible extinction spectroscopy has been used to characterize LSPR resonances of Au nanoparticles both in solution²⁰ and on surfaces¹³ and has been used to study molecular interactions at surfaces based upon LSPR wavelength shifts upon binding.^{38,39} It is well

established that the refractive index of the surrounding environment affects the wavelength of the LSPR.³⁷ To ascertain the optimal conditions for the aptamer-based assays, the sensitivities of LSPR measurements in liquid (buffer) and air were compared by measuring the LSPR wavelength shift (λ) at several points in the assembly and capture process in both PBS and air. Measurements after immobilization of Bt-PEG thiol/PEG thiol, neutravidin, and Bt-aptamer onto the Au nanotriangle array, Supporting Information, Figure S4, produced larger LSPR shifts in air than in PBS. Furthermore, in the bacterial pull-down experiment, bacteria could not be detected at low PAO1 concentrations (10^3 cfu mL⁻¹) in PBS, a small LSPR wavelength shift being detected only at 10^7 cfu mL⁻¹, Supporting Information, Figure S5 and Table S1. These results can be understood based on the larger refractive index difference between air ($n \sim 1.0$) and bacterial cell ($n \sim 1.4$)^{13,42} compared to replacement of buffer ($n \sim 1.35$) with the same bacterium. The presence of bacteria on the sensor surface was verified by SEM images (data not shown). In addition, the variability of LSPR measurements made in PBS, Supporting Information, Figure S6 and Table S2, was significantly higher than those made in air. Thus, given the statistically significant differences in both sensitivity and experimental precision, all LSPR measurements were carried out in air.

Characterization of *P. aeruginosa*-Derived LSPR Shifts

Here, bacterial pull-down was monitored by careful measurements of the UV-visible extinction spectrum. Care was taken to perform the measurements under controlled humidity conditions because the LSPR sensor chips are potentially sensitive to humidity and temperature. Figure 2 shows representative extinction spectra of a bare Au nanotriangle array (curve 1, black), a Bt-PEG thiol/PEG thiol (1:3), neutravidin, and Bt-aptamer modified Au nanotriangle array (curve 2, red), and the same array after exposure to a solution containing 10 cfu mL⁻¹ *P. aeruginosa* (curve 3, blue). Originally, the extinction maximum of the bare Au nanotriangle array was measured at 973.0 nm. Upon surface modification, the LSPR extinction maximum shifted to 1004.5 nm, i.e., $\lambda = 31.5$ nm, indicating successful surface modification. Once bacteria were captured on the sensor surface, the LSPR peak red-shifted again to 1014.5 nm, an additional $\lambda = 10$ nm. Although the specific shifts varied from sample to sample, the general pattern of successive red-shifts upon exposure, first to capture motif, then to target bacteria, was uniformly observed, Supporting Information, Figure S7.

When exposing the sensor surface to bacteria, it is important to simultaneously minimize salt crystal formation and avoid lysis of surface bound bacteria. When dried, salt crystals on sensor surfaces, viz. Supporting Information, Figure S8(a), can contribute to an apparent LSPR wavelength shift, thus confounding the bacterial-derived signal. In addition, lysis of bacteria, Supporting Information, Figure S8(b), could give rise to LSPR signals not correlated with the number of bacteria present in the sample. Thus, substantial effort was devoted to identifying conditions sufficiently stringent to minimize salt crystal formation, yet gentle enough to leave surface-captured bacteria intact. On the basis of extensive optimization experiments, the following protocol was identified. After 1 h incubation with bacteria, sensor chips were washed three times with PBS for 5 min each to remove unbound bacteria, followed by water immersion for 30 s. This combination proved to be a good

compromise capable of removing the majority of salts without causing bacterial lysis, Supporting Information, Figure S8(c).

Sensitivity

The sensitivity of sensor chips was evaluated by exposing them to various concentrations of *P. aeruginosa* and monitoring the LSPR wavelength shift (λ). Before evaluating sensitivity, it is important to understand the various contributions to sample-to-sample variability, which were compared using interbatch and intrabatch variability. Each batch consisted of a microscope slide diced into six small pieces: one chip as a control and the other five chips for testing separate bacterial samples. Each bacterial concentration was studied with 9–14 replicates. The interbatch variation was tested using two (or three in the case of 10 cfu mL⁻¹) microscope slides fabricated in different batches but exposed to the same bacterial concentration. The interbatch variation was analyzed using the difference of means, which ranged from 0.2–4.0 nm (Supporting Information, Figure S9). The small variation indicates that sensor chip fabrication process is reproducible, bacterial preparation protocol is reliable, and batch-to-batch variation is minimal. Intrabatch variation ranged from 1.3–5.5 nm, in statistical agreement with the interbatch measurements, as expected. However, the intrabatch variation was found to increase with increasing bacterial load. Because the number of binding sites grossly exceeds the bacterial load, bacterial binding on the sensor surface is a rare event, which can be described by a Poisson distribution, for which the variance and mean are equal.

