Introduction

Infectious disease outbreaks due to multidrug-resistant bacteria (MDRB) infections are one of the leading causes of death worldwide, which is about one-third of global mortality.[1–5] Due to the biological diversity of the deadly bacteria and quite low infectious dose, there is an urgent need for reliable approaches to identify harmful bacterium with sufficient specificity and sensitivity.[6–9] Just after the development of penicillin in 1940, antibiotics are economic powerhouses for our society.[1–4] They are the most effective life-saving drugs for modern medical procedures. In the USA, antibiotics generate sales of about 42 billion USD per year, which is almost 15–30% of drug expenditures among any therapeutic group of drugs.[5–9] However, due to the heavy use of antibiotics worldwide, deadly human pathogens such as *Tuberculosis*, *Staphylococcus*, and *Salmonella* bacteria have become resistant to many antibiotics.[1–4] Due to the presence of multiple antimicrobial-resistance genes, such as 1) *pse* for ampicillin resistance, 2) *rpoB* for rifampicin resistance, 3) *floR* for chloramphenicol resistance, 4) *str* for streptomycin resistance, 5) *catG* for isoniazid resistance, 6) *sulI* for sulfonamide resistance, and 7) *tetR* or *tetG* for tetracycline resistance, several deadly bacteria are resistant to many commonly used antibiotics.[1–4] According to the World Health Organization (WHO),[6] after 1–2 decades, the current market-existing antibiotics will fail to cure MDRB infectious diseases. Therefore, the development of new approaches for the treatment of infectious bacterial pathogens that do not rely on traditional therapeutic action are urgently required. Driven by this need, in this article we report the development of a hybrid plasmonic shell–magnetic core multifunctional nanotechnology-driven approach for the targeted separation, label-free surface-enhanced Raman spectroscopy (SERS) detection, and selective photothermal destruction of MDR *Salmonella DT104*. The targeted photothermal-lysis experiment, by using 670 nm light at 1.5 W cm$^{-2}$ for 10 min, results in selective and irreparable cellular-damage to MDR *Salmonella*. We discuss the possible mechanism and operating principle for the targeted separation, label-free SERS imaging, and photothermal destruction of MDRB by using the popcorn-shaped magnetic/plasmonic nanotechnology.
titudes at a desired site, plasmonic gold nanotechnology promises to solve the greatest therapeutic challenges of society, which is selective destruction of MDRB infection and cancer. On the other hand, iron-based magnetic nanoparticles have been shown to be a very attractive material for use as contrast agents in magnetic resonance imaging (MRI) and biological separation. Herein, we report the development of core–shell popcorn-shaped nanoparticles and show their capability for magnetic separation, followed by the SERS imaging of MDR. The very high sensitivity, along with the highly informative spectra characteristics of Raman spectroscopy, will enable us to use the SERS-based method as a fingerprint for the label-free detection of MDRB. In the nano-popcorn, the central sphere will act as an electron reservoir, whereas the tips are capable of focusing the field at their apexes. As a result, the sharp tips will provide a huge field enhancement of the SERS-scattering signal. We also report that the same core–shell popcorn-shaped nanoparticle can be used as “light-directed nanohoters” for the hyperthermic destruction of MDR by using NIR light on the gold coating. In the reported popcorn-shaped hybrid nanoparticle, the plasmonic gold coating will be very useful for stabilizing the high-magnetic-moment nanoparticles in corrosive biological conditions. The gold coating will also eliminate the possible toxicity of iron nanoparticle and will also aid easy bioconjugation through the well-understood chemistry of Au-C0. Finally, the plasmonic shell can also be used as photothermal material for the hyperthermic destruction of MDR by using NIR light.

**Results and Discussion**

We synthesized the popcorn-shaped plasmonic gold shell–iron magnetic core nanoparticles through a two-step process, using seed-mediated growth, as shown in Figure 1A (the full details of which are given in the Experimental Section). TEM, a Hitachi 5500 Ultra-high resolution SEM microscope, and UV–visible absorption spectra were used to characterize the core–shell nanoparticles (as shown in Figure 1B–1F). Figure 1B shows the TEM (JEM-2100F) image of iron nanoparticle with a size of about 20 nm. Figure 1D shows the absorption spectra of ferric chloride, iron nanoparticle, magnetic core–gold shell popcorn-shaped nanoparticle. The strong long-wavelength band in the visible region ($\lambda_{PR}=580$ nm) is due to the oscillation of the conduction band electrons of gold. Figure 1D shows the absorption spectra of ferric chloride and the iron nanoparticle. The plasmon band around 580 nm, as shown in

![Figure 1D](image_url) clearly shows the formation of the gold shell. The TEM and SEM images show clear spikes, which indicate the formation of the popcorn-shaped nanoparticle (Fig-
ure 1E and F). As shown in Figure 1G, the EDX data also indicate the presence of both iron and gold.

