Dual Modality FeS Nanoparticles with Reactive Oxygen Species-Induced and Photothermal Toxicity toward Pathogenic Bacteria

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ABSTRACT: Bacterial infections pose a major threat to human health, primarily because of the evolution of mutated strains that are resistant to antibiotic treatment. As a viable alternative, several nanoparticles have emerged as attractive antibacterial agents. Herein, we report the development of iron sulfide (FeS) nanoparticles that show dual-modality therapy: namely reactive oxygen species (ROS)-induced toxicity and red-laser induced photothermal therapy. The aqueous synthesized nanoparticles have been characterized based on their size, shape, crystallinity, and magnetic and optical properties. These nanoparticles showed sustained release of Fe²⁺ ions in an aqueous dispersion. They also have a high absorption cross-section in the visible and near-infrared regions and could be excited by a continuous wave diode laser of wavelength 635 nm leading to significant hyperthermia. Nanoparticle treatment, followed by light irradiation, led to significant cell death in two ghastly pathogenic bacterial strains. Stepwise enhancement of intrabacterial ROS levels, as a result of nanoparticle treatment followed by light activation, has been identified as the primary antibacterial mechanism.

1. INTRODUCTION

It is well known that traditional antibiotics have limited utility in the treatment of bacterial diseases/infections owing to the development of several mutated, antibiotic-resistant strains.1−4 Therefore, alternative strategies are being channelized for treating deadly pathogens. Several metal-based nanoparticles have emerged as a new generation of antibacterial agents due not only to their inherent properties, but also their ability to act as carriers of antibiotics. These include nanoparticles of noble metals (e.g. gold and silver) and their alloys, oxides (e.g. iron oxide and titania), metal chalcogenides (e.g. MoS₂), and so forth.5−20 Although each nanomaterial has its own mode of action, two general antibacterial strategies from them have been identified, which may act separately or in combination. The first modality involves the generation of reactive oxygen species (ROS) from the oxidation of free metal ions.21,22 This occurs as a result of slow degradation of the nanoparticle matrix in biological fluids. The other modality involves localized heat-generation (hyperthermia) as a result of exposure of the nanoparticles to external stimuli, such as light or a magnetic field. This happens as a result of non-radiative relaxation or hysteresis loss of nanoparticles following their excitation with light or a magnetic field, respectively.23−25 Among these, light-activation has emerged as the better strategy owing to its easy availability and simple mode of exposure.

Iron-containing oxides and chalcogenides represent an important family of nanomaterials and have extensively been used in various biomedical applications, such as antimicrobial action, magnetically guided drug delivery, contrast enhancement in MRI, ac magnetic field induced hyperthermia therapy, and so forth.25−32 They are also easy to synthesize and, in general, are found to be safe for biomedical applications. Not only do they have unique magnetic properties (e.g. ferromagnetic, superparamagnetic, etc.), but they also exhibit attractive optical absorption covering the visible and near infrared (NIR) region. As a result, they are also emerging as efficient nanomaterials for photothermal therapy (PTT), which has applications in treating cancer and inflammatory and microbial diseases. PTT is known to inflict physical damage on the bacterial cells when light-absorbing nanoparticles selectively adsorbed on the surface of bacteria are photo-irradiated.33 The resulting local hyperthermia leads to the formation of heat bubbles which destroy the cell wall, followed by bacterial cell death.34 By confining light irradiation to the diseased/infected region pretreated with the nanoparticles, damage to normal cells/tissues can be avoided. The other advantage of PTT is that bacterial cells are not yet known to be resistant to photothermal damage.

Keeping the above facts in mind, we have prepared stable, aqueous-dispersed iron-sulfide (FeS) nanoparticles and have investigated their antibacterial effects. These nanoparticles have been synthesized by the co-precipitation approach using ferric chloride in the presence of sodium dithionite and sodium borohydride as the reducing agent. We have extensively characterized these nanoparticles using transmission electron microscopy (TEM), scanning electron microscopy (SEM), powder X-ray diffraction (XRD) and selected area electron...
diffraction (SAED), which clearly reveal their spherical and crystalline nature. Their optical and magnetic properties have been ascertained by using UV–visible spectrophotometry and vibrating sample magnetometry (VSM), respectively. Next we have explored the combined dual-modality antibacterial therapy involving these nanoparticles in an aqueous dispersion via (a) production of ROS as a result of oxidation of the liberated Fe\(^{3+}\) ions and (b) laser light activated localized hyperthermia. The aqueous dispersion of these nanoparticles upon exposure to red laser light showed a significant rise in temperature and subsequent robust bacterial damage, which make them suitable agents for photothermal applications.

