Multifunctional nanoagents for ultrasensitive imaging and photoactive killing of Gram-negative and Gram-positive bacteria

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Simultaneous imaging and treatment of infections remains a major challenge, with most current approaches being effective against only one specific group of bacteria or not being useful for diagnosis. Here we develop multifunctional nanoagents that can potentially be used for imaging and treatment of infections caused by diverse bacterial pathogens. The nanoagents are made of fluorescent silicon nanoparticles (SiNPs) functionalized with a glucose polymer (e.g., poly[4-O-(α-D-glucopyranosyl)-D-glucopyranose]) and loaded with chlorin e6 (Ce6). They are rapidly internalized into Gram-negative and Gram-positive bacteria by a mechanism dependent on an ATP-binding cassette (ABC) transporter pathway. The nanoagents can be used for imaging bacteria by tracking the green fluorescence of SiNPs and the red fluorescence of Ce6, allowing in vivo detection of as few as $10^5$ colony-forming units. The nanoagents exhibit in vivo photodynamic antibacterial efficiencies of 98% against Staphylococcus aureus and 96% against Pseudomonas aeruginosa under 660 nm irradiation.
The World Health Organization (WHO) released its first Essential Diagnostics List (EDL) in 2018, highlighting the fundamental role of rapid, sensitive, specific, and reasonably priced diagnostics for effective treatments of infectious diseases caused by viruses, parasites, and bacteria. Notwithstanding, it is striking that more than 300 million individuals are still killed by sepsis, endocarditis, and other pathogen-related diseases per year. Currently, a promising cure strategy is to diagnose pathogen in vivo with high specificity and sensitivity and effectively eliminate them at an early stage. Although there are numerous established methods developed for detection of bacteria, such as bacterial culture, biochemistry identification, immunosassays, polymerase chain reaction (PCR), sequencing, and so forth, they generally require complex and time-consuming procedures (e.g., several hours to days for bacterial culture, bacterial metabolites extraction, and bacterial discrimination) to analyze results. As a consequence, even though diagnosis is able to be confirmed via these approaches, the golden time for treatment is possibly missed because bacterial infections may already have caused serious anatomical tissue damage or become systemic. Even in an ideal clinical environment, the physicians would usually perform the broad-spectrum and non-specific anti-bacteria treatment before diagnosis. Fluorescence imaging has been recently considered as a simple, rapid, and sensitive method for bacteria detection. Of note, extensive efforts have been devoted for developing fluorescent nanomaterials-based theranostic agents against microorganisms, which are capable of not only diagnosis of bacterial infections but also curing bacterial infections by virtue of delivering therapeutic agents to the site of interest. However, there are three major barriers limiting their widespread applications: (1) most nanoagents feature poor specificity for bacteria over mammalian cells, resulting in difficulty for distinguishing bacterial infections from other inflammation symptoms. (2) most reported nanoagents could basically determine only one specific group of bacteria (i.e., Gram-negative or Gram-positive bacteria), while clinical bacterial infections (e.g., sepsis, skin burn infection, unsitized disease infection, and so on) are generally caused by both Gram-negative and Gram-positive pathogenic bacteria. (3) currently existing nanoagents usually possess a single emission under a single excitation, their single-emission signals are therefore easily influenced by fluctuation of local probe concentration, leading to difficulty in the accurate and reliable bacterial detection.

As previously reported, silicon nanoparticles (SiNPs) feature benign biocompatibility, bright fluorescence coupled with strong photostability (e.g., SiNPs could preserve strong fluorescence under continuous 180-min UV irradiation) and adjustable drug-loading capacity (e.g., doxorubicin (DOX) loading content onto SiNPs can be up to 21.34%). It is noteworthy that ultrasmall SiNPs (<10 nm) recently have been approved by the Food and Drug Administration (FDA) of USA for the first-in-human clinical trial. In addition, glucose polymer (GP) as the major component of mammalian cell surface, is comprised of ~130 subunits: α-D-glucopyranose. As the targeted ligand is conjugated with SiNPs through the classic Schiff base reaction, in which the aldehyde groups of GP react with amino groups on SiNPs surface with SiNPs through the classic Schiff base reaction, in which the aldehyde groups of GP react with amino groups on SiNPs surface, thereby generating a stable aldehyde -photoluminescence (PL) spectrum. Zeta potential (ZP) -D-glucopyranose through bacteria-specific ABC transporter pathway, manifesting green fluorescence of SiNPs (maximum emission wavelength at 520 nm) and red fluorescence of Ce6 (maximum emission wavelength at 670 nm) under 405-nm excitation. On the other hand, when the infected tissues are exposed to 660-nm irradiation, PDT against bacterial cells is triggered, thus producing reactive oxygen species (ROS) of singlet oxygen ($^1O_2$) from Ce6 based on the photoenergy transferring from Ce6 to surrounding oxygen molecules.

