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Bacterial Killing by Light-Triggered Release of Silver from Biomimetic Metal Nanorods

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Abstract

Illumination of noble metal nanoparticles at the plasmon resonance causes substantial heat generation, and the transient and highly localized temperature increases that result from this energy conversion can be exploited for photothermal therapy by plasmonically heating gold nanorods (NRs) bound to cell surfaces. Here, we report the first use of plasmonic heating to locally release silver from gold core/silver shell (Au@Ag) NRs targeted to bacterial cell walls. A novel biomimetic method of preparing Au@Ag core-shell NRs was employed, involving deposition of a thin organic polydopamine (PD) primer onto Au NR surfaces, followed by spontaneous electroless silver metallization, and conjugation of antibacterial antibodies and passivating polymers for targeting to gram-negative and gram-positive bacteria. Dramatic cytotoxicity of *S. epidermidis* and *E. coli* cells targeted with Au@Ag NRs was observed upon exposure to light as a result of the combined antibacterial effects of plasmonic heating and silver release. The antibacterial effect was much greater than with either plasmonic heating or silver alone, implying a strong therapeutic synergy between cell-targeted plasmonic heating and the associated silver release upon irradiation. Our findings suggest a potential antibacterial use of Au@Ag NRs when coupled with light irradiation, which was not previously described.

Keywords

antibacterial silver release; plasmonic heating; melanin-mimetic polydopamine; antibody; metal nanorods

1. Introduction

The unique optical properties of colloidal gold and silver nanoparticles are closely associated with the localized surface plasmon resonance (LSPR) effect, in which free electrons oscillate collectively on the metal surface when irradiated with particular energies of light[1, 2], causing wavelength dependent absorption and scattering. Due to their strong interaction with light, noble metal nanoparticles have attracted considerable attention for their possible use in diagnostics and therapeutics[3-5]. For example, linking DNA to gold nanoparticles facilitates the use of biological recognition interactions to drive nanoparticle assembly into clusters, resulting in optical changes that can be exploited for diagnostic purposes[6]. Also, gold nanoparticles have the ability to convert light energy into heat[7], a particularly useful property enabling a variety of potential photothermal therapies[5, 8-11]. Recent studies have used these photothermal properties for the selective release of therapeutic molecules[12-14] to treat cancer[15-17].

LSPR wavelength-tuning is useful for matching the optical properties of metal nanoparticles with that of light sources used in the clinic. Furthermore, tissue penetration is a major consideration of *in vivo* optical imaging and therapeutic systems involving nanoparticles, as light transmission through tissues is much higher in the NIR range compared to the visible and ultraviolet ranges[18]. Numerous approaches have been described for tuning metal nanoparticle electromagnetic properties through control of size, shape[19, 20], and composition[3, 21-25]. An important class of shape-controlled metal nanoparticle is the high aspect ratio gold nanorod (NR). The LSPR of gold nanoparticles displays a strong dependence on aspect ratio, exhibiting a dramatic red-shift from 520 nm for spherical nanoparticles to 800 nm or higher for aspect ratios above 5[26-28]. In the case of silver, the LSPR is generally ~400 nm for spherical nanoparticles but can be readily tuned into the NIR through size and shape control[29].

Further control of electromagnetic properties can also be afforded with bimetallic systems that offer unique combinations of optical, photothermal, catalytic and biological properties[30]. Bimetallic gold-silver nanoparticles reported in the literature include binary alloys[31-33], segmented heterometallic nanorods[34], and core-shell structures[35, 36]. Au core-Ag shell (Au@Ag) structures possess blue-shifted LSPRs compared to the bare Au core, with the LSPR wavelength depending strongly on the aspect ratio of the gold core and the thickness of the silver shell[36-43]. In addition, nanoparticulate silver is of great interest for its broad-spectrum and size-dependent antibacterial properties[44-48], which are typically associated with the activity of released silver ions on DNA, enzymes, and cell surface molecules[49-52]. Silver nanoparticle administration has been proposed as an alternative treatment strategy against bacteria compared to classical antibiotics, especially with the emergence of strains resistant to most current clinical regimens[53, 54].