2. EXPERIMENTAL SECTION

2.1. Materials Used. Ferric chloride (FeCl\(_3\)-6H\(_2\)O), sodium dithionite (Na\(_2\)S\(_2\)O\(_4\)), sodium borohydride (NaBH\(_4\)), ammonia solution (25%), dimethyl sulfoxide, ammonium acetate, ethanol, methanol, concentrated hydrochloric acid (HCl), and l-ascorbic acid were purchased from Merck. Ferrozine reagent, phosphate buffered saline (PBS), Triton-X100, neocuproine, fetal bovine serum, Dulbecco's modified Eagle's medium, iodonitrotetrazolium salt (INT), amphoter-icin-B, glucose, and penicillin–streptomycin were purchased from Sigma-Aldrich.

2.2. Synthesis of FeS Nanoparticles. FeS nanoparticles were synthesized by a facile chemical precipitation method using iron chloride (FeCl\(_3\)-3H\(_2\)O) as the iron source and sodium dithionite as the sulfur source. The traditional reducing agent sodium borohydride (NaBH\(_4\)) was used for reduction of Fe\(^{3+}\) to Fe\(^{2+}\). Briefly, 1 g sodium dithionite was dissolved in 1 L of 1 M aqueous NaBH\(_4\) solution. The resulting solution was added drop-wise to a 1 M FeCl\(_3\)-3H\(_2\)O aqueous solution in a volume ratio of 1:1. The solution was decanted to remove the unreacted reactants and side products. The precipitate was thoroughly washed with water and ethanol to purify the nanoparticles. The synthesized FeS nanoparticles were further dried in an oven in the absence of air for 24 h and used for further studies.

2.3. Characterization of FeS Nanoparticles. The as-synthesized FeS nanoparticles were characterized based on their size and morphology. Their size and morphology were observed by the dynamic light scattering (DLS) method using a Malvern Zetasizer (NANO ZS series), TEM using a TECNAI G\(^{2}\)-30 U-TWIN TEM instrument, and SEM using a MIRA3 TESCAN instrument. Powder XRD analysis using a Bruker D8 Discover Y-ray spectrometer and selected area electron diffraction (SAED) using a TECNAI G\(^{2}\)-30 U-TWIN TEM instrument were carried out to confirm the crystalline nature of the nanoparticles. The optical properties of aqueous dispersed FeS nanoparticles (conc. 0.75 g/mL) were monitored by UV–visible spectroscopy using a Shimadzu UV-1601 Spectrophotometer. Their magnetic properties were observed by vibrating sample magnetometry (VSM) using a Microsense EV-7 instrument.

2.4. Temperature Rise (Hyperthermia) Experiment. This experiment was performed by taking various aqueous dispersions of FeS nanoparticles in a 3 mL glass vial, followed by light irradiation with a continuous wave (CW) laser emitting at a wavelength of 635 nm for a maximum time of 30 min. The time-dependent rise in temperature in the dispersions, as a result of photo-induced hyperthermia, was recorded using a digital probe thermometer at regular time-intervals of 5, 10, 15, 20, 25, and 30 min. PBS was used as the blank solution (negative control) in this experiment.