Characterization of GP-Ce6-SiNPs. A series of experiments are performed to characterize the as-prepared GP-Ce6-SiNPs. Figure 2a shows the transmission electron microscopy (TEM) image of GP-Ce6-SiNPs, which appear as spherical particles with excellent monodispersibility. The size distribution (Inset in Fig. 2a), calculated by the measurement of 200 particles, shows that the average size of GP-Ce6-SiNPs is about 2.7 nm, slightly larger than that of unmodified SiNPs (2.3 nm) (see Supplementary Fig. 1). Dynamic light scattering (DLS) measurement of GP-Ce6-SiNPs is given in Fig. 2b, revealing that naked SiNPs exhibit a hydrodynamic diameter of ~3.0 nm, relatively smaller than ~5.6 nm of GP-Ce6-SiNPs.

The modification of GP and Ce6 is further confirmed by UV-vis absorbance -photoluminescence (PL) spectra, Zeta potential and Fourier-transform infrared (FTIR) spectra. As depicted in Fig. 2c, the absorption spectrum of GP-Ce6-SiNPs displays three characteristic absorption peaks at 320 nm (assigned to SiNPs), 405 nm, and 660 nm (assigned to Ce6). It is worth noting that a new distinct absorption peak at 490 nm would appear (Inset in Fig. 2c) when GP-SiNPs are treated with phenol-sulfuric acid. Upon the phenol-sulfuric acid treatment, the linked GP is hydrolyzed into monosaccharide, which is then rapidly
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With regard to Zeta potential in Fig. 2e, the excitation of 405 nm, which are originated from SiNPs and Ce6, characteristic emission peaks at 520 and 670 nm under the SiNPs, while the characteristic Cvibration at 1080 cm (0.4 mV). In FTIR spectra (Supplementary Fig. 5), the typical Si statically adsorbed on the surface of positively charged GP-SiNPs negatively charged Ce6 molecules (−Ce6-SiNPs is similar to that of free Ce6 at the same Ce6 concentration. MgSO4, 10 mM NaHCO3, 2 mM CaCl2, 20 mM glucose, and 1 mM bovine serum albumin (BSA)), as revealed in Supplementary Figs. 3 and 4.

As for PL spectra in Fig. 2d, GP-Ce6-SiNPs display two characteristic emission peaks at 520 and 670 nm under the excitation of 405 nm, which are originated from SiNPs and Ce6, respectively. With regard to Zeta potential in Fig. 2e, the negatively charged Ce6 molecules (−2.1 mV) can be electrostatically adsorbed on the surface of positively charged GP-SiNPs (0.4 mV). In FTIR spectra (Supplementary Fig. 5), the typical Si-O vibration at 1080 cm−1 is observed in both SiNPs and GP-Ce6-SiNPs, while the characteristic C-N vibration at 1640–1690 cm−1 is only shown in GP-Ce6-SiNPs possibly owing to the binding reaction between SiNPs and GP. To investigate PDT effects of GP-Ce6-SiNPs, singlet oxygen sensor green (SOSG) testing is employed. As shown in Fig. 2f, the 1O2 generation ability of GP-Ce6-SiNPs is similar to that of free Ce6 at the same Ce6 concentration under 660-nm irradiation, demonstrating distinct PDT ability of the GP-Ce6-SiNPs.

**In vitro imaging of diverse bacteria.** We first perform experiments to investigate whether GP-Ce6-SiNPs could target different kinds of bacteria in a specific manner. Gram-positive bacteria of *S. aureus* (SA), *M. luteus* (ML), and Gram-negative bacteria of *E. coli* (EC), *P. aeruginosa* (PA) are, respectively, incubated with GP-Ce6-SiNPs at 37 °C for 2 h, and then washed with phosphate buffered saline (PBS) buffer. As shown in the confocal laser scanning microscope (CLSM) images in Fig. 3a, both green fluorescence signals from the SiNPs (first column, λex = 405 nm, λem = 500–550 nm) and red fluorescence signals from the Ce6 (second column, λex = 405 nm, λem = 600–680 nm) can be clearly observed in all treated bacteria, indicating that GP-Ce6-SiNPs can specifically target Gram-positive and Gram-negative bacteria. Furthermore, green fluorescence signals overlap well with red fluorescence signals in the merged channel (third column) with Pearson correlation coefficients more than 0.80, demonstrating good colocalization between SiNPs and Ce6 ascribed to the good stability of GP-Ce6-SiNPs in the cellular environments. Also, there is no significant difference in intensity profiles of green and red signals within the ROI (region of interest, yellow lines in ML of Fig. 3a) across bacteria cells (Fig. 3b). To further test the role of GP in GP-Ce6-SiNPs for targeting bacteria, bacteria incubated with Ce6-modified SiNPs (Ce6-SiNPs) under the same conditions...
are selected for comparison. As shown in Supplementary Fig. 6, no fluorescence signals are detected in bacteria treated with Ce6-SiNPs owing to the absence of GP molecules for targeting bacteria. On the other aspect, GP- and Ce6-modified silicon nanorods (GP-Ce6-SiNRs) are tested as another control group because they have the same surface modifications as GP-Ce6-SiNPs, but the size of GP-Ce6-SiNRs (~100 nm) is much larger than that of GP-Ce6-SiNPs (~2.7 nm)47. As revealed in Supplementary Fig. 7, distinct fluorescence cannot be observed in these four kinds of bacteria incubated with GP-Ce6-SiNRs, indicating larger-size nanoagents modified with GP ligand still cannot be internalized into bacterial cells. Supplementary Fig. 8 shows systematic characterizations of Ce6-SiNPs and GP-Ce6-SiNRs, including TEM images, PL spectra, and UV-vis absorbance (see Supplementary Methods for details of the preparation of GP-Ce6-SiNRs).