It may be possible to more fully realize the antibacterial potential of silver by combining it with the unique optical properties of metal nanoparticles through biomimetic chemistry. Due to its low toxicity and cost, as well as its facile synthetic approach, biologically-inspired formation of silver nanoparticles with mussel-mimetic polymers[55], tea extract[56], seaweed[57], and cells[58] has become an innovative strategy to form antimicrobial agents. Here we describe a new biomimetic coating strategy for creating LSPR-tunable bimetallic Au@Ag nanorods (Au@Ag-NRs) that are designed to kill bacteria through the combined effects of silver release and plasmonic heating. The approach employs a versatile melanin-mimetic coating of polydopamine (PD) on a Au NR[59], in order to both form a silver shell around the gold core as well as conjugate anti-bacterial antibodies to the surface. Photoillumination of *Staphylococcus epidermidis* and *Escherichia coli* bacteria targeted with antibody-bound Au@Ag-NRs (Ab-Au@Ag-NRs) resulted in efficient cell killing due to both plasmonic heating and silver release from the NRs upon irradiation with light. Dark and silver-free controls were far less effective in killing cells, implying strong cooperative antibacterial effects between plasmonic heating and light-induced silver release.

2. Results

Antibody-functionalized Au@Ag-NRs were synthesized from CTAB stabilized Au NRs (CTAB-NRs) using a biomimetic coating strategy as illustrated in Figures 1 and S1. CTAB-NRs were characterized by a strong longitudinal LSPR centered near 800 nm and a less intense transverse LSPR near 520 nm. Deposition of a thin shell of PD onto CTAB-NRs to form PD-coated NRs (PD-NRs) caused a slight red-shift in the longitudinal LSPR (Figure S2a), and the coating was visible by electron microscopy (Figure S2b)[59]. Within seconds of AgNO₃ addition to PD-NRs, a pronounced color change occurred in the suspension, stabilizing within 10 minutes to yield red, yellow, green, purple, and orange colored suspensions as the silver concentration increased (Figure S3a). This color change was accompanied by pronounced increases in intensity, narrowing, and a blue-shift of the longitudinal LSPR (Figure S3b). The magnitude of the blue-shift was independent of the initial wavelength of the longitudinal LSPR but was strongly correlated to the AgNO₃ concentration. Addition of 50, 100, 200, and 300 μ M AgNO₃ to a PD-NR suspension shifted the longitudinal SPR extinction peak from its initial value centered at 804 nm to 693 nm, 629 nm, 565 nm, and 531 nm, respectively. A strong optical backscattering peak, red-shifted compared to overall extinction, was observed from Au@Ag-NRs (Figure S3c).

Transmission electron microscopy (TEM) was performed to confirm the deposition of silver onto PD-NRs. Core-shell NR morphologies were evident under bright field and dark field TEM (Figure 2 and S4). The spatial relationship of gold and silver within the NRs was elucidated by TEM imaging with EDS (Figure 2), revealing a Au NR core (red) surrounded by a Ag shell (teal). The average thickness of the Ag shell under TEM ranged between 1 and 8 nm and was strongly linked to the concentration of AgNO₃ (range: 0-300 μ M) used during NR preparation. The amount of Ag incorporated per NR was quantified by dissolving the NRs in nitric acid and measuring the Ag composition by ICP-OES (Figure S5), confirming the correlation between AgNO₃ concentration and Ag incorporation. For Au@Ag-NRs synthesized from 300 μ M AgNO₃ (all subsequent experiments were performed at this condition unless indicated), detection of an increased Ag 3d signal was observed in XPS

spectra of Au@Ag-NRs compared to PD-NRs (Figure S6a and b). Ellipsometry and compositional depth profiling by XPS of flat surfaces coated with PD and Ag under identical conditions gave evidence of a 3.36 nm thick coating containing both silver and PD (Figure S6c).