For the selective magnetic separation and SERS imaging, we first modified the surface of the hybrid-nanoparticle with a M3038 antibody, which is specific for Salmonella DT104. Ronholm et al. have shown that monoclonal M3038 antibodies are highly specific for Salmonella DT104.[60] As described in the Experimental Section and shown in Figure 2, we used cystamine dihydrochloride and established a glutaraldehyde-spacer method for covalent immobilization of the monoclonal M3038 antibody onto the surface of the core–shell nanoparticle.

To demonstrate the separation capability of MDR Salmonella DT104, we incubated M3038 antibody-conjugated popcorn-shaped hybrid nanoparticles (100 µL) with MDR Salmonella DT104 suspension (2 mL) containing 1.2 × 10^6 colony forming units (CFU) mL⁻¹. After 20 min of incubation at room temperature under gentle shaking, we washed the suspension three times to remove unconjugated nanoparticles. Next, we used a bar magnet to separate and enrich the MDR Salmonella DT104-attached nanoparticles (Figure 3B). During the 10 min magnetic-separation process, the nanoparticle–MDR bacteria conjugates were confined and as a result, the MDR concentration was enriched. In the next step, the supernatant was carefully removed with a pipette. Then, the nanoparticle–MDR bacteria conjugates were resuspended with autoclaved PBS to 100 µL; since we started with a volume of 2 mL and the final volume was 100 µL, the concentration was enriched 20 times during the magnetic separation. Next, suspensions of the nanoparticle–MDR bacteria conjugates and supernatants were characterized by using bacteria colony counting, TEM and SERS techniques, as shown in Figure 3. As shown in Figure 3 D1, we did not see any bacteria in the supernatant, which clearly indicates that most of the bacteria are conjugated with M3038 antibody-conjugated popcorn-shaped plasmonic gold shell–iron magnetic core nanoparticles. To ensure that most of the bacteria were attached to the hybrid nanoparticles, we counted the amount of bacteria from the suspension and compared this value with the original concentration of bacteria. The amount of bacteria in the suspension was 1.15 × 10^7, whereas the initial concentration of the bacteria was 1.2 × 10^8, which indicates that the capture efficiency is about 96%.

Figure 2. A schematic representation showing the synthetic protocol of monoclonal antibody and A9 conjugated popcorn shape magnetic core–gold shell nanoparticle for the selective sensing and killing of Salmonella DT104.