2.5. In Situ Fe\(^{2+}\) Release. The time-dependent cumulative release of Fe\(^{2+}\) ions from the aqueous dispersion of FeS nanoparticles was ascertained by the ferrozine reagent assay.\(^{35}\) Ferrozine reagent is a mixture of four chemicals, namely aqueous solutions of 2,9-dimethyl (1,10-phenanthroline), or neocuproine (6.5 mM), 3-(2-pyridyl)-5,6-bis(phenylsulfonic acid)-1,2,4-triazine, or ferrozine (6.5 mM), ammonium acetate (2.5 M) and l-ascorbic acid (1 M). Ascorbic acid is used to convert any Fe\(^{3+}\) ions into Fe\(^{2+}\) ions. Freshly prepared ferrozine reagent (colorless) reacts with Fe\(^{2+}\) ions to yield a magenta colored solution with an absorption maximum at 562 nm. A fixed concentration of FeS nanoparticles in water (200 μg/mL) was distributed in different vials (1 mL each) and incubated at 37 °C for different time intervals (daily for 7 days). PBS was used as the negative control. Each sample was centrifuged at 10,000 rpm for 5 min and the optically clear filtrate was decanted. Then the filtrate from each vial was treated with 100 μL of ferrozine reagent and incubated for 30 min, and the optical density (OD) of the resulting solution was measured spectrophotometrically at 562 nm. A higher OD corresponds to a higher Fe\(^{2+}\) ion concentration in the solution. The exact amount of Fe\(^{2+}\) ions released in each sample was then determined from a calibration curve obtained using the ferrozine assay with known concentrations of Fe\(^{2+}\) ions (aqueous solution of FeCl\(_2\)). A plot was made between Fe\(^{2+}\) concentration (in μM) and number of days of incubation of the aqueous FeS dispersion.

2.6. Bacterial Culture and Antibacterial Studies. The bacteria Staphylococcus aureus (MTCC 740) {Gram (+)} and Pseudomonas aeruginosa (Gram (−)) were grown in Mueller Hinton broth growth medium at an optimum temperature of 37 °C.

A broth microdilution assay in sterile 24 well cell-culture plates was performed to estimate the antibacterial efficacy of the nanoparticles (using Milli-Q water). The bacterial cells in the cell-culture wells were treated with the aqueous dispersion of the FeS nanoparticles (500 μg/mL, sterile filtered with a Millipore Filter, cut-off size 0.22 μm) and mixed well. Cells treated with only Milli-Q water were used as the negative control. To analyze the photothermal effect, half of the treated bacterial cell wells were irradiated with a diode laser emitting at 635 nm, by focusing the laser beam vertically downward on a particular well for a duration of 7 min each. After the light treatment, the plates were kept in an incubator shaker at 37 °C. After incubation for specific intervals of time (0, 2, 4, 6, 8, 10, and 12 h), 100 μL of the growth indicator INT 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyl-2H-tetrazolium, or INT dye reagent (0.25 mg/mL), were added to each bacterial well and mixed and the cells were further incubated for 30 min. INT upon reduction by live bacteria produces pink formazan dye, with an absorption maximum at 570 nm.\(^{36}\) Therefore, the OD at 570 nm directly correlates with the bacterial growth. The absorption measured at 570 nm for samples from each well was then plotted as a function of time, to analyze the time-dependent bacterial growth upon nanoparticle treatment, without and with light exposure.

2.7. Colony Count Assay. The S. aureus strain grown in LB medium was treated with 500 μg mL\(^{-1}\) FeS nanoparticles, with and without laser irradiation, in a 24 well plate (NUNC) overnight at 37 °C in an orbital shaker at 180 rpm. On the second day, it was diluted serially to 10\(^{-5}\) in fresh LB medium.
and 100 μL of this dilution was plated on LB agar plates. The plates were incubated at 37 °C for 48 h and the colonies were counted manually and used in the equation below.37

No. of colony forming units

= no. of colonies counted/(vol. of dilution plated × dilution factor)

2.8. Interaction of FeS Nanoparticles with Bacterial Cells via TEM Study. The interaction of FeS nanoparticles with bacterial cells was confirmed by TEM analysis. FeS nanoparticles (1 mg/mL) were incubated with Bacillus cereus (fresh culture) for 1 h at 37 °C. After this, the culture was centrifuged and the bacterial cell pellet obtained was rinsed thoroughly with PBS. Then this pellet was fixed with paraformaldehyde (4% solution) and kept at 4 °C for the whole night. Then it was centrifuged and the pellet thus obtained was thoroughly washed with PBS. Next, it was sequentially dehydrated with increasing concentrations of ethyl alcohol such as 10, 20, 40, 60, 80, and 100%. The pellet finally obtained was again resuspended in PBS solution and mounted on a TEM grid for analysis.