The photothermal properties of the NR suspensions were characterized upon irradiation with a visible-NIR light source, producing observable bulk heating of NR suspensions compared to water controls (Figure 3a). The temperature of a PEG-stabilized PD-NR (PEG-NR, Figure S1) suspension increased from 20.6° C to 24.2° C within the first minute of illumination and rose to 41.9° C within 5 minutes before reaching a steady state condition of 44° C after 8 minutes of illumination. Addition of a silver shell (PEG-Au@Ag-NRs) resulted in a slightly lower steady state temperature of 40° C upon irradiation. In a control experiment, the temperature of NR-free water irradiated in an identical manner did not rise significantly above 24° C. Silver release from Au@Ag-NRs was characterized upon irradiation using ICP-MS (Figure 3b). A 115% increase in silver concentration in solution was detected after light irradiation compared to non-irradiated controls ($p < 0.05$).

Antibodies specific to either gram-negative or gram-positive bacteria were reacted to NRs with and without the silver shell. Immobilization of bacterial antibodies onto PD-NRs and Au@Ag-NRs led to a minor red-shift of the LSPR band, in agreement with a previous report that characterized the conjugation of antibodies to PD-NRs[59]. After conjugation, no statistically significant shift in LSPR wavelength occurred upon incubation of antibody-conjugated NRs (Ab-Au@Ag-NRs and Ab-NRs) in 0.85% NaCl over 4 hours (Figure S7). Over the same period of time, 87% of the LSPR intensity was preserved for Ab-Au@Ag-NRs. Further, no LSPR shift was detected in Ab-Au@Ag-NRs over 24 hours.

Incubation of either Ab-NRs or Ab-Au@Ag-NRs with *E. coli* and *S. epidermidis* cells resulted in specific binding of NRs to bacterial cells as confirmed by TEM and optical coherence tomography (OCT). TEM imaging of bacterial cells exposed to Au@Ag-NRs revealed the presence of cell-bound Au@Ag-NRs only when they were functionalized with the antibody specific to gram-negative or gram-positive cells, whereas non-specific antibody-functionalized and antibody-free control NRs were not found to be associated in large numbers with bacterial cells (Figure 4). Similar results were obtained with Ab-NRs (Figure S8). OCT images of *E. coli* and *S. epidermidis* cell suspensions displayed increased brightness and contrast after incubation with Ab-Au@Ag-NRs as compared to PEG-NRs (Figure S9), also implying antibody-mediated co-localization of cells and NRs.

S. epidermidis and *E. coli* were incubated with NRs and illuminated with a 150 mW broadband light source, and then live/dead stained in order to probe the effects of plasmonic heating and the presence of the Ag shell on cell viability (Figures 5, 6, and S10). Importantly, the silver shell, antibody, and light irradiation were all necessary to provide maximal bacterial killing. Under the illumination conditions used in our experiments, we found both *S. epidermidis* and *E. coli* were largely unaffected by illumination in the absence of NRs, maintaining >90% cell viability in control experiments. Further, incubation of *S. epidermidis* with PEG-NRs, PEG-Au@Ag-NRs, and Ab-NRs, both with and without irradiation, did not produce therapeutic responses, all yielding cell viabilities above 90%.

Free silver, non-photothermal heating, or a combination thereof, also did not significantly affect *S. epidermidis* viability (Figure S10). In sharp contrast, viability of *S. epidermidis* cells was dramatically reduced only when they were treated with Ag@Au-NRs functionalized with antibody specific to lipoteichoic acid and also irradiated with light (Figure 5). More specifically, cell viability was reduced to 10% following illumination of cells treated with Ab-Au@Ag-NRs, whereas dark controls maintained cell viability above 80%.

