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Rapid single cell detection of *Staphylococcus aureus* by aptamer-conjugated gold nanoparticles

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Staphylococcus aureus **is one of the most important human pathogens, causing more than 500,000 infections in the United States each year. Traditional methods for bacterial culture and identification take several days, wasting precious time for patients who are suffering severe bacterial infections. Numerous nucleic acid-based detection methods have been introduced to address this deficiency; however, the costs and requirement for expensive equipment may limit the widespread use of such technologies. Thus, there is an unmet demand of new platform technology to improve the bacterial detection and identification in clinical practice. In this study, we developed a rapid, ultra-sensitive, low cost, and non-polymerase chain reaction (PCR)-based method for bacterial identification. Using this method, which measures the resonance light-scattering signal of aptamer-conjugated gold nanoparticles, we successfully detected single *S. aureus* cell within 1.5 hours. This new platform technology may have potential to develop a rapid and sensitive bacterial testing at point-of-care.**

Staphylococcus aureus is a facultative anaerobic, Gram-positive bacterium discovered by Dr. Alexander Ogston in 1880[1]. Literature reports suggest that about 30% to 50% of the population has been carriers of *S. aureus*[2] at one time in their lives and about 20% are long-term carriers[3]. *S. aureus* is widespread in the environment and has become one of the most commonly isolated pathogens in hospital-acquired infections[4]. Moreover, *S. aureus* can cause numerous illnesses, from minor skin infections to life-threatening diseases, such as abscesses[5], pneumonia[6], meningitis[7], endocarditis[8], and septicemia[9]. According to reports of the National Institutes of Health and Centers for Disease Control and Prevention, *S. aureus* infects 500,000 people yearly in America, more than 94,000 of which are cases of life-threatening, antibiotic-resistant *S. aureus* infections[10].

Bacterial culture and metabolic tests are standard protocols for bacterial identification in use by most hospitals. But this process might take days for identification of the pathogenic bacteria—an unacceptable delay in emergency and critically ill situations such as sepsis. For this reason, several ultra-sensitive detection methods based on nucleic acid amplification, such as PCR (polymerase chain reaction)[11], LCR (ligase chain reaction)[12] and SDA (strand displacement amplification)[13], among others, have been introduced. All of these technologies are capable of detecting low numbers of bacterial cells within several hours. However, these technologies require prior isolation of bacterial DNA, preparation of enzyme reaction mix, and expensive instruments for nucleic acid amplification. These high costs and complex procedures limit the widespread use of these technologies for clinical diagnosis. Antibody-based immunoassays for bacterial identification are well established and have been used for many years[14]. However, ultrasensitive detection with such approaches is limited by the fact that antibodies are proteins and thus cannot be amplified. This limitation was circumvented by the development of a technology called immuno-PCR, in which the antibody is cross-linked with a DNA "barcode" for PCR amplification[15]. Although this technology is sensitive, the conjugation and purification of antibody-DNA complexes is still a daunting task.

Aptamers are DNA or RNA molecules that can fold into a variety of structures[16,17]. Like an antibody, a good aptamer can specifically bind to its target with pico- to nanomolar affinity[18]. Importantly, unlike antibodies, aptamers can be directly amplified by PCR. Since their discovery in the late 1990s, aptamers have been widely used in many applications, including target detection, enzyme inhibition, receptor regulation, and drug delivery[19–22]. Several bacterial aptamers had been isolated and recently used in identification of bacteria, including *Escherichia coli*[23], *Mycobacterium tuberculosis*[24], *Salmonella enterica*[25], and *Bacillus anthracis*[26]. However, none of these studies reported showed the capability of identifying extremely low numbers of target bacteria without a PCR reaction.

Gold nanoparticles (GNPs) are gold particles that range in size from 1 nm to several hundred nanometers and possess strong light-scattering properties[27,28]. The intensity of light scattering is based on the size of the particle. Moreover, GNPs can be easily conjugated with protein or modified DNA molecules through sulfhydryl linkages. These properties make GNPs a useful tool for ultrasensitive molecular detection. Moreover, a GNP-based amplification method has been developed and the system was shown to be capable of detecting prostate-specific antigen with sensitivity in the attomolar range[29].

In the current study, we determined the resonance light-scattering intensity of different sizes and concentrations of GNPs in a liquid-phase system under 638 nm diode laser beam stimulation. *S. aureus*-targeting aptamers were identified by cell-based SELEX (Systematic Evolution of Ligands by Exponential Enrichment), and dissociation constants and binding specificity were characterized. Two of the isolated aptamers, SA17 and SA61, recognized *S. aureus* with high specificity and nanomolar affinity. Using these aptamers, we developed a rapid, ultra-sensitive, low cost, and non-PCR–based method that combines aptamer-conjugated GNPs and a resonance light-scattering–detection system. In this method, the number of SA17 and SA61 aptamers or aptamer-conjugated GNPs bound to single *S. aureus* cells is quantified by quantitative PCR (qPCR). For ultrasensitive detection of *S. aureus* cells, aptamers are conjugated onto GNPs followed by bead-based amplification. After amplification, one bacterial cell was capable of generating more than 10^4 GNPs, and amplified GNPs could be detected by the light-scattering–sensing system. Single cell detection was reached within 1.5 hours without expensive equipment such as thermal cyclers or centrifuges.