2.9. ROS Generation Assay. Generation of hydroxyl, peroxyl and other ROS was measured using 2,7-dichloro-dihydrofluorescein diacetate (DCFH-DA), a fluorescent dye. For ROS detection, S. aureus was treated without and with FeS NPs (0.5 and 1 mg/mL) and incubated for 5 h. Later it was centrifuged and washed with PBS. The pellet was incubated with dye (50 μM) for 30 min at 37 °C. The fluorescence of 2′,7′-dichlorofluorescein was measured using a TECAN Multimode Microplate Reader at excitation: 485 nm and emission: 525 nm. The results were expressed in relative fluorescence units.

3. RESULTS AND DISCUSSION

3.1. Characterization Studies. In this study, we have synthesized FeS nanoparticles by a simple micellar approach using ferric chloride (FeCl₃·3H₂O) and sodium dithionite, with sodium borohydride (NaBH₄) as the reducing agent. The iron precursor and the dithionite were used in a 1:1 ratio and NaBH₄ was added in a small amount and heated at 120 °C for 8–10 h. After the completion of the reaction a dark brown precipitate of FeS nanoparticles was obtained, which was used for further studies. We first recorded the hydrodynamic size of the synthesized nanoparticles using DLS, which yielded an average hydrodynamic diameter of 102 nm (Figure 1A). The hydrodynamic diameter takes into account the hydration layer of the nanoparticles. The actual size of the nanoparticles was obtained using TEM, which showed an approximate size range of 60–80 nm (Figure 1B). SEM images of FeS nanoparticles provided additional insight into their structure, shape and surface morphology (Figure 1C). Here, FeS nanoparticles exhibited quasi-spherical morphology with an average grain size of 70 nm.

Powder XRD data revealed that the synthesized FeS nanoparticles are crystalline in nature (Figure 2A). On comparing this data with the corresponding XRD pattern of pure tetragonal phase FeS nanoparticles (JCPDS card no. 860389), it was evident that there was a good agreement between the observed peaks, namely (101), (110), (102), (201), and (213).38 In the selected area electron diffraction (SAED) pattern of the nanoparticles, the bright spotted area and concentric rings confirmed their crystallinity (Figure 2B). These observations were in accordance with the XRD pattern obtained for the nanoparticles. The concentric rings observed can be correlated with the (110), (102), (201), and (113) diffraction peaks.38

The magnetic behavior of the nanoparticles was elucidated by using a vibrating sample magnetometer (VSM). The observed magnetization curve (Figure 3A) shows that the synthesized FeS nanoparticles were ferromagnetic (Figure 3A, inset) in nature and might behave like soft magnets. Soft

Figure 1. (A) DLS data (average hydrodynamic size distribution), (B) TEM micrograph (scale bar = 200 nm), and (C) field emission SEM image (scale bar = 0.5 μm) of FeS nanoparticles.

Figure 2. (A) XRD pattern of powdered FeS nanoparticles showing sharp peaks, indicating the crystalline nature of nanoparticles. The overlay pattern below shows the standard tetragonal phase FeS nanoparticles. (B) SAED pattern of the nanoparticles, confirming their crystalline nature.

Figure 3. (A) VSM data (inset: magnified near zero-field) and (B) UV−visible spectrum of FeS nanoparticles (0.75 mg/mL). (C) Temperature rise (hyperthermia) pattern of aqueous dispersed FeS nanoparticles as a function of time of irradiation with CW laser light (wavelength 635 nm).
magnets are those materials which show high magnetic permeability and low coercivity. Iron based nanoparticles are used widely as soft magnetic materials and these particles attain magnetic saturation in a relatively low applied magnetic field. Although we have not exploited the magnetic properties of these nanomaterials for antibacterial action in this work, magnetic targeting as well as magnetic hyperthermia are nevertheless attractive antibacterial strategies which have not yet been completely explored.

Then, the optical properties of FeS nanoparticles were monitored by UV-visible spectroscopy. In the recorded spectrum (Figure 3B), it was evident that the FeS nanoparticles showed a broad absorption in the visible region (400–700 nm). This property makes them a suitable photothermal agent for broad visible light excitation. We tested their photothermal (light-excited heating effect) efficiency by irradiating aqueous dispersions of these nanoparticles at various concentrations (0.125 and 0.75 mg/mL) with continuous-wave laser light of wavelength 635 nm (power density 20 W/cm²). A significant rise in the temperature from 32 °C to a maximum of 58 °C was observed after 30 min of light irradiation (Figure 3C). This experiment showed that the FeS nanoparticles can act as good photothermal agents following visible light excitation, which can be applied for anticancer or antibacterial hyperthermia therapies.