Figure 3. A) A schematic representation showing the separation procedure for Salmonella DT104 by using M3038 antibody-conjugated core–shell nanoparticles. B) The separation of Salmonella DT104-attached magnetic/plasmonic nanoparticles by using a magnet. B) 1) Salmonella DT104-attached core–shell nanoparticle in the absence of a bar magnet and 2) Salmonella DT104-attached core–shell nanoparticle in the presence of bar magnet. C) A schematic representation showing the selective SERS imaging and targeted photothermal destruction of Salmonella DT104 after magnetic separation. D) Colonies of MDR Salmonella DT104: 1) Colonies shows absence of bacteria in the supernatant solution after magnetic capture and 2) colonies show the presence of bacteria in the suspension of magnetic/plasmonic nanoparticle-conjugated-MDR Salmonella DT104 bacteria. The TEM image also shows the formation of bigger microbial clusters in presence of 6.2 × 10^6 CFU mL⁻¹ MDR Salmonella DT104 bacteria; scale bar, 1 µm.
As shown in Figure 3E, in the presence of MDR Salmonella DT104 bacteria, several M3038 antibody-conjugated popcorn-shaped core–shell nanoparticles conjugate with one MDR bacteria, thereby producing nanoparticle aggregates. Since the MDR Salmonella DT104 bacteria is more than an order of magnitude larger in size (1–2 μm) than the anti-conjugated core–shell nanoparticles, in the presence of MDR Salmonella bacteria, several nanoparticles conjugate with one Salmonella bacteria through an antigen–antibody interaction. As a result, the absorption maximum for the plasmon absorption band of the popcorn-shaped core–shell gold nanoparticles at 580 nm was redshifted to about 650 nm and became much broader (Figure 4A). This redshift might be due to two factors: one is the change in the local refractive index on the nanoparticle surface caused by the specific binding of the antibody-conjugated core–shell nanoparticles on the MDR bacterium cell surface; the other is the interparticle interaction resulting from the assembly of nanoparticles on the cell surface. As shown in Figure 4A, our experimental results also show the formation of MDR microbial clusters in the presence of M3038 antibody-conjugated nanoparticles. MDR microbial clusters form because the M3038 antibody-conjugated popcorn-shaped core–shell nanoparticles act as binding agents between the cells to give a cellular network. There are multiple antibodies present on the surface of each nanoparticle; thus, when an M3038 antibody from one side of the core–shell nanoparticle binds to a specific binding-site on the surface of the target MDR Salmonella DT104 bacterium, there are other M3038 antibodies on the other side of nanoparticle that could be unoccupied, which enables each nanoparticle to bind to more than one MDR Salmonella bacteria cell. Therefore, the distance between the nanoparticles in such a cellular network is expected to be much smaller than the distance between nanoparticles that are not conjugated with bacteria. As a result, an intense redshift in the absorption spectra is observed.

The optical properties of the multifunctional popcorn-shaped magnetic–plasmonic gold nanoparticles also depend on the refractive index near the nanoparticle surface. As the refractive index near the nanoparticle surface increases, the nanoparticle extinction spectrum shifts to longer wavelengths. The refractive index near surface of the nanostructure is readily influenced by analyte binding. The plasmon properties of a metal nanostructure functionalized with a chemical receptor change when a molecule binds to the receptor and significantly alters the refractive index of the medium directly surrounding the metal nanostructure. In our case, when the antibody-attached nanoparticles conjugate with one Salmonella bacteria through an antigen–antibody interaction, the local refractive index on the nanoparticle surface is expected to increase as a result of a redshift in the plasmon mode.

As we discussed before, the high sensitivity afforded by the SERS detection enabled us to detect bacteria by using SERS without the use of a tagged dye. Also, as reported before by several groups, the largest Raman scattering enhancements are observed when molecules are residing in the fractal space between aggregated colloidal nanoparticles. As shown in Figure 3E, it is evident that MDR Salmonella DT104 helps to generate “hot spots” by aggregation of the multifunctional M3038-antibody nanoparticles through an antigen–antibody interaction. These are the perfect conditions in which to use the SERS spectra as “fingerprint” for the whole MDR organism. Figure 4B shows the SERS spectra from the suspensions of the nanoparticles.
monella conjugates after magnetic separation. Since the bacterial cell wall consists of proteins, lipids, and carbohydrates, one can expect to see the SERS spectra from the vibrational mode of the above compositions. Table 1 shows the vibrational assignments of the observed SERS peaks as shown in Figure 4B. Our observed SERS bands are in good agreement with the Raman bands reported for different microorganisms in the literature.\textsuperscript{[42–44]}

The Raman enhancement, \( G \), is measured experimentally by direct comparison with normal and SERS spectra as shown in Equation (1):\textsuperscript{[51–54]}

\[ G = \frac{I_{\text{SERS}}}{I_{\text{Raman}}} \times \frac{M_{\text{bulk}}}{M_{\text{ads}}} \]  

(1)

in which \( I_{\text{SERS}} \) is the intensity of a 1460 cm\(^{-1}\) vibrational mode in the surface-enhanced spectrum in the presence of Salmonella DT104, and \( I_{\text{Raman}} \) is the intensity of the same mode in the bulk Raman spectrum from only bacteria. \( M_{\text{bulk}} \) is the number of bacteria used in the bulk, \( M_{\text{ads}} \) is the number of bacteria adsorbed and sampled on the SERS-active substrate. \( M_{\text{bulk}} \) was calculated by counting colonies. For the \( M_{\text{ads}} \) calculation, we also counted the colonies after magnetic separation. All spectra are normalized for the integration time. An enhancement factor estimated from the SERS signal and normal Raman signal ratio for 1460 cm\(^{-1}\) band is approximately 8.5/C148107. No significant changes in the Raman bands reported for different microorganisms in the literature.\textsuperscript{[42–44]}