To examine if the uptake of GP-Ce6-SiNPs is mediated by energy-dependent transporter pathway, the accumulation of GP-Ce6-SiNPs within bacterial cells under 4 °C is observed by CLSM. As observed in Supplementary Fig. 9, neither green nor red fluorescence signals could be clearly observed when the bacteria are incubated with GP-Ce6-SiNPs at 4 °C for 2 h. Such temperature-dependent internalization process indicates GP-Ce6-SiNPs transport into bacterial cells through energy-dependent transporter pathway. Furthermore, the inhibition assay as well as competition assay have been performed to confirm the uptake mechanism of GP-Ce6-SiNPs into bacteria via ABC transporter pathway. For the inhibition assay, sodium azide (NaN₃) is incubated with bacteria in order to inhibit the ATP-dependent ABC transporter pathway since NaN₃ can serve as the inhibitor of the respiratory chain of bacteria48. As a result, fluorescence is undetectable in the NaN₃-treated EC and SA (Fig. 3c), indicating the accumulation of GP-Ce6-SiNPs into bacteria has been drastically inhibited. As for a competition assay, the EC and SA solutions are incubated with GP with concentrations of 0, 2, and 20 mg mL⁻¹ for 5 min and then incubated with GP-Ce6-SiNPs.
**Fig. 3** In vitro imaging of Gram-negative and Gram-positive bacteria. 

- **a** Confocal fluorescence images of four different kinds of bacteria (EC, SA, ML, and PA) after incubation with GP-Ce6-SiNPs. Scale bar: 10 μm.
- **b** Intensity profiles of ROI across the line shown in ML in panel (a) with confocal microscope.
- **c** Confocal fluorescence images of EC and SA treated with NaN₃ and then incubated with GP-Ce6-SiNPs for 2 h. Scale bar: 10 μm.
- **d** Confocal fluorescence images of EC and SA incubated with GP with different concentrations (0, 2, 20 mg mL⁻¹) for 5 min and then incubated with GP-Ce6-SiNPs for 2 h. Scale bar: 25 μm.
- **e** TEM (scale bar: 1 μm) and zoom-in TEM (scale bar: 30 nm) images of intact EC and cell membrane of EC treated with GP-Ce6-SiNPs.
- **f** TEM (scale bar: 20 nm) and zoom-in TEM (scale bar: 5 nm) images of GP-Ce6-SiNPs nanoparticles in cell lysate of EC.
- **g** Confocal fluorescence images of the mixture of ARPE cells and EC, pure ARPE cells, the mixture of human blood and EC, pure human blood after incubation with GP-Ce6-SiNPs. Scale bar: 25 μm.
- **h** Histogram quantifying the level of GP-Ce6-SiNPs transport. Statistical analysis was performed using a one-way ANOVA analysis. Error bars represent the standard deviation obtained from three independent measurements (**p < 0.001, n = 3**). In the above experiments, the final concentration of GP-Ce6-SiNPs is 10 mg mL⁻¹ (3.6 mg mL⁻¹ of GP and 100 μg mL⁻¹ of Ce6). Source data are provided as a Source Data file.
for 2 h. Since GP molecules are internalized into bacterial cells also through ABC transporter pathway\(^{20}\), they would compete with GP-C6-SiNPs for ABC transporter. As a consequence, the fluorescence observed in bacteria becomes gradually weaken with the increase of GP concentrations (Fig. 3d), suggesting the uptake of GP-C6-SiNPs into bacteria is greatly competitively inhibited by GP.

On the other side, TEM characterizations of GP-C6-SiNPs stained EC are further performed to confirm whether GP-C6-SiNPs specifically enter into bacterial intracellular volume rather than nonspecifically adsorb on the bacteria cell surface. Typically, the surface of cell membrane of stained EC is smooth (see TEM images in Fig. 3e), while numerous nanoparticles are observed in the lysate of stained EC cells (see TEM images in Fig. 3f), demonstrating GP-C6-SiNPs are internalized into bacterial cells, but not binding to the bacteria cell surface.

In order to investigate the specificity of GP-C6-SiNPs for bacteria over mammalian cells, HeLa cells, ARPE cells and human blood samples are selected for study. As revealed in confocal images in Fig. 3g, no fluorescence signals can be detected in pure ARPE cells, while green and red fluorescence signals are only observed in EC when ARPE cells are mixed with EC, followed by 2-h incubation with GP-C6-SiNPs. Moreover, green and red fluorescence signals are only observed in EC cells when human blood samples spiked with EC are treated with GP-C6-SiNPs for 2 h, suggesting the potential use of GP-C6-SiNPs for imaging of bacteria in clinical blood samples. Figure 3h shows higher level of GP-C6-SiNPs transporting in bacterial cells than in mammalian cells. Quantitatively, the amount of GP-C6-SiNPs accumulated into both Gram-positive and Gram-negative bacteria is at least 12 times more than that in ARPE and human blood cells. On the other aspect, the observed non-specific internalization of the nanoagents by mammalian cells might be mediated by the other pathway of endocytosis\(^{19}\).