Results

To identify specific aptamers against *S. aureus*, we developed a cell-based method based on SELEX, as described in **Supplementary Figure S1**. The details of the protocol are described in Supplementary Information. Briefly, 10^7 *S. aureus* cells were first incubated with an aptamer pool and bound aptamers were isolated after washing five times. After removing non-specific aptamers, bound aptamers were refolded and incubated with 10^8 *Staphylococcus epidermidis* for counter-selection. Like *S. aureus*, *S. epidermidis* is a common flora on human skin and belongs to the same genus as *S. aureus*. We hypothesized that if the isolated aptamer could distinguish *S. aureus* from *S. epidermidis,* it might target a specific structure of *S. aureus* and should therefore not cross-react with bacteria from other genera. The aptamers that survived counter-selection were amplified and purified as single-stranded DNA (ssDNA) aptamers. Purified ssDNA aptamers were added into a fresh batch of *S. aureus* cells to start the next SELEX round. After eight rounds of SELEX, the pool was cloned and sequenced. The dendrogram of the isolated sequences is shown in **Supplementary Figure S2** and complete sequence information is presented in **Supplementary Table 1**. Aptamer clones SA17 and SA61 were selected for their high specificity and relatively strong total binding signal against *S. aureus*.

The specific binding of SA17 and SA61 was verified by fluorescence microscopy (**Fig. 1a**) and scanning electron microscopy (SEM) (**Fig. 1b**), and quantified in immunofluorescence assays (IFAs) (**Fig. 1c**). Other than weak IFA signals in assays of SA61 with *S. epidermidis* and *P. aeruginosa* (**Fig. 1c**), SA17 and SA61 did not cross-react with 13 other bacterial species, including *Bacillus subtilis*, *Citrobacter freundii*, *E. coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Moraxella catarrhalis*, *Salmonella enterica*, *Shigella boydii*, *Shigella flexneri*, *Streptococcus bovis Streptococcus pneumoniae Staphylococcus saprophyticus,* and *Staphylococcus haemolyticus* (**Fig. 1**, **Supplementary Fig. S3**, and **Supplementary Table 2**). We also analyzed six different *S. aureus* strains (ATCC: 6538, 6538DR, 6538P, 12600, 25923, 29213) for their interactions with SA17 and SA61 in IFAs. The results indicated that SA17 and SA61 were able to recognize all six strains of S. aureus (**Fig. 1c**). Although SA61 cross-reacted with *S. epidermidis* and *P. aeruginosa*, these interactions were significantly weaker than those with *S. aureus* strains. The successful detection of all six *S. aureus* strains by SA17 and SA61 suggested that these aptamers might be capable of very high detection rates in tests of clinical *S. aureus* samples. The results of qPCR, which was also used to study the binding specificity, were consistent with IFA findings (data not shown). The affinities of SA17 and SA61 for *S. aureus* cells, measured as dissociation constants (K_ds), were determined using a qPCR-based total binding assay (**Supplementary Fig. S4a**). The results showed that the K_ds of SA17 and SA61 aptamers for *S. aureus* cells were 35 and 129 nM, respectively. The secondary structures of SA17 and SA61, predicted by mfold software, are shown in **Supplementary Figure S4b**. Immobilization of the aptamers on GNPs might alter the aptamer functional structure. For this reason, the affinities of SA17-GNPs and SA61-GNPs for *S. aureus* must be re-evaluated. The result showed that the K_ds of SA17-GNPs and SA61-GNPs for *S. aureus* were 3.03 and 9.9 nM, respectively, a significant enhancement compared to the free aptamer forms (**Supplementary Fig. S4c**). We also estimated the number of SA17 and SA61 molecules bound to single *S. aureus* cells by qPCR. The results showed that 900 to 1200 molecules of SA17 and 1500 to 2300 molecules of SA61 bound a single *S. aureus* cell (**Supplementary Fig. S5**). Collectively, these data suggest that one bacterium can generate thousands of DNA sequences and, unlike immuno-PCR, these sequences can directly serve as a DNA barcode for detection.

Two strategies were used for detecting the interaction between aptamer-GNPs and *S. aureus:* a direct detection method and a bead-amplification method (**Fig. 2**). For both methods, SA17 and SA61 aptamers were first conjugated to 60-nm GNPs by a thio-modified adaptor sequence (**Fig. 2a**).

The aptamer-GNP–binding capacity capacity of a single bacterial cell was then analyzed directly using a binding assay based on size separation employing 0.22-μm filters, as shown in **Figure 2b**. In this assay, the aptamer-GNP complex was incubated with different numbers of *S. aureus* cells for 30 minutes on ice. After washing away the unbound aptamer-GNPs, bacteria-bounded GNPs were eluted by NaOH and collected for the analysis of resonance light-scattering signals (see below).