Synergistic effects between plasmonic heating, silver release, and antibody targeting were also observed in the case of *E. coli*. In the absence of Ag, *E. coli* cells treated with Ab-NRs maintained viability above 50% even upon exposure to light, whereas their viability when treated with Ab-Au@Ag-NRs dropped from >80% to 8% upon exposure to light (Figure 6). In general, *E. coli* cells were more sensitive than *S. epidermidis* cells to treatment, as antibody free PEG-NRs were moderately toxic to *E. coli* cells (73% viability, reduced to 63% when irradiated with light). Silver shells increased toxicity to *E. coli*, as treatment with PEG-Au@Ag-NRs resulted in 58% viability, which was further reduced to 15% upon exposure to light. *E. coli* cells were also sensitive to free silver, non-photothermal heating, or a combination thereof (Figure S10). Overall, while *E. coli* viability was reduced with all treatments, incubation with Ab-Au@Ag-NRs followed by irradiation provided the greatest therapeutic response compared to all other controls.

3. Discussion

The electromagnetic properties of gold nanoparticle systems, which offer diagnostic and therapeutic functionality, are highly tunable through composition and shape control[60, 61]. Gold NRs with longitudinal plasmon extinction peaks in the NIR window are particularly attractive for medical imaging and therapeutic applications because tissue penetration depth is high in the NIR[61, 62]. Plasmonic heating of gold nanoparticles has been extensively investigated for localized photothermal treatment of mammalian cells and cancerous tissues[39, 63-68], however few studies have extended this approach for bacterial cell killing[11, 66]. In this study, antibody-functionalized gold NRs were used to target bacterial cell walls and release silver upon light irradiation to kill both gram-negative and gram-positive bacterial cells. We envisioned a powerful outcome for bacterial killing through the integration of silver with plasmonic heating, as silver is expected to contribute broad-spectrum antibacterial properties[44-47] over and above the plasmonic heating effects. Our results revealed a surprisingly strong synergy between plasmonic heating and irradiation-induced silver release leading to high bacterial toxicity by Au@Ag-NRs.

Previous approaches for synthesis of Au@Ag-NRs involve addition of silver salts and reducing agent to a suspension of Au NRs[36-39, 41-43], which upon neutralization results in reduction of Ag(I) and overgrowth of a metallic Ag(0) shell on top of the Au NR core. In contrast, our synthetic strategy employed a thin coating of PD, a mussel and melanin-mimetic coating which deposits as a nanometers-thick adherent film on objects during alkaline oxidative polymerization of dopamine[69]. PD then served as a chemically reactive 'primer' on the surface of gold NRs, onto which silver, antibody, and passivating polymer were deposited in a simple stepwise manner (Figure 1). Importantly, the chemical versatility

of the PD layer allowed for adhesion to a gold NR surface, reduction of silver ions and formation of a silver shell around the gold NR core, and covalent conjugation of bacterial-targeting antibodies and non-fouling PEG polymers through Michael-type addition reactions.

In the first step, CTAB-NRs were dispersed in an alkaline dopamine solution for 30 min, resulting in deposition of a several nanometer thick PD film on the Au-NR surface as detected by a slight red-shift of the SPR and by EM analysis (Figure S2), agreeing with a previous study[59]. During this step the CTAB ligand is essentially replaced by PD, which has the added benefit of eliminating nonspecific cytotoxicity associated with CTAB[59, 70, 71]. The redox activity of soluble and surface-immobilized catechol-containing polymers with reducible metal ions has been previously exploited for spontaneous *in situ* formation of polymer-stabilized gold and silver nanoparticles[55], formation of silver-releasing gels from catechol-functionalized polymers[72], and metallization of a surface with silver[69, 73-75]. Therefore, we hypothesized that catechol functional groups found within the PD shell would possess sufficient reductive capacity to reduce Ag(I) to Ag(0), acting as a template for Ag metallization of the PD shell around the gold NR. Indeed, core-shell Au@Ag-NRs spontaneously formed upon addition of AgNO₃ to the PD-NR suspension, as confirmed by TEM analysis with EDS spectral imaging (Figures 2 and Figure S4), ICP-OES (Figure S5), and the detection of metallic Ag by XPS (Figure S6). The thickness of the Ag shell ranged between 1 and 8 nm, and was readily controlled by varying the amount of AgNO₃ added to the NR solution. XPS depth profiling of macroscopic gold substrates coated with a 3.4 nm thick layer of PD and Ag under identical conditions indicated colocalization of PD and Ag in the outer portion of the hybrid coating (~ 0.6 nm) and a pure silver portion interfacing with the gold surface. The optical properties of the PD-NRs changed upon formation of an Ag shell, as evidenced by a visible change of the colloidal suspension color and by changes in the wavelength and appearance of the LSPR peak. Through control of AgNO₃ concentration during synthesis, longitudinal LSPR wavelength tuning between 520 nm and 860 nm was achieved. The observed blue-shift of the LSPR extinction (Figures 2 and S3) is consistent with Au@Ag-NR formation[36, 37, 39, 41-43]. Likewise, the narrowing of the LSPR peak with increasing silver concentration is consistent with the 'plasmonic focusing' effect observed in Au@Ag core-shell nanostructures[38].