**In vivo imaging of diverse bacteria.** To demonstrate that the as-prepared GP-C6-SiNPs have the potential to realize imaging of Gram-negative and Gram-positive bacteria in vivo, the corresponding bacteria-infected mice models are constructed. Micrograph of the histology of SA-infected muscles of mice in Fig. 4a shows that SA cells exist in infected tissues. In Fig. 4b and Fig. 4c, 50 \(\mu\)L SA with 0.9 \(\times\) 10\(^7\) CFU and 50 \(\mu\)L PA with 0.7 \(\times\) 10\(^7\) CFU are, respectively, injected into the right and left caudal thigh of mice. After 24-h infection, the treated mice are intravenously injected with 100 \(\mu\)L of vancomycin-modified SiNPs (Van-SiNPs) (Fig. 4b) and GP-C6-SiNPs (Fig. 4c) with the same amount of SiNPs (10 mg mL\(^{-1}\)) through the tail vein, which are then imaged by an in vivo optical imaging system (IVIS Lumina III) under two channels of \(\lambda_{ex} = 460\) nm, \(\lambda_{em} = 520\) nm and \(\lambda_{ex} = 460\) nm, \(\lambda_{em} = 670\) nm at 24 h post injection. The actual amount of SA or PA at the infection site during imaging is 1.0 \(\times\) 10\(^7\) CFU, which is determined via tissue harvesting, homogenization, and culturing with CFU count\(^{7,50}\). As shown in Fig. 4b, Van-SiNPs can image SA infection while are unable to image PA infection, this result is consistent with the previous report, in which Van-SiNPs could only be specifically against Gram-positive bacteria based on the strong affinity between vancomycin and cell wall of Gram-positive bacteria\(^{51}\). As supported by corresponding histograms in Fig. 4b, Van-SiNPs-treated mice, SA-infected site has a \(\sim 3.7\)-fold increase in fluorescence intensity compared with the PA-infected site. On the contrary, in GP-C6-SiNPs-treated groups, green and red fluorescence signals can be observed at both two infected sites (Fig. 4c). And there is no significant difference of fluorescence intensity between the two sites, as revealed in corresponding histograms in Fig. 4c. In Fig. 4d, 50 \(\mu\)L PBS buffer and 50 \(\mu\)L the mixture containing PA and SA (PA + SA) with 0.8 \(\times\) 10\(^7\) CFU are, respectively, injected into the left and right caudal thigh of mice. After 24-h infection, the infected mice are intravenously injected with 100 \(\mu\)L of GP-C6-SiNPs and then imaged at 24 h post injection. The actual amount of mixed bacteria at the infection site during imaging is 1.0 \(\times\) 10\(^7\) CFU, which is determined by CFU counting method as mentioned above. Distinct green and red fluorescence signals can only be observed at the mixed bacteria-infected site instead of the PBS-treated site, as shown in Fig. 4d. In particular, the corresponding histograms in Fig. 4d show that the site of mice infected with PA + SA have a \(\sim 6.2\)-fold increase in fluorescence intensity compared with PBS-treated site. These experimental results indicate the GP-C6-SiNPs can achieve in vivo imaging of both Gram-negative and Gram-positive bacterial infections. Also, there exist negligible non-specific interactions between nanoparticles without GP (Ce6-SiNPs) and biological tissues, as demonstrated in Supplementary Fig. 10.

The minimum number of bacteria imaged by GP-C6-SiNPs in vivo is determined in the following experiments. Fifty microliters of PBS buffer and 50 \(\mu\)L SA with 1.4 \(\times\) 10\(^7\) CFU or PA with 1.2 \(\times\) 10\(^7\) CFU are, respectively, injected into the left and right thigh of the mice and imaged by GP-C6-SiNPs. The actual amount of SA or PA at the infection site during imaging is determined as 1.0 \(\times\) 10\(^7\) CFU by using the same CFU counting method as mentioned above. Figure 4e presents that GP-C6-SiNPs can discriminate as few as 10\(^5\) CFU of SA or PA in vivo under green and red emission channels. As revealed in corresponding histograms in Fig. 4e, the site of mice infected with 10\(^5\) CFU of SA or PA have a \(\sim 2.7\)-fold or \(\sim 3.5\)-fold increase in fluorescence intensity compared with PBS-treated site.

Furthermore, the developed GP-C6-SiNPs can be employed for long-term tracking bacterial infections in vivo by virtue of their good photostability and high specificity, as demonstrated in Fig. 4f. Typically, 50 \(\mu\)L SA (0.9 \(\times\) 10\(^7\) CFU) is injected into the right thigh muscle of mice. The mice are intravenously injected with 100 \(\mu\)L of GP-C6-SiNPs after 24-h infection. The actual amount of SA at the infection site during imaging is 1.0 \(\times\) 10\(^7\) CFU. As displayed in Fig. 4f, strong fluorescence signals from both SiNPs and Ce6 channels can still be observed at the infected site even at 4th day post injection. The corresponding time-dependent histograms in Fig. 4f show that fluorescence intensities from SiNPs and Ce6 keep steady at the infected site during 4-day treatment (e.g., the fluorescence intensity only drops by 12% at the 4th day compared with that at the 1st day), suggesting long retention ability of GP-C6-SiNPs at the infected site.