Figure 3 shows the magnitude of LSPR wavelength shift (λ) as a function of bacterial concentration from 0 to 10⁵ cfu mL⁻¹. In contrast to other surface-based LSPR bacterial sensing studies,¹³ we observed a correlation between the magnitude of the LSPR wavelength shift and log bacterial concentration with a limit of detection (LOD) of 10 cfu mL⁻¹ *P. aeruginosa*, a level at which the expectation loading at the sensor surface is $\langle n \rangle = 1$ bacterium. Control experiments (0 cfu mL⁻¹) showed no statistically significant response compared to the unexposed pull-down surface, with a wavelength shift $\lambda = -1.3 \pm 2.3$ nm. Experiments performed at the ultralow surface bacterial loading ($\langle n \rangle = 0.1$) of 1 cfu mL⁻¹ resulted in a wavelength shift of $\lambda = 2.1 \pm 1.7$ nm, not statistically distinguishable from the control, as expected.

Single bacterium experiments performed at 10 cfu mL⁻¹ gave rise to a wavelength shift of $\lambda = 8.9 \pm 3.9$ nm, which is statistically different than the control and the 1 cfu mL⁻¹ result at the 99.9% confidence level. This exquisite sensitivity may originate from: (i) the inherent sensitivity of the LSPR sensor responding to small refractive index changes near the Au nanotriangle array surface,⁴³ combined with the relatively thin capture layer which brings bacteria closer to the LSPR sensing volume,²⁷ or (ii) a single bacterial cell on surface could distort local electric field around Au nanotriangles and provided an extraordinarily large signal. The LSPR wavelength shift saturated at concentrations higher than 10³ cfu mL⁻¹, although as suggested by the inset of Figure 3, bacterial samples in the 10–10³ cfu mL⁻¹ can all be distinguished both from the control and the 1 cfu mL⁻¹ result at the 99.9% confidence level. Saturation of the LSPR signal at the relatively small loading of 10³ cfu mL⁻¹ is

consistent with the extraordinary LOD observed and suggests mechanisms which enhance the LSPR response in the proximity of whole cell bacteria.

Selectivity

LSPR-based sensors are not inherently selective; their selectivity comes from the choice of affinity reagent, in our case, an aptamer specially selected to recognize fixed *P. aeruginosa* cells. Very few *Pseudomonas*-specific aptamer sequences are available, and the aptamer used in this study was previously identified by selection against inactivated *P. aeruginosa* using whole-cell SELEX,³² although the molecular nature of the interaction between the aptamer and the *P. aeruginosa* cell envelope has not been delineated. The selectivity of the aptamer was tested with two different strains of *P. aeruginosa* (PAO1, PA14), *E. coli* DH5 α , and *S. aureus* RN4220 using the LSPR-based sensing platform. All four bacteria were harvested in the exponential growth phase (Supporting Information, Figure S10). Figure 4 shows that bacterial binding to sensor surfaces resulted in average LSPR wavelength shifts of 0.4 ± 1.6 , 19.3 ± 7.2 , 3.5 ± 2.3 , 4.7 ± 2.8 , and 10.2 ± 5.8 nm for control, PAO1, PA14, *E. coli*, and *S. aureus*, respectively. The Student's *t* test (Supporting Information, Table S3) shows that there is a significant difference between PAO1 and control, PA14, and *E. coli* at the 99.9% confidence level, as well as a significant difference between PAO1 and *S. aureus* at the 99% confidence level. These results indicate that this aptamer has strong affinity to the PAO1 strain of *Pseudomonas* and negligible affinity to PA14 and *E. coli*, indicating that overall the aptamer is specific to recognizing *P. aeruginosa* strain PAO1. The specificity of the aptamer for PAO1 over *S. aureus* is somewhat lower. Noting that *S. aureus* exhibits different morphology than *Pseudomonas*, the wavelength-shift sensitivity to spherical *S. aureus*, viz. Supporting Information, Figure S11, may differ from the rod-shaped *Pseudomonas* and *E. coli*. In addition, some cross-reactivity may arise from *S. aureus* surface binding motifs that are inherently cross-reactive with the *Pseudomonas* aptamer. The selectivity of the sensor chip may be improved by: (1) using a modified SELEX process to identify an aptamer with higher specificity, and (2) changing the geometry of the nanotriangles to minimize sensitivity to spherical bacteria. In addition, the clinical and environmental potential of this LSPR sensing platform may be expanded by applying it to different samples, for example, environmental isolates and clinical samples at various stages of infection, e.g., planktonic vs biofilm. Such samples will provide the impetus for screening new aptamers, from which a potential library can be developed.

