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1. Introduction

Overuse and abuse of antibiotics by humans for a long time led to the emergence and prevalence of antibiotic-resistant bacteria, which has reduced the therapeutic efficacy of antibiotics for human and animal pathogens. It is estimated that by 2050, approximately 10 million people will die or indirectly die from a multidrug resistant infection. Despite enormous efforts in research, the development of new antimicrobial drugs cannot catch up with the emergence of antibiotic resistant pathogens. Pursuit of alternative strategies and drugs to overcome antibiotic resistance is thus highly desirable.

In the past few decades, near-infrared (NIR) laser-induced photothermal therapy (PTT) has been used as a powerful strategy to combat cancers and bacterial infection because of its non-invasive manipulation, good controllability, and high tissue penetration. Various photothermal materials have been developed such as gold nanoparticles, gold nanorods (AuNRs), carbon nanotubes, two dimensional MoS$_2$ and MnO$_2$, as well as graphene nanoribbons and their supermolecules. Mesoporous silica-coated gold nanorods (AuNR-SiO$_2$) have aroused great attention as photothermal materials because of its tenable surface plasmon and excellent light-to-heat energy conversion efficiency as well as light controllable delivery of biomolecules into the cytoplasm. Besides, the large specific surface area of mesoporous silica guarantees a high drug payload and optimizes light-transparent window in the NIR region. Therefore, AuNR-SiO$_2$ seems to be a desirable candidate for highly stable and NIR laser-induced antibacterial applications. Turcheniuk et al. reported that AuNR-SiO$_2$ loaded with verteporfin could be used as an efficient near infrared nanostructure to eradicate Escherichia coli infection.

Photodynamic therapy (PDT) against microbial cells is also considered to be an alternative high-efficiency strategy to eliminate bacteria both in vitro and in vivo. The antibacterial strategy uses a specific wavelength light to activate photosensitizers (PSs), which react with oxygen to produce reactive oxygen species (ROS) to kill bacteria. Phthalocyanines (Pcs) and metal phthalocyanines (MPCs) have been used as promising photosensitisers for PDT of cancerous and noncancerous diseases. Recently, a series of new Pcs and their nano-formulations have exhibited excellent anticancer and antimicrobial activities. However, the properties of Pcs such as easily aggregating in water, lacking to target specific tissue, and limiting optimal wavelength for tissue penetration hinder their applications for PDT. It is desired to synthesize novel Pcs to make up for the drawbacks of traditional Pcs.

In this work, we synthesized a novel silicon Pc with mercapto-tetrazolyl functional groups, named bis-(1-(4-hydroxyphenyl)-5-mercapto-tetrazolyl) silicon (IV) phthalocyanine (Tet-SiPc), and used it as a photosensitizer for antibacterial research (Scheme 1). Mercapto-tetrazolyl group, a pharmaceutical synthetic intermediate, is a main pharmacophore of antibacterial and anti-inflammatory drugs. Introducing mercapto-tetrazolyl functional groups to axial position of Pc rings is expected to reduce its aggregation and improve its photodynamic antibacterial performance. To achieve the synergistic PDT and...
PTT, a light-controlled nano-switch was assembled through the mesoporous silica-coated gold nanorods with bis-(1-(4-hydroxyphenyl)-5-mercaptop-tetrazolyl) silicon (IV) phthalocyanine. The effect of the nano-switch against a variety of antibiotic-resistant *E. coli* strains were evaluated.

2. Materials and methods

2.1. Materials and instruments

The organic solvents used for the preparation of the nano-switch (Tet-SiPc@AuNR-SiO$_2$) were reagent grade. Sodium borohydride (NaBH$_4$), silver nitrate (AgNO$_3$), sodium hydroxide (NaOH) and hydrochloric acid (HCl) were purchased from Sinopharm Group Chemical Reagent Co., Ltd. Hydrogen tetra-chloroaurate (III) trihydrate (HAuCl$_4$·3H$_2$O) was purchased from Shanghai Bailingwei Chemical Technology Co., Ltd. Cetyltrimethylammonium bromide (CTAB) was obtained from Sigma Aldrich (Mainland, China). 1-(4-Hydroxyphenyl)-5-mercaptop-tetrazolium, tetraethyl orthosilicate (TEOS) and 3-ammonopropyltriethoxysilane (APTES) were purchased from Energy Chemical. Ascorbic acid (AA) was purchased from the Xinning Chemical Plant in Shantou (Guangdong, China).