**In vitro antibacterial activity of GP-C6-SiNPs.** The in vitro antibacterial activity of GP-C6-SiNPs is evaluated by scanning electron microscope (SEM) characterizations, agar plate experiments, and liquid medium turbidity assays. In these antibacterial assays, EC and SA are chosen as the representative Gram-negative and Gram-positive bacteria. As shown in SEM images in Fig. 5a, smooth cell walls of EC and SA incubated with GP-C6-SiNPs for 2 h can be observed prior to light irradiation. On the contrary, rough and wrinkled cell walls and lysed debris of such EC and SA are found after treatment with light irradiation for 15 min (660 nm, 12 mW cm\(^{-2}\)). As shown in Fig. 5b, luria-bertani (LB) liquid media in GP-C6-SiNPs-treated groups are pulled due to the destruction of bacteria under constant 15-min irradiation, while the media become turbid in the control groups due to the rapid growth of bacteria. Nearly no bacterial colony of EC and SA exists in the group under constant 15-min irradiation. In contrast, numerous bacterial colonies are observed in control groups under the identical conditions (Fig. 5c). For a more reliable quantitative
measurement, irradiation time-dependent CFU counting is performed, as shown in Fig. 5d. The bacterial counts of groups of EC and SA treated with GP-Ce6-SiNPs under constant 15-min irradiation drop dramatically compared to control groups under the same conditions. These experimental results demonstrate the in vitro photodynamic antimicrobial activity of GP-Ce6-SiNPs.

**In vivo antibacterial activity of GP-Ce6-SiNPs.** To further evaluate the antibacterial ability of GP-Ce6-SiNPs in vivo, 50 μL of SA (1.0 × 10^7 CFU) and PA (1.0 × 10^7 CFU) are injected into the right thigh of the mice, respectively. Then these two groups of bacteria (SA, PA)-infected mice are intravenously injected with 100 μL of PBS and GP-Ce6-SiNPs under 660-nm irradiation,
respectively; the other two groups of infected mice are suffered from the same treatments but without 660-nm irradiation. The representative photographs of four groups of bacteria (SA, PA)-infected mice are shown in Fig. 6a. Typically, among these four groups, the earliest and fastest infection wound healing and scarring is observed in the mice treated with GP-Ce6-SiNPs coupled with irradiation at 660 nm (12 mW cm$^{-2}$) for 40 min. Moreover, the corresponding infection wound area is measured every day and the relative wound area (S/S0) is plotted in Fig. 6b. The comparisons of the relative wound area among the experimental groups and control groups have been made for both SA- and PA-infected mice. Particularly, in SA-infected mice, a significant difference ($p < 0.01$) exists among the nanoagent + irradiation, the PBS and nanoagent alone. On the contrary, no significant difference exists between the nanoagent + irradiation and PBS + irradiation, which might be due to the adaptable...
self-healing ability of mice toward SA infections. On the other aspect, for PA-infected mice, a significance comparison ($p < 0.05$) in the relative wound area is made among the nanoagent+irradiation, the PBS+irradiation, the PBS and nanoagent alone. The corresponding hematoxylin-eosin (H&E) staining images of infection tissues of four groups from SA- and PA-infected mice at 7th (SA) and 9th (PA) day treatment are exhibited in Fig. 6c.

Normal morphological features with blood vessels and hair follicles can be detected in all groups treated with GP-Ce6-SiNPs under light irradiation while the tissues from other three control groups are damaged, emerging a large number of neutrophils. Additionally, the SA- and PA-infected skin tissues are excised from the mice at the 7th (SA) and 9th (PA) day post injection and the amounts of bacteria collected from the excised tissues are measured to assess antibacterial rates. As depicted in Fig. 6d, the amounts of bacteria in the "nanoagent+irradiation" groups are significantly ($p < 0.001$) less than the other control groups. Quantitatively, the antibacterial rate of GP-Ce6-SiNPs is calculated as 98% against SA and 96% against PA. These experimental results suggest significant photodynamic antimicrobial activity of GP-Ce6-SiNPs in vivo.