In the bead-based amplification method for *S. aureus* detection (**Fig. 2c**), magnetic beads were pre-coated with SA17 (SA17-MAGs), and 60-nm GNPs were pre-coated with dual biotin-labeled SA61 (b-SA61-GNPs). The SA61 aptamer was selected for conjugating with GNPs because of its higher binding capacity for *S. aureus* compared to SA17. SA17-MAG and b-SA61-GNPs were incubated with *S. aureus* cells and separated by a magnet. After removing the unbound b-SA61-GNPs, the b-SA61 sequences coated onto beads were eluted and incubated with adaptor-GNPs and streptavidin-MAG. The b-SA61 aptamer acts as a bridge that allows adaptor-GNPs to be captured by streptavidin-MAG, increasing the total number of GNPs for the detection system. The total duration from amplification to detection was 1.5 hours. With this amplification method, one aptamer-GNP can generate 1500 aptamer sequences (**Supplementary Fig. S6**), resulting in the amplification of the number of GNPs by several orders of magnitude.

To investigate the possibility of using the resonance light-scattering property of GNPs in ultrasensitive bacterial detection, we constructed an instrument consisting of a 638-nm laser light source, an objective lens, a photodiode, an amplifier and a digital voltmeter, as described in **Figure 3a**. Samples containing GNPs are excited by 638-nm laser, and the generated resonance light-scattering signals are converted into an electrical signal by the photodiode. The electrical signals are further amplified by an amplifier and read by the voltmeter. **Supplementary Figure S7a** shows the light-scattering signal of 3×10^5 60-nm GNPs/μl with serial 2-fold dilutions. The light-scattering intensities increased linearly with GNP concentration (**Supplementary Fig. S7b**) and exponentially with the sixth power of the