Antibodies specific to gram-negative and gram-positive bacteria were immobilized to NR surfaces under mildly basic conditions. To target gram-negative cells, antibodies for endotoxin were chosen, whereas antibodies specific to lipoteichoic acid were employed for gram-positive cells. Minor LSPR red-shifts were detected upon exposure of PD-NRs and Au@Ag-NRs to antibody solution, indicating immobilization of antibodies onto the NR surface[2, 59]. It is hypothesized that nucleophiles in the antibodies, such as amines and thiols, covalently react with the quinones of the PD surface layer through Michael-type addition[76-78], although we cannot rule out contributions from physisorption of antibody onto the NR surface. To prevent aggregation in high ionic strength media and avoid nonspecific interactions, NR surfaces were 'back-filled' with PEG, rendering Ab-NRs and Ab-Au@Ag-NRs stable in isotonic saline over a period of 4 hours (Figure S7), as indicated by an unchanging longitudinal LSPR.

TEM and OCT imaging confirmed antibody-specific NR targeting to bacterial cells. Incubation of *E. coli* and *S. epidermidis* with Ab-NRs and Au@Ag-NRs functionalized with antibodies that specifically target gram-negative or gram-positive cells, respectively, resulted in significantly increased binding of NRs to cells as compared to non-specific antibody and antibody-free controls (Figure 4 and S8). Cell imaging by OCT produced similar findings, as suspensions of cells exposed to Ab-Au@Ag-NRs were significantly brighter compared to cells incubated with control PEG-NRs (Figure S9), which we attribute to backscattering from metal NRs bound to cells since OCT intensity is correlated to the number of NRs present, as absorption and scattering by NRs leads to contrast in OCT[79, 80].

The photothermal property of gold nanoparticles arises from absorption of light energy at the LSPR, transferring this energy into heat that is released into the local environment surrounding the nanoparticle[7, 81]. It has been estimated that the temperature rise occurring during plasmonic illumination of nanoparticles can be several thousand °C and highly localized to the close surroundings of the nanoparticle[82]. The intense local heating is believed to lead to cytotoxic effects through denaturation of biomolecules, bubble formation, metal nanoparticle explosion, and physical damage to cell membranes[10, 11, 66, 82-84]. In the present study, plasmonic heating was used to release silver in a controlled manner from the surface of NRs targeted to bacterial cells. A substantial bulk temperature increase of 20-25° C was recorded upon irradiation of a suspension of Au@Ag-NRs (Figure 3a), causing the release of silver from the NR surface (Figure 3b). It is interesting to speculate that PD may enhance the conversion of light to heat in this case, as PD is similar in structure to melanin, a biological photopigment that evolved to protect organisms from light damage by converting light energy to heat[85]. We can likely rule out direct nanoparticle-specific cytotoxic effects, as a recent study has shown that ionic silver is the active toxic species rather than the nanoparticles themselves[52].