As shown in Figure 5B, our colony-counting results indicate that the bacteria-capture efficiency was less than 1%; on the other hand, the bacteria-capture efficiency was 96% for MDR Salmonella DT104. This result clearly shows that our bioconjugated popcorn-shaped magnetic core–plasmonic shell nanoparticle is capable of selective separation, enrichment, and imaging of the MDR organism. The novelty of our approach is that we used a simple methodology to capture, separate, and enrich the MDR Salmonella DT104 bacterium by using a bioconjugated popcorn-shaped magnetic core–plasmonic shell nanoparticle.

After successful targeted magnetic separation and imaging, we performed NIR irradiation experiments to determine whether the popcorn-shaped core–shell magnetic/plasmonic nanoparticles can be used for photothermal destruction of MDR selectively through the gold shell. Gold nanoparticles are known to be capable of converting NIR to vibrational energy, and generate sufficient heat that can kill microorganisms and cancer cells.\textsuperscript{[15–17,19,20,25,27]} For the photothermal destruction of MDR bacteria, we used red light (670 nm OEM laser at 1–2 W cm\(^{-2}\)) for 10 min. When the MDR Salmonella DT104-conjugated M3038 antibody-conjugated nanoparticles were incubated with an E. coli suspension (2 mL) containing 1.2×10\(^7\) CFU/mL, we incubated M3038 antibody-conjugated nanoparticles (100 \( \mu \)L) with an E. coli suspension (2 mL) containing 1.2×10\(^7\) CFU/mL. After 20 min incubation at room temperature with gentle shaking, we used a bar magnet to separate and enrich the sample. As shown in Figure 5A and B, no E. coli bacteria were captured by the magnetic nanoparticles.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|}
\hline
Vibration mode & Raman peak position [cm\(^{-1}\)] \\
\hline
\( \nu(\text{COO}^-) \) anti-symmetric & 1585 \\
\( \delta(\text{CH}_2) \) saturated lipids & 1460 \\
\( \nu(\text{COO}^-) \) symmetric & 1380 \\
\( \nu(\text{NH}_2) \) Stretch for adenine and guanine & 1340 \\
\( \nu(\text{CC}) \) ring stretch, n-alkanes & 1150 \\
\( \delta(\text{CCH}) \), aliphatic & 830 \\
\( \rho(\text{CH}_3) \) & 720 \\
\( \delta(\text{CCC}) \) ring deformation & 590 \\
\hline
\end{tabular}
\caption{Analysis for the observed SERS vibrational modes from Salmonella DT104.}
\end{table}
ture. This local temperature increase produces sufficient heat for the destruction of MDR bacteria, which could therefore be the perfect solution to kill MDR bacteria without the use of antibiotics. Following photothermal lysis, the amount of MDR Salmonella DT104 bacteria that was destroyed was confirmed by colony plating. Figure 6A shows the number of colonies after 10 min exposure to 670 nm light for MDR Salmonella DT104 with and without magnetic/plasmonic nanoconjugation. As shown in Figure 6, our experimental results clearly demonstrate that the localized heating that occurs during NIR irradiation is able to perform irreparable cellular-damage. Therefore, our study shows that bioconjugated core–shell magnetic/plasmonic nanoparticles could be the next avenue for exploration to selectively target and destroy MDR microbial cells.

Since SERS is capable to find the fine structures of the bacterial cell wall, we wanted to understand whether SERS could be used to monitor any change in cell wall during the photothermal destruction of bacteria. For this purpose, we performed an SERS experiment after the photothermal-lysis experiment. As shown in Figure 6B, there was no appreciable change in the SERS vibrational modes even after 20 min of laser exposure (670 nm). This may indicate that during the photothermal destruction of bacteria, there may not be any occurrence of significance change in the MDR cell wall. The mechanism of MDR Salmonella DT104 death could be due to numerous factors including thermal stresses, shock waves, bubble formation and nanoparticle explosion.