Next, we tested the time-dependent release of Fe²⁺ ions from the aqueous dispersion of FeS nanoparticles (200 μg/mL) using the ferrozine assay, as described earlier. As shown in Figure 4, Fe²⁺ ions are found to be released in solution in a time-dependent manner, with an initial burst release followed by a steady release. This release is attributed to partial surface erosion of the nanoparticles. The released Fe²⁺ ions have been reported to be responsible for the production of various ROS under oxic conditions.

As discussed earlier, a promising antibacterial strategy is photo-induced hyperthermia, which involves localized heating of the bacterial surface as a result of light-irradiation of certain nanoparticles accumulated on the surface of the bacteria. We first ascertained the interaction of the nanoparticles with the bacterial cell surface using TEM (Supporting Information S1). FeS nanoparticles are robust visible/NIR light induced photothermal agents and thus promising transducers for antibacterial hyperthermia therapy. As shown in Figure 5A,B, it was evident in both the bacterial cultures (SA and PA) that while untreated bacteria shows ample growth, the growth is highly suppressed for the bacteria treated with the FeS nanoparticles. This effect can be termed as “bacteriostatic”, where bacterial growth is greatly reduced, although some remnant viable cells remain. On the other hand, near-complete suppression of bacterial growth was observed when the nanoparticle-treated bacteria were exposed to laser light, which can be termed as “bactericidal” owing to the death of bacteria. The bacteriostatic effect of the FeS nanoparticles (no light exposure) can be attributed to the attachment of the nanoparticles to the bacterial surface and local production of ROS, thus stifling their cell division and growth. When the nanoparticle-attached bacteria were exposed to light, photo-induced hyperthermia was further responsible for the death of the bacterial cells. This experiment directly proves the effect of the antibacterial visible-light induced photothermal effect of FeS nanoparticles. The antibacterial effect of the nanoparticles, in combination with light irradiation, is further confirmed by a colony counting assay (Supporting Information S2). The nanoparticles did not show any appreciable toxicity upon interaction with normal mammalian fibroblast cells in culture (Supporting Information S3), indicating their general biocompatibility.

Finally, we probed the intra-bacterial ROS production using the DCFH-DA assay by comparing the ROS generated by the bacteria without and with nanoparticles as well as light treatment. We have used DCFH-DA in our study as it can detect a broad spectrum of ROS species, like hydroxyl radicals, singlet oxygen, super oxide, and hydrogen peroxide. In Figure 6, substantial enhancement in intra-bacterial ROS can be observed upon treatment of the cells with the nanoparticles. The ROS levels further increase with light irradiation of the treated cells. This enhancement in ROS upon nanoparticle treatment and light activation directly correlated with their antibacterial properties, implying that ROS enhancement is the primary mechanism in the killing of bacterial cells.

4. CONCLUSIONS

FeS nanoparticles have been synthesized by using a simple aqueous co-precipitation method. A broad absorption pattern
of these aqueous dispersed nanoparticles was obtained in the visible region; subsequent irradiation with laser light emitting at 635 nm led to an increase in temperature (up to 25 °C) owing to photo-induced hyperthermia. Time-dependent release of Fe²⁺ ions was observed from these aqueous dispersed nanoparticles. Their dual-modality antibacterial effect was observed in both Gram positive and Gram negative bacterial strains, owing to (a) the bacteriostatic effect due to the produced ROS and (b) the bactericidal effect due to photo-induced hyperthermia. Stepwise enhancement in intra-bacterial ROS levels, as a result of nanoparticle treatment followed by light activation, has been identified as the primary antibacterial mechanism. Such new antibacterial approaches have the potential to replace traditional antibiotic based approaches.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03177.

The manuscript contains Electronic Supporting Information (ESI), which contains data on bacteria–nanoparticle interaction studied by TEM, the antibacterial effect of nanoparticles and light treatment using the colony counting assay, and cell-viability analysis of the nanoparticles in normal cells (PDF)

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**Notes**

The authors declare no competing financial interest.

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**ABBREVIATIONS**

ROS, reactive oxygen species; PTT, photothermal therapy; SA, Staphylococcus aureus; PA, Pseudomonas aeruginosa

**REFERENCES**

(1) Alanis, A. J. Resistance to antibiotics: are we in the post-antibiotic era? *Arch. Med. Res.* 2005, 36, 697–705.