A particularly noteworthy outcome of our study was the discovery of a strong therapeutic synergy between plasmonic heating and silver-induced bacterial toxicity (Figures 5 and 6). Ab-NRs without the silver shell did not induce significant toxicity to *S. epidermidis* cells upon illumination, suggesting that under the conditions of our experiments, plasmonic heating alone did not lead to significant cell toxicity. The addition of a silver shell (Ab-Au@Ag-NRs) also did not significantly increase the therapeutic efficacy in the absence of illumination. Importantly, only *S. epidermidis* cells treated with Ab-Au@Ag-NRs and illumination were efficiently killed due to plasmonic heating and release of silver upon irradiation. When combined with targeting to the bacterial membrane and wall components, light-induced Ag release should significantly enhance the uptake of silver into the cell where it exerts its toxic effects in the usual manner when in ionic form by binding to DNA, respiratory enzymes, and cell surface molecules and receptors[48]. These effects may be further accentuated by physical cell membrane damage created by plasmonic heating of NRs bound to the cell surface[11] which likely facilitates cell entry, thereby significantly enhancing the therapeutic effect of silver.

Similar trends were observed for *E. coli*, however these cells were more susceptible to the singular effects of heating or silver compared to *S. epidermidis* (Figures 6 and S10), which is

attributed to the differences in their cellular wall structure. The cell walls of gram-negative bacteria are much thinner than those of gram-positive bacteria that have a thick outer peptidoglycan layer that obscures the cell membrane; measurements of the peptidoglycan layer thickness of bacteria range from ca. 6 nm for *E. coli* to 30-60 nm for *S. epidermidis*[86-88]. Therefore, this thicker cell wall of *S. epidermidis* can provide better protection against the individual effects of photothermal heating or silver than the relatively thin cell walls of *E. coli*.

Coupling plasmonic heating with small molecule[12, 14] or DNA[13] release is an emerging strategy to locally treat cancer[15-17]. However, this study is the first of its kind to use plasmonic heating to release silver from metal nanoparticles for an antibacterial effect. Importantly, the biomimetic synthetic strategy that uses melanin-mimetic polymer coatings affords a chemical versatility that is vital to the integration of a gold NR, silver shell, and antibody into a single, multifunctional antibacterial agent. With the ability to coat gold NR surfaces, deposit silver around the gold core, and react with antibodies, PD can tune the optical and biological properties simultaneously. Further, the use of gold in the core of the bimetallic NR has particular advantages compared to pure silver rods including easier synthesis, greater colloidal stability, red-shifted plasmons for greater tissue penetration for translation into clinical settings, and reduced nonspecific toxicity due to its relative inertness. We envision this platform to be capable of incorporating an array of multicomponent, organic-inorganic, and multifunctional hybrid nanoparticles for biomedical applications, such as the treatment of cancer[59] and bacterial infections.

Our PD-based method can conceivably accommodate a variety of organic and inorganic therapeutic agents such as siRNA, DNA aptamers, antibodies and their fragments, polysaccharides[77], peptides, and metals. Further, the use of inorganic silver has particular advantages compared to organic materials with regard to plasmonic heating-induced drug release, since organic therapeutic agents attached to the nanoparticle surface are more susceptible to damage from transient but extreme temperatures achieved during plasmonic heating.

4. Conclusions

Bacterial antibody labeled gold core-silver shell nanorods were synthesized and employed for bacterial cell killing by plasmonic heating and irradiation-induced silver release. A simple yet versatile synthetic approach was described, exploiting a polydopamine 'primer' to facilitate deposition of the silver shell and for surface conjugation of passivating polymer and antibodies for specific targeting of gram-negative or gram-positive bacteria. The presence of a silver shell substantially synergized the antibacterial effect of Au nanorod plasmonic heating against both *S. epidermidis* and *E. coli* due to the release of antibacterial silver upon light irradiation, which can potentially be exploited for therapy against bacterial infections.