Next, to understand whether our antibody-conjugated hybrid nanoparticle-based magnetic separation and photothermal destruction is highly selective, we performed the same experiment with E. coli. For this purpose, we incubated M3038 antibody-conjugated hybrid nanoparticles (100 µL) with of E. coli suspension (2 mL) containing 1.2 x 10^5 CFU mL^1. After 20 min of incubation at room temperature with gentle shaking, we used a bar magnet to separate and enrich the sample. As we have discussed before, because almost all the E. coli remained in the supernatant solution, after separation we treated the supernatant with light (670 nm at 1.5 W cm^-2) for 10 min. As shown in Figure 6C, during photothermal lysis, the time-interval live-bacteria test indicated that within 10 min, all the MDR Salmonella DT104 were killed when exposed to light (670 nm at a power of 1.5 W cm^-2); however, almost 97% of the E. coli bacteria remain live even after 20 min of treatment with the same exposure conditions. Thus, our result clearly shows that the conjugation of nanoparticles with bacteria is necessary for photothermal destruction by using light (670 nm). As shown in Figure 5B, because the M3038 antibody-conjugated nanoparticles do not bind with E. coli, the bacteria in the supernatant do not have enough absorption at 670 nm. Therefore, during photothermal destruction by using 670 nm light, the effective temperature increase in bacteria will be very little, which will be insufficient to kill these cells. Next, to understand how the temperature increases during photothermal destruction, we performed thermal imaging at one-minute intervals during the therapy process by using a MikroShot Camera. We found that the temperature increased to about 48°C when nanoparticle-bound MDR Salmonella DT104 were exposed to a 670 nm laser at 1.5 W cm^-2 power for 10 min. Conversely, under the same conditions, the temperature increased to only 30°C for E. coli bacteria without any nanoparticles.

**Conclusion**

We have reported the synthesis and characterization of a multifunctional popcorn-shaped iron magnetic core–shell gold nanoparticle and demonstrated the use of M3038 anti-

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**Figure 6.** A) Colonies of MDR Salmonella DT104 bacterium demonstrating the amount of live bacteria after exposure to 670 nm light for 10 min. 1) MDR Salmonella DT104 bacteria (1.2 x 10^5 CFU mL^-1) without NP. 2) MDR Salmonella DT104 bacteria (1.2 x 10^5 CFU mL^-1) in the presence of M3038 antibody-conjugated magnetic core–plasmonic shell popcorn-shaped gold nanoparticles. B) Plot shows SERS intensity variation from MDR Salmonella DT104, before and after photothermal lysis. We did not observe any significance change. C) Plot demonstrating the percentage of live bacteria during the time-dependent photothermal lysis by using 670 nm light at a power of 1.5 W cm^-2. An amount of 1.2 x 10^5 CFU mL^-1 bacteria was used for each case.
body-conjugated popcorn-shaped core–shell nanoparticles for the targeted magnetic separation and enrichment, SERS imaging, and the photothermal destruction of MDR Salmonella DT104. Our experimental results show that the M3038 antibody-conjugated hybrid nanoconjugate is capable of selective label-free SERS detection of MDR Salmonella DT104 with an excellent detection limit (10^2 CFU mL⁻¹). Our experimental results shown that when the antibody-conjugated core–shell nanoparticles are attached to MDR Salmonella DT104 bacterial cells, the localized heating that occurs during 670 nm light irradiation is able to cause irreparable cellular damage and kill approximately 100% of MDR Salmonella bacteria within 10 min of exposure even at a power of 1.5 W cm⁻². The reported bioassay using the popcorn-shaped magnetic core–plasmonic shell nanoparticles is rapid, takes about 30 min from bacterium binding to separation and enrichment, selective detection, and photothermal cell death. Since the prevalence of multidrug-resistant bacteria is on the rise, our results demonstrated that the bioconjugated multifunctional hybrid-nanoparticle approach to selectively target and destroy MDRB could be the next avenue for exploration. Even though our experimental observation have demonstrated promising results for MDRB separation and enrichment, followed by SERS imaging and therapy, it is fair to admit that we are at a relatively early stage of development. After the optimization of different parameters, we believe that this multifunctional popcorn-shaped hybrid nanotechnology-driven assay could have enormous potential for applications in the rapid detection and photothermal destruction of MDRB in clinical samples.