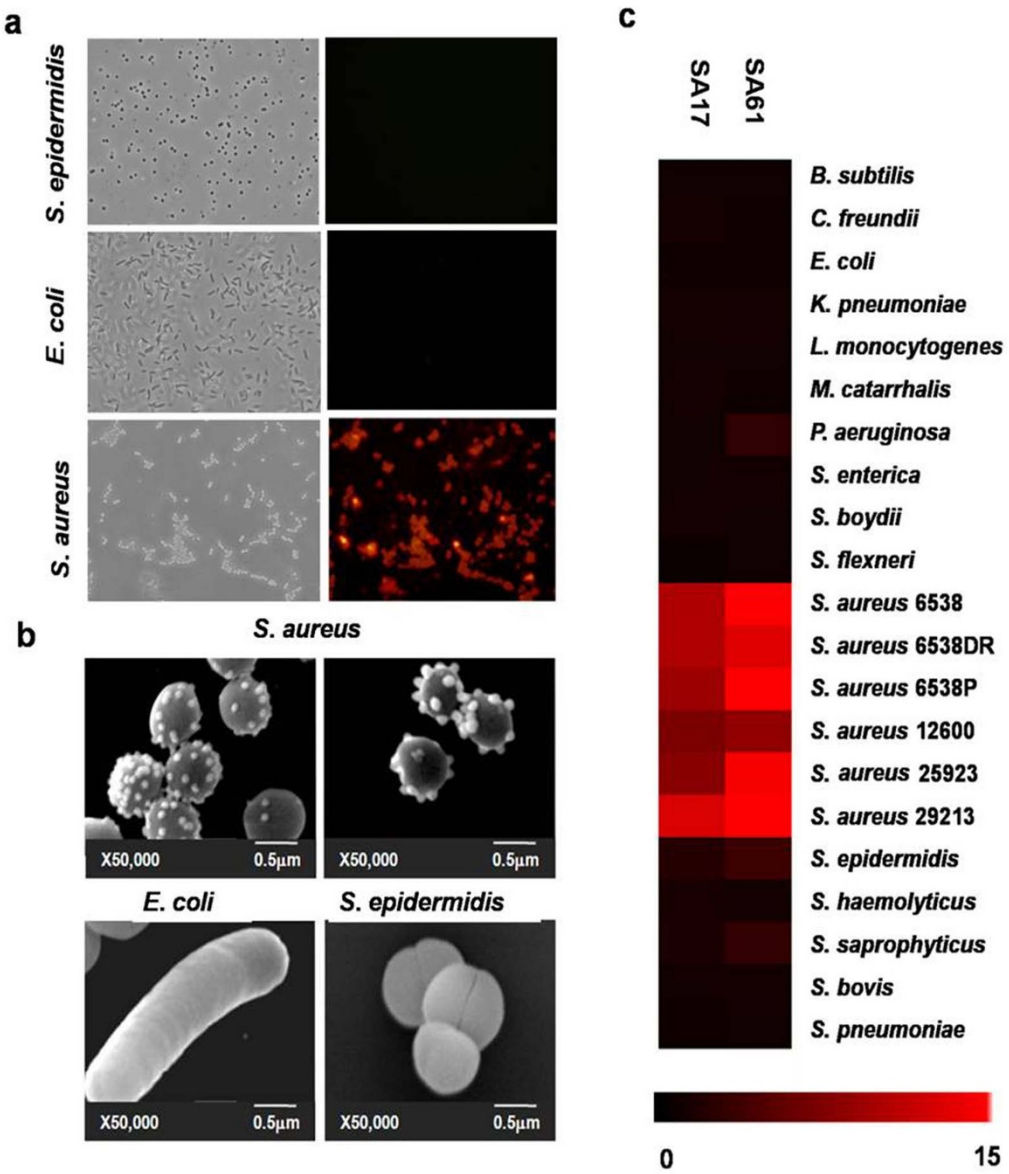

Figure 1 | Characterization of the light-scattering signal of GNPs. (a), Fluorescence microscopic detection of SA17 binding to different bacteria. Biotin-labeled SA17 (500 nM) was incubated with *S. aureus* cells at 4°C for 30 minutes and stained with streptavidin-PE. Bright field (left) and fluorescent (right) images of each of the tested bacterial strains are shown. (b), SEM images (50,000× enlargement) show the interaction of aptamer-GNPs and *S. aureus*. *Top panel:* interaction of SA17-coated 60- or 100-nm aptamer-GNPs and *S. aureus*;. *Bottom panel*: absence of interaction of 60-nm aptamer-GNPs with *S. epidermidis* and *E. coli*. (c), Quantification of IFA results showing interactions of SA17 and SA61 with 21 bacterial strains. FAM-labeled SA17 or SA61 (500 nM) were incubated with 100 μl suspensions of different bacterial strains (OD_{600} = 1) at 4°C for 30 minutes. Bound aptamers were eluted and analyzed using a florescence reader.

particle radius (**Supplementary Fig. S7c**). This result is consistent with the previous finding that the light-scattering intensity of GNPs is enhanced with increases in GNP size[27]. The lower limit of detection of the instrument was 63 ± 21 GNPs/μl for 100-nm particles and 508 ± 176 GNPs/μl for 60-nm particles. At these levels, SA17- and SA61-GNPs could detect as few as 312 and 1250 bacterial cells, respectively (**Fig. 3b**). Particle numbers were quantified from light-scattering signals in **Figure 3b** by reference to the standard curve of 60-nm GNPs shown in **Supplementary Figure S7b**. The equation was: particle numbers/μl = (signal intensity − 10.913) × 10^3. According to the equation, a single bacterial cell could bind 14.5 molecules of SA17- or 35.5 molecules of SA61-conjugated 60 nm-GNPs, respectively. The relative sizes of GNPs and *S. aureus* cells are shown in SEM images (**Fig. 1b**). Tests of samples containing extremely low numbers of bacteria revealed that analysis of light-scattering signals following the amplification procedure shown in **Figure 2c** was able to detect as few as 10 bacterial cells (**Fig. 3c**). However, at dilutions approaching a single bacterium, variations in detection were significant, possibly reflecting the unequal distribution of bacterial cells in the solution during the dilution process.

For accurately demonstrating if this bead-based amplification system could detect as few as single bacterial cell, *S. aureus* suspension was serially diluted and plate counted revealing that 1 OD_{600} of suspension contained 1.5 × 10^8 cells/ml. Ten bacterial cells roughly estimated by optical density was further determined by plate count and bead-based amplification assay. The suspension containing approximately 10 bacteria cells was divided into 30 independent samples. After analyzed by bead-based amplification assay, the positive samples were marked and served as containing one bacterial cell each (**Supplementary Fig. S8b**). In four independent assays, the cell