**Toxicity assessment of GP-Ce6-SiNPs.** Cytotoxicity of GP-Ce6-SiNPs is evaluated by an established methyl thiazolyl tetrazolium (MTT) assays. As shown in Fig. 7a, the cell viability of HeLa and ARPE cells remains above 90% when they are incubated with PBS.
GP-Ce6-SiNPs with different concentrations for 24 h. The corresponding images of morphologies of treated cells are presented in Fig. 7b, showing there is no obvious morphological change in HeLa or ARPE cells incubated with GP-Ce6-SiNPs. These results suggest feeble cytotoxicity of GP-Ce6-SiNPs. Afterward, in vivo biocompatibility of GP-Ce6-SiNPs is investigated. The main organs of heart, liver, spleen, lung, and kidney are resected from PBS/nanoagents-treated healthy mice with or without irradiation after 10-day treatment. As revealed in Fig. 7c, normal morphological features are found in biopsy sections in all resected organs, implying the negligible toxicity of GP-Ce6-SiNPs in vivo. To further evaluate the biodistribution of GP-Ce6-SiNPs, ex vivo imaging of heart, liver, spleen, lung, and kidney resected from healthy mice after 24 h post injection of GP-Ce6-SiNPs is performed. As indicated in Fig. 7d, bright green and red fluorescence signals are only observed in liver and kidney rather than in other organs, indicating GP-Ce6-SiNPs are mainly metabolized by liver and not retained in other organs. Moreover, ex vivo imaging of the urine collected from the healthy mice after 4 h post injection of nanoagents is performed. As shown in Supplementary Fig. 11, green and red fluorescence signals are observed in urine from the mice treated with nanoagents, confirming that nanoagents can be eliminated from the mice through renal clearance owing to the small size of nanoagents (~2.7 nm), which is in a good agreement with previous reports. These results demonstrate negligible in vitro and in vivo toxicity of GP-Ce6-SiNPs. Besides, the irradiation of PDT produces feeble toxicity in mammalian cells in vivo (Supplementary Fig. 12).

Discussion

The presented multifunctional nanoagents are made of SiNPs functionalized with GP and loaded with Ce6. In the as-prepared nanoagents, GP, serving as ideal targeted ligand for imaging bacteria, is covalently linked to SiNPs. Of note, the structural modifications at the reducing end of GP would not influence the...
internalization of probes by ABC transporters because the non-reducing end of GP was hypothesized as the recognized site of ABC transporters.36,37 Another noticeable feature of nanoagents is that the green fluorescent emission from SiNPs under 405-nm UV excitation is not desirable for in vivo imaging due to its relatively poor penetration depth. To circumvent this issue, Ce6 loaded on SiNPs serves not only as a PDI agent but also as an imaging agent to provide stable red fluorescence signal (maximum emission wavelength at 670 nm), facilitating the improvement of the penetration depth. Notwithstanding, it is also important to develop near-infrared light-emitting SiNPs-based probes in the future, facilitating the improvement of the penetration depth of tissues in vivo.

There are two prerequisites for nanoagents to access bacterial cells through the ABC transporter pathway: (1) surface modification of nanoagents with glucosyl residues and (2) relative small size of nanoagents. In this case, the as-prepared GP-Ce6-SiNPs can be selectively internalized into both Gram-negative and Gram-positive bacterial cells, while hardly entering mammalian cells. The high specificity of GP-Ce6-SiNPs for bacteria over mammalian cells is mainly ascribed to two factors: (1) GP-mediated ABC transporters are only presented in bacteria rather than in mammalian cells, and thus mammalian cells can not internalize SiNPs conjugated to GP ligand; (2) GP is composed of hydrophobic a (1–4)-linked glucose oligomer, which is unable to penetrate mammalian cellular membrane, resulting in low levels of non-specific uptake in mammalian cells.36,54 Occasionally, the nanoagents might be nonspecifically internalized into mammalian cells by the pathway of endocytosis. However, the uptake rate mediated by endocytosis is much lower than that mediated by ABC-transporter pathway. Importantly, the minimum number of bacteria discriminated in vivo by nanoagents is 104 CFU. Bacteria at a concentration <105 CFU are hardly discriminated, which is probably due to the fact that 105 CFU of bacteria are too few to establish a stable infection in immune competent mice. Normally, the amount of bacteria would fluctuate in the intervening time between infection and imaging since the bacteria reproduce and are fought by the immune system. A reliable way to determine the amount of bacteria at the infection site during imaging in this study is tissue harvesting, homogenization and culturing with CFU count.50 Moreover, the nanoagents can afford a long-term imaging (4 days) of bacterial infections in vivo, exhibiting stable green and red fluorescence signals. Finally, the antibacterial efficiency is up to ca. 98% against SA and ca. 96% against PA under 660-nm irradiation for constant 40 min with a relative low power density of 12 mW cm−2. Such significant therapeutic effect is also attributed to selective and robust internalization of GP-Ce6-SiNPs into bacteria, resulting in a relative high local concentration of Ce6 at infection sites. The mechanism of the nanoagents against bacteria is based on photodynamic effect of Ce6, which is different from that of conventional antibiotics. It is worth noting that nanoagents amount used in our case is relatively high (10 mg mL−1), which is based on the following two considerations: (1) providing strong fluorescence signals even at ultralow concentrations of bacteria (e.g., 1.0 × 105 CFU mL−1); (2) achieving desirable treatment effects within a relatively short irradiation time to eliminate possible skin damage. Based on further optimizing experimental parameters (e.g., nanoagents amount) and systematical biosafety assessment, we envision that the developed multifunctional nanoagents with appropriate injection dose would hold potential applications for diagnosis and therapy of bacterial infections.