**Experimental Section**

**Materials**: Hydrogen tetrachloroaurate (HauCl₄·3H₂O), NaBH₄ sodium citrate, and iron chlorides were purchased from Sigma–Aldrich and used without further purification. Ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline antibiotics, drug-resistant Salmonella typhimurium DT104, and other bacteria strains were obtained from the American Type Culture Collection (ATCC, Rockville, MD: ATCC 700408).

**Synthesis of popcorn-shaped iron magnetic core–gold shell nanoparticles**: We synthesized the popcorn-shaped iron nanoparticle magnetic core–gold shell nanoparticles through a two-step process, by using seed-mediated growth, as shown in Figure 1A. In the first step, very small spherical iron nanoparticles were synthesized by using tri-sodium citrate as a stabilizer and sodium borohydride as a strong reducing agent. For this purpose, an aqueous solution of NaBH₄ (2.5 mM) was added dropwise into FeCl₃·NH₄Cl·3H₂O, deionized (DI) water. Next, we added an aqueous solution of trisodium citrate (TSC, 0.25 mM) at room temperature with vigorous stirring. TSC helps the formation of iron nanocrystals through the nucleation of the monoclonal M3038 antibody onto the amine-coated popcorn-shaped magnetic core–gold shell nanoparticle, we used the highly established glutaraldehyde-spacer method.[16,19–21] To remove the excess antibody, we washed the antibody-conjugated popcorn-shaped magnetic core–gold shell nanoparticle several times with PBS. To measure the number of M3038 antibody molecules in each core–shell nanoparticle, we performed a KCN dissolution procedure, as reported previously.[16,34] In this case, we used an Rh-6G-modified antibody. We estimated that there were about 100–120 Rh-6G-modified M3038 antibodies per popcorn-shaped magnetic core–gold shell nanoparticle. This experiment has been performed 5 to 6 times and the average values are reported in this manuscript. During immobilization of the antibody, we did not note any aggregation of popcorn-shaped magnetic core–gold shell nanoparticles as examined by TEM and UV–visible absorption spectroscopy. Surface-enhanced Raman spectroscopy (SERS) probe for the targeted sensing of cancer cells: For the SERS experiment, we designed a SERS probe, as we have reported recently.[16] In short, we used a continuous wavelength DPSS laser from laser glow technology (LUD-670) operating at 670 nm, as an excitation light source. For excitation and data collection, we used InPhotonics 670 nm Raman fiber optic probe, which is a combination of 90 μm excitation fiber and 200 μm collection fiber with filtering and steering micro-optics. For Raman signal collection, we used a miniaturized QE65000 Scientific-grade Spectrometer from Ocean Optics as a Raman detector. The spectral response range of this mini Raman spectrometer is 220–3600 cm⁻¹. It is equipped with TE cooled 2048 pixel CCD and interfaced to computer through a USB port. The Hamamatsu FFT-CCD detector used in the QE65000 provides 90% quantum efficiency with high signal-to-noise and rapid signal processing speed as well as remarkable sensitivity for low-light level applications. The Raman spectrum was collected with Ocean Optics data acquisition SpectraSuite spectroscopy software. Bacteria sample preparation and incubation with magnetic/plasmonic nanoparticles: Salmonella typhimurium DT104, which is resistant to five different antibiotics such as Ampicillin, chloramphenicol, streptomycin, CTAB (10 mg) in DI water (50 mL). HauCl₄ (2 mL 10⁻² M) was added into the solution and stirred for 1 min. After that, CTAB acts as a shape-templating surfactant so that the seeds can grow into the larger particles of particular morphology we desired. Then we continued stirring for 24 h. After 24 h, hybrid magnetic nanoparticles were separated and washed with DI water and ethanol by using a magnet. A TEM and Hitachi 5500 Ultra-high resolution SEM microscope and UV–visible absorption spectrum were used to characterize the core–shell nanoparticles (as shown in Figure 1C–F). A plasmon band around 580 nm, as shown in Figure 1D, clearly shows the formation of gold shell. The TEM and SEM images show clear spikes, which indicate the formation of popcorn-shaped nanoparticles. As shown in Figure 1G, energy dispersion X-ray (EDX) data also indicate the presence of both iron and gold. We also observed a Cu and an Al peak in the EDX data, which originate from the support grid.