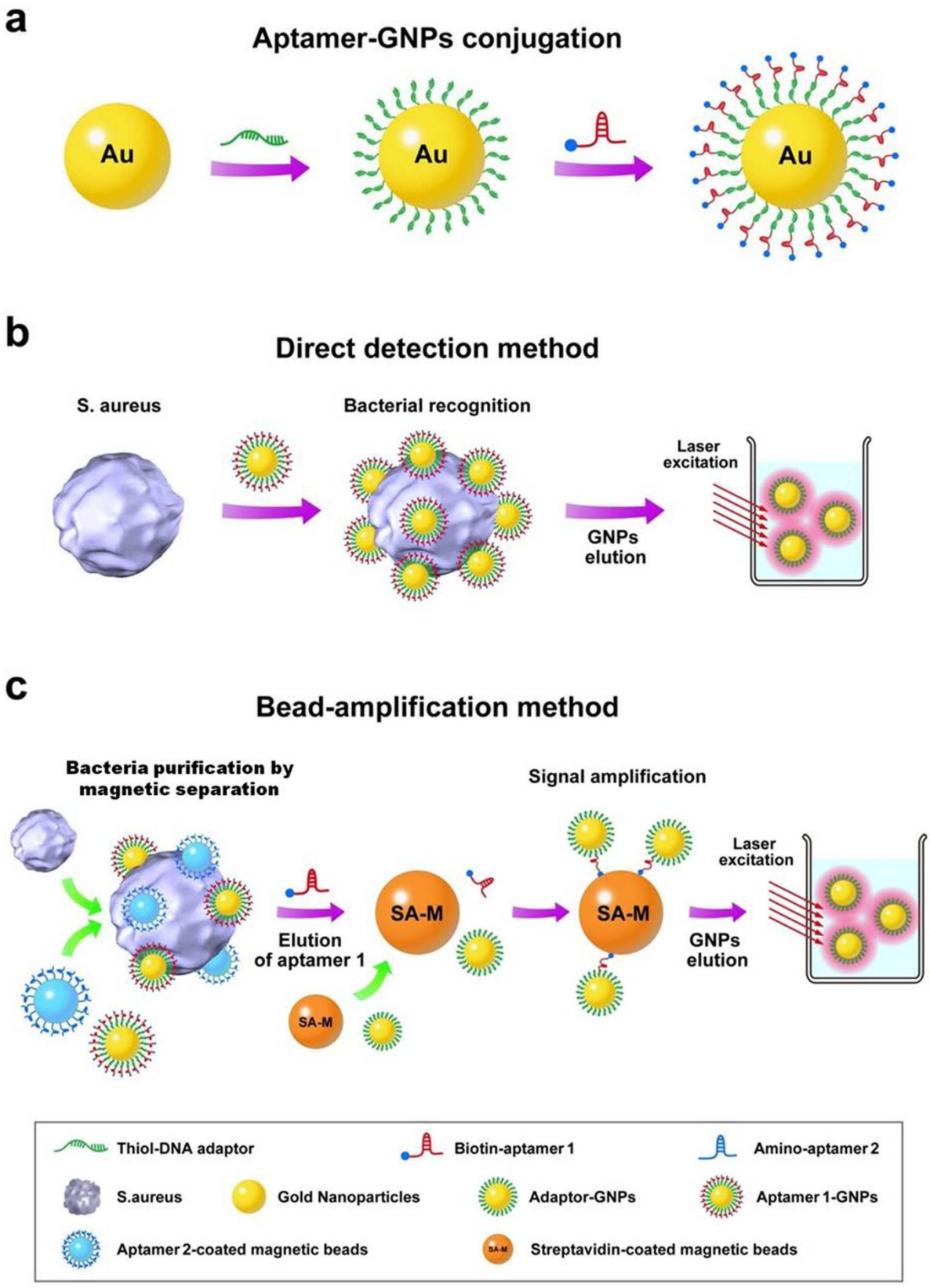

Figure 2 | Flowchart of *S. aureus* detection using aptamer-conjugated GNPs. (a), Aptamers were conjugated onto 60-nm GNPs with thio-DNA adaptors. (b), Aptamer-GNPs in the direct detection of *S. aureus*. 10^9 aptamer-GNPs were incubated with *S. aureus* cells. After removal of unbound aptamer-GNPs, bound aptamer-GNPs were eluted and their light-scattering signals were analyzed. (c), Bead-based amplification in the detection of *S. aureus*. SA61-aptamers (biotin-aptamer 1) were conjugated onto 60-nm GNPs, and SA17-aptamers (aptamer 2) were conjugated onto magnetic beads. Aptamer 1-GNPs and aptamer 2-magnetic beads interacted with *S. aureus* and the resulting complexes were isolated with a magnet. Bound biotin-aptamer 1 was eluted by heating and further incubated with an excess of reporter-GNPs (conjugated with DNA adapter) and streptavidin (SA)-coated magnetic beads. The reporter-GNPs were then captured with SA-magnetic beads in the presence of biotin-aptamer 1. The bound reporter-GNPs were eluted with NaOH and their light-scattering signals were analyzed.