**Methods**

**Chemicals and reagents.** (3-aminopropyl) trimethoxysilane (C3H7NO3Si), 1,8-naphthalimide, vancomycin (Van), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide-hydrochloride (EDC), N-hydroxysuccinimide (NHS), trisodium citrate, citric acid and NaBH4 were purchased from Sigma-Aldrich. E. coli, B. subtilis, S. aureus, P. aeruginosa (PA), S. mutans and B. subtilis (ATCC 11303) were purchased from American Type Culture Collection (ATCC). S. aureus (SA) was obtained from the First Affiliated Hospital of Soochow University. M. luteus (ML) (BNCC 102589) and P. aeruginosa (PA) (BNCC 125486) were purchased from BeNa Culture Collection (BNCC). The human blood samples were provided by a healthy volunteer. All basic culture reagents (LB medium) were obtained from Sangon Biotech (Shanghai, China), and reagents for cellular culture were provided by Gibco (Grandisland, USA). HeLa and ARPE-19 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) and Roswell Park Memorial Institute (RPMI)-1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS) and relevant antibiotics (100 µg mL−1 streptomycin and 100 U mL−1 penicillin). Both cell lines were cultured at 37 °C in a 5% CO2 incubator with humidified atmosphere. Six-week-old female nude mice (18–25 g in weight) were provided by Suzhou Pengsheng Biological Company. Propidium iodide (PI), SYTO9, chlorin e6 (Ce6), and glucose polymer (GP) of poly(4-O-(a-D- glucopyranosyl)-D-glucopyranose) were purchased from Sigma-Aldrich (Shanghai, China).

All animal experimental procedures were performed according to the Guideline for Animal Experimentation with the approval of the animal care committee of Soochow University.

**Instruments.** The morphology and size of nanoagents were examined by transmission electronic microscopy (TEM, Philips CM 200) with 200 kV. A 750 UV-Vis near-infrared spectrophotometer (Perkin-Elmer lambda) was used for the measurement of UV-vis absorption spectra. A spectrofluorometer (HORIBA JOBIN YVON FLUORMAX-4) was employed for recording photoluminescence (PL). DLS and Zeta potential measurements (Brookhaven HYPERION) were used for the characterization of FTIR spectra. Delta+ nano submicron particle size and Zeta potential particle analyzer (Beckman Coulter, Inc) was employed for the analysis of dynamic light scattering (DLS) and Zeta potentials. Fluorescence imaging experiments were performed by a confocal laser scanning microscope (CLSM, Leica, TCS-SP5 II). In vivo fluorescence images were obtained by an in vivo optical imaging system (IVIS Lumina III). The number of bacterial colonies were counted by a colony counting instrument (Czone 8).

**Fabrication of GP-Ce6-SiNPs.** The SiNPs were synthesized in the presence of C8H16NO3Si and 1,8-naphthalimide molecules under 40 min of continuous UV irradiation at 365 nm at room temperature. To remove residual reactants, the resulted solution was purified by centrifugation at 6000 rpm for 15 min and then dialysis (MWCO, 1000, Spectra/Por). The final SiNPs solution was collected through evaporation and stored at 4 °C for future use. Next, the SiNPs solution (150 µL, 25 mg mL−1) was mixed with GP dissolved in deionized water (100 µL, 10 mg mL−1). The dispersion was continuously stirred at 70 °C for 6 h, and 0.01 mg of NaBH4 was added and reacted for another 12 h at room temperature to obtain the stable GP-modified SiNPs. To remove the unreacted GP, the reaction solution was filtered by using Nanosep centrifugal devices (MW cutoﬀ, 3 kDa; Millipore) through centrifugation at 7500 rpm for 15 min. To further fabricate the GP-Ce6-SiNPs, the Ce6 solution (50 µL, 200 µM) was added in the above prepared GP-SiNPs solution and stirred at room temperature overnight. After the excess unreacted Ce6 was removed using Nanosep centrifugal devices (MW cutoﬀ, 3 kDa; Millipore) through centrifugation at 7000 rpm for 10 min. Then the product was collected and stored at 4 °C in the dark for the following experiments.

**Preparation of Van-SiNPs.** Hundred microliters of EDC (50 mg mL−1) and 25 µL of NHS (50 mg mL−1) was mixed with 5 mg Van powder at 4 °C under stirring for 15 min in order. Then, 50 µL of SiNPs solution (25 mg mL−1) was added into the activated Van solution for another 12 h at 4 °C in dark to obtain Van-SiNPs. To remove the excess unreacted Van, the resulting mixture was filtered by using Nanosep centrifugal devices (MW cutoﬀ, 10 kDa; Millipore) through centrifugation at 7500 rpm for 15 min. The final mixture was stored at 4 °C in the dark.

**Bacterial culture.** Gram-negative EC and PA, Gram-positive SA and ML, were employed in experiments. Bacterial cells were grown in LB medium at 230 rpm and 37 °C and then obtained at the exponential growth phase. Finally, the bacterial suspensions were washed twice and re-suspended in PBS buffer for the next use. The concentration of bacteria was detected by measuring the optical density (OD) at 600 nm.