(2) (a) Wright, G. D.; Sutherland, A. D. New strategies for combating multidrug-resistant bacteria. *Trends Mol. Med.* 2007, 13, 260–267. (b) Fair, R. J.; Tor, Y. Antibiotics and bacterial resistance in the 21st century. *Perspect. Med. Chem.* 2014, 6, 25–64.

(3) Hernandez, V.; Crépin, T.; Palencia, A.; Cusack, S.; Akama, T.; Baker, S. J.; Bu, W.; Feng, L.; Freund, Y. R.; Liu, L.; Meehan, M.; Mohan, M.; Mao, W.; Rock, F. L.; Sexton, H.; Sheoran, A.; Zhang, Y.; Zhang, Y.-K.; Zhou, Y.; Nieman, J. A.; Anugula, M. R.; Keramane, E. M.; Savariraj, K.; Reddy, D. S.; Sharma, R.; Subedi, R.; Singh, R.; O’Leary, A.; Simon, N. L.; de Marsh, P. L.; Mushtaq, S.; Warner, M.; Livermore, D. M.; Alley, M. R. K.; Plattner, J. J. Discovery of a novel class of boron-based antibacterials with activity against gram-negative bacteria. *Antimicrob. Agents Chemother.* 2013, 57, 1394–1403.

(4) Fuchs, A. D.; Tiller, J. C. Contact-active antimicrobial coatings derived from aqueous suspensions. *Angew. Chem., Int. Ed.* 2006, 45, 6759–6762.

(5) Xia, Y.; Li, W.; Cobley, C. M.; Chen, J.; Xia, X.; Zhang, Q.; Yang, M.; Cho, E. C.; Brown, P. K. Gold nanocages: from synthesis to theranostic applications. *Acc. Chem. Res.* 2011, 44, 914–924.

(6) Liu, H.; Chen, D.; Li, L.; Liu, T.; Tan, L.; Wu, X.; Tang, F. Multifunctional gold nanoshells on silica nanorattles: a platform for the combination of photothermal therapy and chemotherapy with low systemic toxicity. *Angew. Chem., Int. Ed.* 2011, 50, 891–895.

(7) Chuang, Y.-C.; Lin, C.-J.; Lo, S.-F.; Wang, J.-L.; Tsou, S.-C.; Yuan, S.-S.; Wang, Y.-M. Dual functional AuNRs@MnMEOIs nanoclusters for magnetic resonance imaging and photothermal therapy. *Biomaterials* 2014, 35, 4678–4687.

(8) Wang, S.; Huang, P.; Nie, L.; Xing, R.; Liu, D.; Wang, Z.; Lin, J.; Chen, S.; Niu, G.; Li, G.; Chen, X. Single continuous wave laser induced photodynamic/plasmonic photothermal therapy using photosensitizer-functionalized gold nanostars. *Adv. Mater.* 2013, 25, 3055–3061.

(9) Zhang, Z.; Wang, J.; Nie, X.; Wen, T.; Ji, Y.; Wu, X.; Zhao, Y.; Chen, C. Near infrared laser-induced targeted cancer therapy using thermoresponsive polymer encapsulated gold nanorods. *J. Am. Chem. Soc.* 2014, 136, 7317–7326.

(10) Huang, X.; Tang, S.; Mu, X.; Dai, Y.; Chen, G.; Zhou, Z.; Ruan, F.; Yang, Z.; Zheng, N. Freestanding palladium nanosheets with plasmonic and catalytic properties. *Nat. Nanotechnol.* 2011, 6, 28–32.

(11) Tang, S.; Chen, M.; Zheng, N. Sub-10-nm Pd nanosheets with renal clearance for efficient near-infrared photothermal cancer therapy. *Small* 2014, 10, 3139–3144.

(12) Sethi, K.; Roy, I. Organically modified titania nanoparticles for sustained drug release applications. *J. Colloid Interface Sci.* 2015, 456, 59–65.

(13) Fujishima, A.; Ohtsuki, J.; Yamashita, T.; Hayakawa, S. Photocatalysis: Principles and applications. *Photomed. Photobiol.* 1986, 8, 45–46.