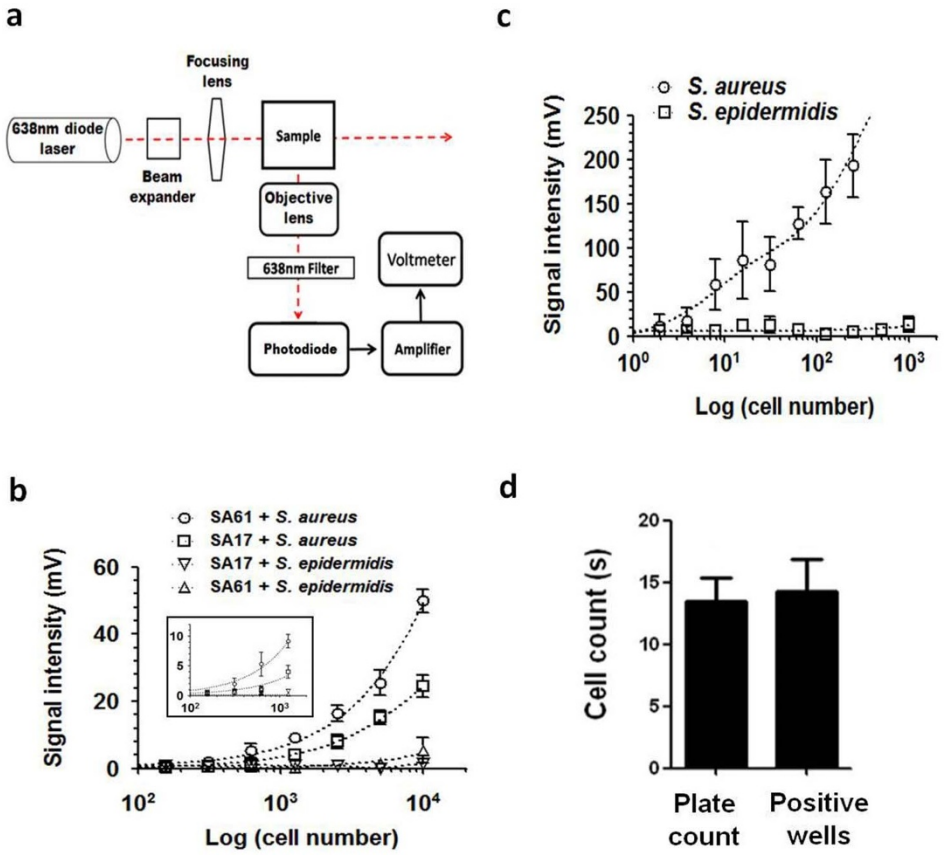

Figure 3 | The light-scattering system and application of aptamer-GNPs in the detection of *S. aureus*. (a), Schematic diagram of the instrument. The instrument consists of a 638-nm diode laser for excitation, an objective lens for collection of scattered-light signals, and a photodiode for transducing the light into electrical signals. The data are collected with a voltmeter. (b), Results of direct detection of *S. aureus* using SA17-GNPs and SA61-GNPs. The numbers of bacteria tested in the study were 10^4 and 2-fold serial dilutions in selection buffer. In this study, SA17 and SA61 aptamers were conjugated with 60-nm GNPs (10^7/μl). Filled diamond: SA61-GNPs incubated with *S. aureus*; filled square: SA17-GNPs incubated with *S. aureus*; filled triangle: SA61-GNPs incubated with *S. epidermidis*; filled inverted triangle: SA17-GNPs incubated with *S. epidermidis*. (c), Results of bead-based amplification in the ultrasensitive detection of *S. aureus*. The starting number of bacteria was 10^3 followed by 2-fold serial dilutions. Biotin-SA61 (b-SA61)-conjugated 60-nm GNPs (10^7/μl) and SA17-conjugated magnetic beads (5×10^6/μl) were incubated with *S. aureus* for 30 minutes. Bound b-SA61 aptamers were eluted and incubated with SA-magnetic beads and reporter-GNPs. Reporter-GNPs were eluted and analyzed for light-scattering signal. Filled circle: *S. aureus*; filled square: *S. epidermidis*. (d), Results of single bacterial cell detection. The number of bacterial cells was determined by plate count and bead-based amplification The mean bacteria number was 13.5 and 14.3, respectively. Two assay plateforms show good correlation with R^2 of 0.89.

numbers determined by the bead-based amplification assay and plate-count method were 11 : 12, 16 : 19, 11 : 8 and 16 : 18. The R^2 for the results of two assays was 0.89. These data were combined and are shown in **Figure 3d**.

Discussion

A number of molecular technologies had been developed for bacterial detection. However, few have been widely used in clinical applications, primarily because of the associated high costs and complex protocols, which are cumbersome for the clinical operator. Most rapid and sensitive technologies, such as qPCR and the Verigene system, are based on the detection of bacterial DNA[30]. Detection of bacterial DNA requires bacterial cell lyses, which is a laborious and time-consuming process, especially for Gram-positive bacteria such as *S. aureus* that required lysostaphin to breakdown the thick cell wall. Moreover, unlike bacterial surface antigens, which are numerous, the number of DNA targets is limited. This difference in copy numbers can be several orders of magnitudes, and higher target numbers suggest a lower limit of detection. Immuno-PCR is a technology that can ultrasensitively detect bacterial surface antigen using an antibody chimera with a DNA barcode[15]. However, conjugating DNA molecules onto a specific site of an antibody without affecting its interaction can be problematic. Moreover, immuno-PCR requires an expensive qPCR machine for amplification and analysis of the sample.

In this report, DNA aptamer and GNPs technology were combined to demonstrate an ultrasensitive bacteria detection system. *S. aureus,* a well-known human pathogen, was chosen for aptamer selection. Aptamers that specifically recognize *S. aureus* were identified, and an ultrasensitive method for rapid bacterial detection was developed that uses aptamer-conjugated GNPs. A determination of the K_ds of free aptamer forms and aptamer-GNPs for *S. aureus* showed that the K_ds of aptamers were significantly enhanced upon conjugation with GNPs. This increased affinity might be caused by an avidity effect reflecting multiple aptamers and targets interactions. Using aptamer-GNPs, we developed a bead-based amplification method for detecting *S. aureus*, and demonstrated that it is capable of rapidly detecting single bacterial cells. Despite a large variation in the signal intensity in this assay, a statistical analysis confirmed a strong correlation between the bead-based amplification assay and the traditional plate-count method. This signal variation might be caused by cell aggregation, which is a common phenomenon among Staphylococcus species.