**Fluorescence imaging of bacteria and cells in vitro.** The 20 µL of purified and re-suspended bacterial suspension (1.0 × 108 CFU) was incubated with GP-Ce6-SiNPs (200 µL, 10 mg mL−1) for 2 h in a shaking incubator (200 rpm) at 37 °C. The bacteria were harvested by centrifuging the mixture at 8000 rpm for 10 min in Eppendorf (EP) tubes. The resulting bacteria were re-suspended and washed with PBS for three times. Then 10 µL of the washed bacteria solution was transferred onto a microscope slide covered by a coverslip, and then imaged by a confocal laser microscope with 30% power of diode laser. For the fluorescence imaging of mixture sample of bacteria and cells, ARPE cells were cultured in Dulbecco’s modified
quantitative polymerase chain reaction (qPCR) and treated with serial concentrations of SiNPs (0, 5, 2.5, 1.25, 0.625 mg mL\(^{-1}\)) before and after 10-day treatment including the heart, liver, spleen, lung, and kidney were collected, fixed with paraformaldehyde in 4% PFA solution for the following H&E staining. After fixed in 4% paraformaldehyde solution at 37 °C for 2 h, and then collected by centrifugation at 10,000 rpm for 10 min. Afterward, UV absorbance of nanoagents in the supernatant was determined to measure the concentration of nanoagents (Ce6) based on the calibration curve. On the other aspect, the bacterial protein mass (M\(_{\text{protein}}\)) was quantified by the bicinchoninic acid (BCA) assay. As a consequence, the Y-axis in Fig. 3b was generated, which was a normalized value of concentrations of nanoagents in bacterial supernatant (Mean\(_{\text{nanoagent}}\)/M\(_{\text{protein}}\)).

### In vitro imaging of bacterial infections

To construct the bacteria-infected mouse model, 50 µL of PA (0.7 \(\times 10^5\) CFU) and SA (0.9 \(\times 10^5\) CFU) were subcutaneously injected into the left and right caudal thigh of the mice. The actual amount of bacteria at the infection site during imaging was determined via tissue homogenization, hemoglobinization and culturing with CFU count. Specifically, the harvested tissues were first homogenized in sterile PBS buffer (1 mL). Then, the bacteria suspension was collected from the tissue dispersions by centrifugation (10,000 rpm) removing tissue fragments. Finally, the collected bacteria were diluted by PBS buffer and cultured on an agarose medium at 37 °C for 12 h, followed by counting bacterial colonies by using a colony counting instrument (Cone 8). By using this method, the actual amount of SA or PA at the infection site during imaging was determined as 1.0 \(\times 10^5\) CFU. On the other aspect, 50 µL of PBS and 50 µL of mixture bacterial solution (PA + SA, 0.8 \(\times 10^5\) CFU) were subcutaneously injected into the left and right caudal thigh of the mice; the actual amount of mixed bacteria at the infection site during imaging is 1.0 \(\times 10^5\) CFU, which is determined by CFU counting method as mentioned above. Fifty microliters of PBS and 50 µL of SA (1.4 \(\times 10^5\) CFU) or PA (1.2 \(\times 10^5\) CFU) were subcutaneously injected into the left and right caudal thigh of the mice. The actual amount of SA or PA at the infection site during imaging is determined as 1.0 \(\times 10^5\) CFU by using the same CFU counting method as mentioned above. After 24 h post infection, bacteria-infected mice were intravenously injected with 100 µL GP-Ce6-SiNPs (10 mg mL\(^{-1}\)) or 100 µL Van-SiNPs (10 mg mL\(^{-1}\)) and then imaged by an in vivo optical imaging system (IVIS Lumina III) equipped with two laser channels of \(\lambda_{\text{em}} = 660\) nm, \(\lambda_{\text{ex}} = 460\) nm and \(\lambda_{\text{ex}} = 670\) nm at 24 h post injection. Meanwhile, the infected tissues were fixed in 4% paraformaldehyde (PFA) solution, mounted with paraffin, sliced, and stained with hematoxylin and eosin, and then imaged by a microscope. To investigate the biodistribution of GP-Ce6-SiNPs, the ex vivo fluorescence imaging was carried out. The nanosensors (100 µL, 10 mg mL\(^{-1}\))-treated healthy mice were sacrificed after 24 h post injection to collect the main organs for in vivo optical imaging. The urine of nanosensors (100 µL, 10 mg mL\(^{-1}\))-treated healthy mice was collected after 4 h post injection and imaged by an in vivo optical imaging system.

### Statistical analysis

Error bars represent the standard deviation obtained from three independent measurements. All the statistical analyses were performed using Origin software and GraphPad Prism software. The images were processed by Image J software. The statistical significance of differences was determined by a one-way ANOVA analysis or paired two-tailed t-test. p < 0.05 (**), p < 0.01 (***) were used to indicate statistical difference.

### Reporting information

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

### Data availability

The data that support the findings of this study are available within the paper and its Supplementary Information. Source data underlying Figs. 2b–f, 3b, 3h, 4b–f, 5d, 6b, 6d and 7a, and Supplementary Fig. 2, 3, 4, 5, and 8 are provided as a Source Data file, which is also deposited in figshare (https://doi.org/10.6084/m9.figshare.9169973.v1). Any other data are available from the corresponding authors upon request.

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