5. Experimental Section

Gold NR Synthesis

CTAB-stabilized gold NRs (CTAB-NRs) were synthesized according to previously described protocols[89]. Aqueous CTAB (0.2 M, 5.0 mL, heated to 30° C) was mixed with 0.5 mM NaAuCl₄ (5.0 mL). Ice-cold 0.01 M NaBH₄ (0.6 mL) was added to this solution and sonicated for 5 minutes to form a brownish-yellow seed solution. 50.0 mL of 0.2 M CTAB was then gently mixed with 50.0 mL 1.0 mM NaAuCl₄ and 0.1 mL 0.1 M silver nitrate to form a growth solution. Ascorbic acid was added as a mild reductant (78.8 mM, 0.7 mL), followed by addition of 120 µL of the seed solution. After 45 minutes, 100 mL was mixed with 100 mL 0.2 M glycine (pH 8.0) and left unstirred overnight at ambient temperature.

Surface Modification of Nanorods

Schematic illustrations of NR surface modifications are presented in Figures 1 and S1. First, PD was deposited onto CTAB-NRs as follows[59]. 500 µL of CTAB-NR suspension was centrifuged at 9000 rpm for 10 minutes and the supernatant was decanted. The NR pellet was resuspended in 1 mL of 10 mM TRIS buffer (pH 8.5). Dopamine hydrochloride was added to a final concentration of 0.555-1.11 mM and sonicated for 30 minutes to form polydopamine-coated NRs (PD-NRs). Au@Ag core-shell nanorods (Au@Ag-NRs) were formed by addition of AgNO₃ in ultrapure DI with stirring (final [Ag] = 50-300 µM), and samples were sonicated for 10 minutes. Au@Ag-NRs were isolated by centrifugation at 9000 rpm for 10 minutes, and supernatant solution was decanted. To immobilize antibodies specific to either lipoteichoic acid in gram-positive bacterial cell walls or endotoxins in gram-negative *E. coli* J5 mutant cell surfaces, 12.5 µL of 0.1 mg/ml antibody solution was added to Au@Ag-NR or PD-NR suspensions (pH 8.5) and sonicated for 30 minutes. Then, 10 µL 1 mg/ml mPEG-SH (pH 8.5) was added and the mixture sonicated for an additional 30 minutes to passivate the remaining surface area with PEG, yielding Ab-Au@Ag-NRs and Ab-NRs, respectively. Antibody-free PEG-stabilized NRs were fabricated by reacting mPEG-SH with PD-NRs and Au@Ag-NRs as described above to yield PEG-NRs and PEG-Au@Ag-NRs.

Optical Spectroscopy

A Hitachi (Hitachi City, Japan) U-2010 Spectrophotometer was used to acquire optical spectra in a two-beam geometry. 10 mM TRIS buffer (pH 8.5) or 0.85% NaCl was used for the reference beam. Spectral scans were performed at a resolution of 1 nm over the 200-1000 nm range of wavelengths.

Electron Microscopy

Pelleted NRs (5µL) were deposited on ultrathin holey carbon or lacey carbon EM grids (Ted Pella, Redding, CA) and allowed to air dry. Bacterial suspensions (10µL) were deposited on EM grids for 1 min, followed by 3 ultrapure deionized water rinse steps, staining with 2% uranyl acetate, and drying. Transmission EM (TEM) was performed at 200 kV on a Hitachi

HD-2300 Ultra High Resolution FE-STEM (Hitachi City, Japan) equipped with a detector for energy dispersive X-ray spectroscopy (EDS) spectral imaging.

Photothermal Heating

1 mL of NR suspension was centrifuged, supernatant decanted, and the pellet resuspended in 50 μ L ultrapure deionized water. Samples were irradiated for 10 minutes with a NKT photonics SuperK Versa pulsed laser source (480 nm – 850 nm) with a 1 mm spot size and 150 mW average power. This corresponded to a beam intensity of 60 W/cm² and an energy/area of 36 kJ/cm². At 600 nm, the pulse width was 40 ps and the power density was 0.14 W/nm; at 800 nm, the pulse width was 31 ps and power density was 0.18 W/nm. Suspension temperature was monitored with a Luxtron I652 Industrial Fluoroptic Thermometer (Lambda Photometrics; Hertfordshire, UK) with a fiber optic thermocouple STF (Shanghai Thermostat Factory) probe.