Using this ultrasensitive method, we achieved PCR-like sensitivity and quantified bacterial numbers within 1.5 hours without the need for any expensive instruments. The protocol is simple and the cost of

the method is low. This new platform technology may have potential for development as a rapid and sensitive multiplex detection system for common pathogens in clinical settings such as intensive care units. Taken together, these advantages make this technology an appealing choice for future development of point-of-care pathogen testing.

Methods

Primers and aptamers. A ssDNA library composed of 30-nucleotide (nt) long, randomized probe sequences flanked by 16-nt PCR priming sequences at both 5′- and 3′-ends (TCCCTACGGCGCTAAC–$[N]_{30}$–GCCACCGTGCTACAAC) was synthesized by Integrated DNA Technologies (Coralville, IA, USA). All other primers and aptamers were from Purigo Biotech (Taipei, Taiwan). The bacteria-bound probes isolated during the SELEX process were amplified by PCR primers (designated R9 primers) with the sequences 5′-TCC CTA CGG CGC TAA C-3′ (forward) and 5′-GTT GTA GCA CGG TGG C-3′ (reverse). Proper folding of aptamers was attained by denaturing at 95°C for 2 minutes followed by gradual cooling to 37°C at a rate of 2°C per 40 seconds using a thermocycler. The aptamers were then stored at −20°C until ready for assay. Biotin- labeled aptamers were used for phycoerythrin (PE)-staining and fluorescence microscopy.

Cell-based SELEX. A total of 10^7 *S. aureus* (ATCC: 6538DR) cells were incubated in SELEX buffer with an aptamer library containing 10^{15} randomized DNA sequences for 30 minutes on ice. After washing away the unbound aptamers, bound aptamers were eluted with SELEX buffer and heated at 95°C for 2 minutes. The isolated aptamers were refolded by thermal cycling, as described above, and counter-selected with 10^8 *S. epidermidis* cells on ice for 30 minutes. The supernatant was collected and PCR-amplified with R9 forward primers and biotin-labeled R9 reverse primers in PCR buffer containing 50 mM NaCl, 10 mM Tris–HCl (pH 8.9), 10 mM betaine, 1% dimethyl sulfoxide, 200 μM each dNTP, 1 mM $MgCl_2$, 200 nM each primer, and 2 units of *Taq* DNA polymerase. The PCR amplicons were rendered single-stranded and purified with streptavidin-coated magnetic microspheres (Chemogen, So. Portland, ME, USA). The isolated ssDNA pool was refolded and incubated with a new batch of *S. aureus* to start a new SELEX round.

Bacterial cultures and harvest conditions. All bacteria were purchased from Food Industry Research and Development Institute (FIRDI, Hsin-Chu, Taiwan). The bacterial strains used in the study were *B. subtilis* (ATCC: 21336), *C. freundii* (ATCC: 8090), *E. coli* (ATCC: 43896), *K. pneumonia* (ATCC: 13883), *L. monocytogenes* (ATCC: 19112), *M. catarrhalis* (ATCC: 25238), *P. aeruginosa* (ATCC: 27853), *S. enteric* (ATCC: 13314), *S. boydii* (ATCC: 8700), *S. flexneri* (ATCC: 29903), six strains of *S. aureus* (ATCC: 6538DR, 6538P, 12600, 25923, 29213, 6538), *S. epidermidis* (ATCC: 155), *S. haemolyticus* (ATCC: 29970), *S. saprophyticus* (ATCC: 15305), *S. bovis* (ATCC: 43077) and *S. pneumoniae* (ATCC: 6301). *Staphylococcus spp.* were cultured with Brain-Heart infusion broth (Oxoid, Basingstoke, England) at 37°C; *B. subtilis* was cultured with LB broth (Difco, Detroit, MI, USA); the remaining bacteria were cultured with nutrient broth (Difco, MI, USA). The concentration of *S. aureus* was determined by serial dilution with subsequent plating on agar plates and measurement of colony forming units (CFUs). CFUs were also determined by measuring optical density (OD) at 600 nm (an OD_{600} of 1.0 ≈ 1.5×10^9 CFU/ml). Muller-Hinton broth (Difco) was used in antimicrobial susceptibility testing.

Measurement of K_ds for SA aptamers. *S. aureus* cells were incubated with serially diluted aptamers for 30 minutes at 4°C with gentle shaking. The bacteria were washed with 3× SELEX buffer by centrifugation. Bound aptamers were eluted with 95°C distilled H_2O (dH_2O) and mixed with SYBR Green Master Mix containing 200 nM R9 primer pair. qPCR was performed using an ABI-7900 system (Applied Biosystems, Alameda, CA, USA). K_d was calculated according to the equation, $Y = B_{max} \times X/(K_d + X)$.

Immunofluorescence assay. FAM-labeled SA aptamers (250 nM) were incubated with 100 μl of bacterial suspension with an OD_{600} of 1.0. The mix was incubated on ice for 30 minutes and washed several times with SELEX buffer to remove unbound aptamers. To avoid interference due to autofluorescence of bacterial cells, bound aptamers were eluted by heating, and eluates were analyzed using a SpectraMAX PLUS fluorescence microplate reader (Molecular Devices, Union City, CA, USA).