(14) Cai, R.; Kubota, Y.; Shuin, T.; Sakai, H.; Hashimoto, K.; Fujishima, A. Induction of cytotoxicity by photoexcited TiO₂ particles. *Cancer Res.* 1992, 52, 2346–2348.

(15) Fujishima, A.; Rao, T. N.; Tryk, D. A. P-Doped titania xerogels as efficient UV-Visible photocatalysts. *J. Photochem. Photobiol., C* 2000, 1, 1–21.

(16) Paunesku, T.; Vogt, S.; Lai, B.; Maser, J.; Stojic’ević, N.; Thurn, K. T.; Osipo, C.; Liu, H.; Legnini, D.; Wang, Z.; Lee, C.; Wolochask, G. E. Intracellular distribution of TiO₂-DNA oligonucleotide nanoconjugates directed to nucleus and mitochondria indicates sequence specificity. *Nano Lett.* 2007, 7, 596–601.

(17) Endres, P. J.; Paunesku, T.; Vogt, S.; Meade, T. J.; Wolochask, G. E. DNA-TiO₂ nanoconjugates labeled with magnetic resonance contrast agents. *J. Am. Chem. Soc.* 2007, 129, 15760–15761.

(18) Yang, K.; Feng, L.; Shi, X.; Liu, Z. Nano-graphene in biomedicine: theranostic applications. *Chem. Soc. Rev.* 2013, 42, 530–547.

(19) Nair, L. V.; Nagaoka, Y.; Maekawa, T.; Sakkthikumar, D.; Jayasree, R. S. Quantum dot tailored to single wall carbon nanotubes: a multifunctional hybrid nanoconstruct for cellular imaging and targeted photothermal therapy. *Small* 2014, 10, 2771–2775.
Akhavan, O.; Ghaderi, E. Graphene nanomesh promises extremely efficient in vivo photothermal therapy. Small 2013, 9, 3593–3601.

Keenan, C. R.; Goth-Goldstein, R.; Lucas, D.; Sedlak, D. L. Oxidative Stress Induced by Zero-valent Iron Nanoparticles and Fe(II) in Human Bronchial Epithelial Cells. Environ. Sci. Technol. 2009, 43, 4555–4560.

Wang, B.; Yin, J.-J.; Zhou, X.; Kurazh, I.; Chai, Z.; Zhao, Y.; Feng, W. Physicochemical origin for free radical generation of iron oxide nanoparticles in bio-microenvironment: catalytic activities mediated by surface chemical states. J. Phys. Chem. C 2013, 117, 383–392.

Riley, R. S.; Day, E. S. Gold nanoparticle-mediated photothermal therapy: applications and opportunities for multimodal cancer treatment. Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol. 2017, 9, No. e1449.

Estelrich, J.; Busquets, M. Iron oxide nanoparticles in photothermal therapy. Molecules 2018, 23, 1567–1592.

Cheng, J.; Teply, B. A.; Jeong, S. Y.; Yin, C. H.; Ho, D.; Sherifi, I.; Jon, S.; Farokhzad, O. C.; Khademihoosseini, A.; Langer, R. S. Magnetically responsive polymeric microparticles for oral delivery of protein drugs. Pharm. Res. 2006, 23, 557–564.

Yang, Y.; Jiang, J.-S.; Du, B.; Gan, Z.-F.; Qian, M.; Zhang, P. Preparation and properties of a novel drug delivery system with both magnetic and biomolecular targeting. J. Mater. Sci.: Mater. Med. 2009, 20, 301–307.

Gou, M. L.; Qian, Z. Y.; Wang, H.; Tong, Y. B.; Huang, M. J.; Kan, B.; Wen, Y. J.; Dai, M.; Li, X. Y.; Gong, C. Y.; Tu, M. J. Preparation and characterization of magnetic poly(epsilon-caprolactone)-poly(ethylene glycol)-poly(epsilon-caprolactone) microspheres. J. Mater. Sci.: Mater. Med. 2008, 19, 1033–1041.

Brähler, M.; Georgieva, R.; Buske, N.; Müller, A.; Müller, S.; Pinkernelle, J.; Teichgräber, U.; Voigt, A.; Bäumler, H. Magnetite-loaded carrier erythrocytes as contrast agents for magnetic resonance imaging. Nano Lett. 2006, 6, 2505–2509.