Stability

The stability of the sensor chip in ambient conditions is important to its potential use in the field and in point-of-care applications. An ideal sensor chip would be stable over a wide range of temperatures and humidities for a long period of time. In this study, the stability of unmodified, Bt-PEG thiol/PEG thiol (1:3), and Bt-PEG thiol/PEG thiol (1:3)/neutravidin modified sensor chips was monitored for 2 months in ambient conditions by following the shifts in the extinction maxima. Figure 5 shows the temporal evolution of LSPR signals arising from unmodified Au nanotriangle arrays (group 1), arrays with Bt-PEG thiol/PEG thiol (1:3) (group 2), and arrays with subsequent modification of Bt-PEG thiol/PEG thiol (1:3) with neutravidin (group 3). Each set was tested using three different sensor chips. Comparing day 1 to day 56, the LSPR wavelength shifts observed for groups 1–3 were 12.5

± 1.8 , 0.2 ± 0.3 , and 4.0 ± 2.2 nm, respectively. Overall, sensor chips coated with Bt-PEG thiol/PEG thiol with or without neutravidin were significantly more stable than unmodified Au nanotriangles, and both were observed to be stable in ambient conditions for at least 2 months. Additionally, all sensor chips, including the unmodified surface, were stable for at least 2 months when stored in N₂. The stability of these surface-modified sensor chips in ambient conditions suggests the possibility of mass production and long-term storage of these sensor chips for field and clinical applications.

CONCLUSIONS

An LSPR sensing platform was developed for the detection of whole-cell microorganisms, using *P. aeruginosa* strain PAO1 as a model system. The sensor chip was designed to pull down whole-cell *P. aeruginosa* cells from solution via a surface-confined aptamer. Capture of bacteria was monitored through the resulting red-shift in wavelength of the LSPR extinction maximum. This LSPR whole-cell microbial sensing platform is (a) rapid (~3 h), (b) highly sensitive, down to the level of a single bacterium, (c) selective against other strains of *Pseudomonas* and *E. coli*, and (d) stable in ambient conditions for 2 months. Importantly, the general whole-cell LSPR sensing scheme demonstrated here could be translated to the detection of other microorganisms, including other bacteria as well as viruses, by switching affinity agents. This robust LSPR sensing platform has the potential to be used in clinical diagnostics and therapeutics, including in-the-field detection and point-of-care applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(a) Schematic illustration of LSPR sensor chip (left) with legend (right). (b,c) Representative SEM images of Au nanotriangle arrays before (b), and after (c), exposure of the sensor surface to *P. aeruginosa* strain PAO1. The cell shown was likely captured during cell division.

Figure 2. Representative LSPR extinction spectra of Au nanotriangle array (curve 1, black), the same array after Bt-PEG thiol/PEG thiol, neutravidin, and Bt-aptamer modification (curve 2, red), and after exposure of the modified surface to 10 cfu mL⁻¹ PAO1 (curve 3, blue).

Figure 3.

Box plot showing LSPR wavelength shift as a function of bacterial concentration. Each color represents a different bacterial concentration with 9–14 individual data points (solid diamonds are results of individual experiments). The central box line represents 50th percentile. The lower and upper boundary lines represent the 25th and 75th percentiles, respectively. The circle represents the mean of all data. The whisker extends to one standard deviation. Diagonal crosses (x) represent the 1st and 99th percentiles. Inset shows the linear range of the calibration curve, where error bar represents the standard deviation of all data points at a specific bacterial concentration. A linear fit of LSPR shift vs log bacterial concentration gives $y = 6.0x + 2.2$ with $R^2 = 0.99$.

Figure 4.

Box plot demonstrating LSPR wavelength shift obtained from different bacteria employing the PAO1-specific aptamer. A bacterial load of 10^3 cfu mL⁻¹ was tested in each case. Each color represents a different bacterial sample with 8–10 individual data points (solid diamonds are results of individual experiments). The central box line represents 50th percentile. The lower and upper boundary lines represent the 25th and 75th percentiles, respectively. The circle represents the mean of all data. The whisker extends to one standard deviation. Diagonal crosses (x) represent the 1st and 99th percentiles.

Figure 5. LSPR wavelength stability of sensor chips stored in ambient conditions as a function of time. Groups 1, 2, and 3 represent Au nanotriangle array, Bt-PEG thiol/PEG thiol modified Au nanotriangle array, and Bt-PEG thiol/PEG thiol/neutravidin modified Au nanotriangle array, respectively. Three different sensor chips were used to test each condition.