Silver release quantification with ICP-MS

Au@Ag-NRs were prepared and resuspended in 30 μ L of ultrapure deionized water, 5 μ L of which were added to 600 μ L Eppendorf tubes. The samples were irradiated with NIR light for 10 minutes, followed by immediate addition of 395 μ L of ultrapure deionized water and centrifugation using 10,000 MW cutoff centrifugal filters (Ultrafree-MC, Millipore, Billerica, MA) to separate the nanoparticles from solution. 300 μ L of filtrate were collected and analyzed via Inductively Coupled Plasma Mass Spectrometry (ICP-MS, X Series 2; Thermo-Fisher Scientific, Pittsburgh, PA) for silver content.

Bacterial Assays

1 mL of bacterial suspension in 0.85% NaCl (10⁹ CFU/mL) was added to 40 μ L PEG-NR, PEG-Au@Ag-NR, Ab-NR, or Ab-Au@Ag-NR pellets, incubated under shaking for 20 minutes at 37° C, and then centrifuged at 2000 rpm at 4° C for 5 minutes. Supernatant was removed, and the cell pellets were resuspended in 50 μ L sterile 0.85% NaCl solution. 5-10 μ L were placed onto a glass slide and imaged with an optical coherence tomography (OCT) system.

Photothermal treatments were performed as described above on 5 μ L of the cell suspension placed in a 600 μ L eppendorf tube and irradiated for 10 minutes. Cells were stained with Syto-9 and propidium iodide (Invitrogen) according to manufacturer's specifications, and imaged with an epifluorescent microscope. Cell viability was quantified by counting individual numbers of live and dead cells with ImageJ software (6 fields of view per condition, ~ 100 cells per field of view). Statistical significance was determined via one-way ANOVA and the Bonferroni post-test with SPSS software.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Schematic illustration of the polydopamine-based strategy used to synthesize Ab-Au@Ag-NRs.

Figure 2.

Electron microscopic and spectroscopic characterization of Au@Ag-NRs formed upon mixing PD-NRs with 0 μM (a-e), 100 μM (f-j), and 300 μM (k-o) AgNO_3 . Electron microscopy was performed in dark field mode (a,f,k) with energy dispersive x-ray spectroscopy (EDS) spectral imaging of silver (b,g,l), gold (c,h,m) and merged silver/gold (d,i,n). Optical extinction spectra of each sample are shown at right (e,j,o) with color photographs of suspensions (inset). Scale bars = 20 nm.

Figure 3.

(a) Bulk heating profiles of aqueous PEG-NR and PEG-Au@Ag-NR suspensions upon irradiation with light. (b) Silver released from Au@Ag NRs with and without light irradiation.

Figure 4.

TEM images of *S. epidermidis* (right) and *E. coli* (left) cells treated with Au@Ag-NRs without antibodies (top row), Au@Ag-NRs functionalized with gram-positive bacteria targeting antibodies (middle row), and Au@Ag-NRs functionalized with gram-negative bacteria targeting antibodies (bottom row). Within each panel, the image pairs show the same field of view under bright field (left) and dark field (right) imaging modes. Scale bar = 500 nm.

Figure 5. *S. epidermidis* toxicity after incubation with Ab-NRs or Ab-Au@Ag-NRs and irradiation with light. (a) Fluorescence microscopy images of live/dead stained cells (green=live; red=dead) and (b) quantitative cell viability determined from image analysis.

Figure 6.

E. coli toxicity after incubation with Ab-NRs or Ab-Au@Ag-NRs and irradiation with light.

- (a) Fluorescence microscopy images of live/dead stained cells (green=live; red=dead) and
 (b) quantitative cell viability determined from image analysis.