Conjugation of GNPs. Adaptor sequences (A_{20}) were conjugated onto GNPs (BBInternational, Cardiff, UK) by adjusting the pH of the gold colloid solution to 8.5–9.1 using 100 mM K_2CO_3 and incubating overnight at 25°C with 5 μM thio-labeled adaptor sequences, pre-activated with 10 mM tris(2-carboxyethyl)phosphine (TCEP). After the adaptor sequences had been conjugated, NaCl was added to a final concentration of 200 mM. Unbound adaptor sequences were removed by washing six times with adaptor-GNPs with stabilizing buffer containing 20 mM Tris-HCl (pH 8.5), 1% bovine serum albumen (BSA), 5 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$ and 150 mM NaCl by centrifugation. The adaptor-GNPs were stored at 4°C. GNPs were conjugated with aptamer sequences by incubating 10 μM aptamer sequences (with poly-T linker) with 1 ml of adaptor-GNP solution containing 10^{10} particles. The mixture was then heated to 65°C for 5 minutes, gradually cooled to 4°C at a rate of 2°C per 40 seconds using a thermocycler, and incubated overnight at 4°C. Aptamer-GNPs were washed six times with stabilizing buffer and stored at 4°C before use.

Aptamer-GNPs for the detection of *S. aureus*. For the direct detection of *S. aureus*, 10^8 aptamer-GNPs were incubated with bacteria samples in 25 μl of SELEX buffer (40 mM HEPES buffer pH 8.0, 5 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, and 150 mM NaCl). After washing away the unbound aptamer GNPs using a 0.45-μm filter column (Millipore, Billerica, MA, USA), bound aptamer-GNPs were eluted with 0.1 M NaOH and quantified using the light-scattering–detection system. For bead amplification, M270 beads were coated according to the manufacturer's instructions. Briefly, 10^8 M270 epoxy beads (Invitrogen, Carlsbad, CA, USA) were incubated with 1 μmole of amine-labeled SA17 for 48 hours in pH 9.0 borate buffer. After blocking with 1% BSA, the coated M270 beads were stored in stabilizing buffer at 4°C. Then, 10^8 biotin-SA61-GNPs and 10^7 SA17-M270 were incubated with bacterial samples for 30 minutes on ice. After separation with a magnet, bound biotin-SA61 was eluted with 95°C dH_2O. Streptavidin-Magnetic beads (Chemogen) were blocked with hybridization buffer (20 mM Tris-HCl pH 9.0, 1% BSA, 100 mM NaCl) for 1 hour at 37°C and then incubated with 10^8 adaptor-GNPs and previously eluted biotin-aptamers for 30 minutes at 37°C. The GNPs captured by magnetic beads were eluted with 0.1 M NaOH and analyzed using the light-scattering–detection system.

For single cell detection, *S. aureus* was inoculated in Brain heart infusion media and incubated at 37°C for expansion. Bacterial cells were collected while OD_{600} reached 0.5–0.8. The collected cells were repetitively resuspended by pipetting and washed twice by SELEX buffer containing 2% of PEG 2000 and 0.02% of Tween-20 to reduce cell aggregates. Bacterial cells were resuspended to 1 OD_{600} with SELEX buffer containing 1% of BSA and 0.02% of Tween-20. The cell density of 1OD_{600} was determined by plate count. The cell suspension was further serially diluted to one cell per μl. The 10 μl diluted bacterial suspensions containing approximately ten bacterial cells was confirmed by plate count and bead-based amplification assay simultaneously. In bead-based amplification assay, 10 μl of diluted suspension was further diluted to 300 μl and equally divided into 30 wells. Each well was further analyzed by bead-based amplification to determine whether it contained bacteria or not. The positive wells were recorded and served as containing one bacteria cell each.

Scanning electron microscopy. For SEM observations, bacteria were incubated with 10^8 60-nm aptamer-GNPs or 10^7 100-nm aptamer-GNPs at 4°C for 30 minutes. The mixtures were filtered using a 0.45-μm filter column and spotted onto a poly-L-lysine–coated cover glass to allow bacterial attachment (10 minutes at 4°C). The samples were fixed by incubating with 1% formaldehyde and 2% glutaraldehyde at room temperature for 2 hours, then postfixed with 2% osmium tetroxide for 1 hour, dehydrated with ethanol, critical-point dried, and coated with gold-palladium alloy. Finally, bacterial surfaces were photographed using a Jeol JSM T330A scanning electron microscope (Jeol, Inc., Peabody, MA, USA) at 15 kV acceleration.

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Author contributions

Dr. Pan-Chyr Yang was director of the project. Ms. Chia-Ying Yang and Ms. Ruei-Lin Sun contributed to aptamer identification. Dr. Yi-Feng Cheng and Mr. Wei-Chen Kao contributed to aptamer characterization. Dr. Yi-Chung Chang contributed to the experimental set-up and wrote the manuscript.

Additional information

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