Denis, M. C.; Mahmood, U.; Beneist, C.; Mathis, D.; Weissleder, R. Imaging inflammation of the pancreatic islets in type 1 diabetes. Proc. Natl. Acad. Sci. U.S.A. 2004, 101, 12634–12639.

Bulte, J. W. M. Intracellular endosomal magnetic labeling of cells. Methods Mol. Med. 2006, 124, 419–439.

Matteucci, M. L.; Anyarambatla, G.; Rosner, G.; Azuma, C.; Fisher, P. E.; Dewhirst, M. W.; Needham, D.; Thrall, D. E. Hyperthermia increases accumulation of technetium-99m-labeled liposomes in feline sarcomas. Clin. Cancer Res. 2000, 6, 3748.

Majors, I. J.; Myc, A.; Thomas, T.; Mehta, C. B.; Baker, J. R. PAMAM dendrimer-based multifunctional conjugate for cancer therapy: synthesis, characterization, and functionality. Biomacromolecules 2006, 7, 572–579.

Araújo, M.; Pal, S.; Samantarrai, D.; Panigrahi, T. K.; Mallick, B. C.; Pramanik, K.; Mallick, B.; Jha, S. Antimicrobial activity of iron oxide nanoparticle upon modulation of nanoparticle-bacteria interface. Sci. Rep. 2015, 5, 14813–14824.

Milenbaugh, N.; Baskin, J.; DeSilva, M.; Elliott, W. R.; Glickman, R. Photothermal killing of Staphylococcus aureus using antibody-targeted gold nanoparticles. Int. J. Nanomed. 2015, 10, 1953–1960.

Kim, E.-J.; Kim, J.-H.; Azad, A.-M.; Chang, Y.-S. Facile synthesis and characterization of Fe/FeS nanoparticles for environmental applications. ACS Appl. Mater. Interfaces 2011, 3, 1457–1462.

Riemer, J.; Hoepken, H. H.; Czerwinski, H.; Robinson, S. R.; Dringen, R. Colorimetric ferrozine-based assay for the quantitation of iron in cultured cells. Anal. Biochem. 2004, 331, 370–375.

Agnihotri, S.; Pathak, R.; Jha, D.; Roy, I.; Gautam, H. K.; Sharma, A. K.; Kumar, P. Synthesis and antimicrobial activity of aminoglycoside-conjugated silica nanoparticles against clinical and resistant bacteria. New J. Chem. 2015, 39, 6746–6755.

Al-Sharqi, A.; Apun, K.; Vincent, M.; Kanakaraju, D.; Bilung, L. M. Enhancement of the Antibacterial Efficiency of Silver Nano-particles against Gram-Positive and Gram-Negative Bacteria Using Blue Laser Light. Int. J. Photoenergy 2019, 2019, 1–12.

Dutta, A. K.; Maji, S. K.; Srivastava, D. N.; Mondal, A.; Biswas, P.; Paul, P.; Adhikary, B. Synthesis of FeS and FeSe nanoparticles from a single source precursor: a study of their photocatalytic activity, peroxidase-like behavior, and electrochemical sensing of H2O2. ACS Appl. Mater. Interfaces 2012, 4, 1919–1927.

Cullity, B. D.; Graham, C. D. Introduction to Magnetic Materials; Wiley-IEEE Press: NJ, 2009.

Yang, K.; Yang, G.; Chen, L.; Cheng, L.; Wang, L.; Ge, C.; Liu, Z. FeS nanoparticles as a multipurpose nanotheranostic for magnetic resonance imaging guided photothermal therapy. Biomaterials 2015, 38, 1–9.

Cheng, D.; Yuan, S.; Liao, P.; Zhang, P. Oxidizing Impact Induced by Mackinawite (FeS) Nanoparticles at Oxic Conditions due to Production of Hydroxyl Radicals. Environ. Sci. Technol. 2016, 50, 11646–11653.

Lara, H. H.; Ayala-Núñez, N. V.; Ixtepan Turrent, D. C.; Rodríguez Padilla, C. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World J. Microbiol. Biotechnol. 2010, 26, 615–621.

Zou, L.; Wang, J.; Gao, Y.; Ren, X.; Rottenberg, M. E.; Lu, J.; Holmgren, A. Synergistic antibacterial activity of silver with antibiotics correlating with the upregulation of the ROS production. Sci. Rep. 2018, 8, 11131.

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