**Preparation of monoclonal antibody-conjugated popcorn-shaped magnetic core–gold shell nanoparticles**: For selective sensing and killing of MDR DT104, we modified the surface of the popcorn-shaped magnetic core–gold shell nanoparticle by using the monoclonal M3038 antibody,[30] as shown in Figure 2. As we discussed in the last section, the popcorn-shaped magnetic core–gold shell nanoparticles were synthesized by using seed-mediated growth procedure in the presence of CTAB. As a result, the as-synthesized core–shell nanoparticles have a CTAB coating. However, CTAB is known to be cytotoxic, and as a result, it will not be ideal for in vivo diagnosis. Furthermore, CTAB is positively charged at physiological pH and therefore it will be able to attract negatively charged bacteria easily to its surface.[31–33] It is now evident that CTAB-coated popcorn-shaped magnetic core–gold shell nanoparticles are coated with specific binding proteins. To overcome this problem, we modified the surface of the gold-shell nanoparticle with amine groups (as shown in Figure 2). For this purpose, we added cystamine dithirolcholester (30 mM) to the popcorn-shaped magnetic core–gold shell nanoparticle (50 mL) and the solution was kept at 50°C for several hours under constant sonication. After that, the excess cystamine dithirolcholester was removed by centrifugation at 4000 rpm for several minutes. For coating immobilization of the monoclonal M3038 antibody onto the amine-coated popcorn-shaped magnetic core–gold shell nanoparticle, we used the highly established glutaraldehyde–spacer method.[16,19–21] To remove the excess antibody, we washed the antibody-conjugated popcorn-shaped magnetic core–gold shell nanoparticle several times with PBS. To measure the number of M3038 antibody molecules in each core–shell nanoparticle, we performed a KCN dissolution procedure, as reported previously.[16,34] In this case, we used an Rh-6G-modified antibody. We estimated that there were about 100–120 Rh-6G-modified M3038 antibodies per popcorn-shaped magnetic core–gold shell nanoparticle. This experiment has been performed 5 to 6 times and the average values are reported in this manuscript. During immobilization of the antibody, we did not note any aggregation of popcorn-shaped magnetic core–gold shell nanoparticles as examined by TEM and UV–visible absorption spectroscopy.
sulfonamides, and tetracycline, was purchased from the ATCC (ATCC 700468) and then cultured in our laboratory. The MDR bacteria were cultured by following the ATCC protocol as instructed. Initially, the sup- plied pellet of DT104 was rehydrated on bacto tryptic soy broth (BD: 5 to 6 mL) and incubated at 37°C for 24 h. Next, from the growth culture, a loop of bacteria were streaked on tryptic agar plate and incubated for 24 h at 37°C, as instructed by ATCC. A tryptic agar plate was made with Difco tryptic soy agar (BD). A single colony from tryptic agar plate was inoculated into tryptic soy broth (TSB; 10 mL) and incubated (37°C for 16 h) in a shaker at 150 rpm, which have an inoculum of 10^7 CFU/mL. We then diluted the stock bacteria several times to vary the concentra- tion of Salmonella DT104 from 10^{-1} to 10^{-6} CFU/mL. All the growth medium and Agar were autoclaved at 121°C for 15 min at high pressure (0.1 MPa) before the experiment. Different amounts of bacteria were then immersed into the antibody-conjugated magnetic core–plasmonic shell pop-corn-shaped nanoparticle solution at room temperature before performing the magnetic separation experiment. After magnetic separa- tion, we performed TEM and colony counting (Figure 3). The absorption data show that Salmonella DT104 bound to the magnetic/plasmonic pop- corn-shaped nanoparticles have quite broad absorption (Figure 3E); this may be due to the aggregation of nanoparticles on Salmonella DT104 bacteria surfaces as shown in the TEM picture in Figure 3.

**Photothermal cell-death and determination of live bacteria percentages:** For photothermal lysis, we used a portable continuous wavelength OEM laser operating at 670 nm, with a power of 1 W cm^{-2} as an excitation- light source for 10–20 min. After that, the bacteria were transferred to tryptic agar plate, which was incubated for 24 h at 37°C and colony number for each countable plate was counted with a colony counter.

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Popcorn-Shaped Multifunctional Nanoparticles


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