The infrared spectra (KBr pellets) were recorded on a PE-983G spectrometer. $^1$H NMR spectra were recorded on a Bruker Avance $^1$H NMR (400 MHz, DMSO-d$_6$) δ/ppm: 8.95 (2H, s, SH, D$_2$O exchange, H$_4$), 7.37 (2H, t, J = 8Hz, H$_3$), 7.28 (4H, d, J = 8Hz, H$_1$), 7.22 (4H, d, J = 8Hz, H$_1$), 3.85~3.86 (8H, m, H$_6$), 9.68~9.70 (8H, m, H$_5$); ESI-MS Calcd. for m/z =926.16, found: m/z = 925.63 [M$^+$].

2.2. Synthesis of bis-(1-(4-hydroxyphenyl)-5-mercaptop-tetrazolyl) silicon (IV) phthalocyanine (Tet-SiPc)

A mixture of dichloro-phthalocyanine silicon (SiPcCl$_2$) (0.061g, 0.1 mmol), $^1$-(4-hydroxyphenyl)-5-mercaptop-tetrazole (Tet) (0.05826 g, 0.3 mmol) and potassium carbonate (0.028g, 0.2 mmol) in toluene (30 mL) was heated at 110 °C for 48 h. The mixture was cooled to room temperature and the solvent was removed by filtration. After evaporation under reduced pressure, the crude product was purified twice by alumina column chromatography using acetonitrile and hexane (v:v = 1:5) and methanol and ethyl acetate (v:v = 1:10) as eluents, respectively. The obtained product was further purified twice by chromatography on a silica gel column using acetonitrile and hexane and methylene chloride (v:v = 1:10) as eluent. A dark blue-green solid Tet-SiPc was obtained in a yield of 18 %.

2.3. Preparation of mesoporous silica-coated gold nanorods loaded with bis-(1-(4-hydroxyphenyl)-5-mercaptop-tetrazolyl) silicon (IV) phthalocyanine (Tet-SiPc@AuNR-SiO$_2$)

The AuNRs was synthesized according to the seed-mediated growth method as describe by Babak et al. Firstly, CTAB solution (10 mL, 0.10 M) mixed with HAuCl$_4$ solution (50 μL, 50 mM). Subsequently, ice-cold NaBH$_4$ solution (600 μL, 0.01 M) was added to the mixture followed by stirring at 25 °C for 3 min for the formation of a brown seed solution. In order to grow the gold seeds, the seed solution was allowed to stand for 2 h. The
nanorod growth solution was prepared by mixing HAuCl₄ solution (300 μL, 50 mM) and CTAB solution (30 mL, 0.10 M) with gentle stirring, then AgNO₃ solution (300 μL, 0.10 M), HCl solution (300 μL, 1.0 M) and ascorbic acid (240 μL, 0.10 M) were added in sequence to prepare growth solution.

To grow the gold nanorods, the seed solution (75 μL) was added to the growth solution under slow stirring, and the mixture was continuously stirred at 25 °C overnight to obtain a purple-red gold nanorod mixture, then the mixture was centrifuged at 8,000 rpm for 10 min, and washed with ultra-pure water three times. Finally, the product, AuNRs, was dispersed in 30 mL of ultra-pure water for further use.

For preparing the mesoporous silica-coated gold nanorods, NaOH solution (0.10 M) was added to the above prepared AuNRs solution with stirring to adjust the pH of the mixed solution to 10. Next, tetraethyl orthosilicate (TEOS) (20 %, 30 μL) in methanol and 10 μL of 2% 3-aminopropyltriethoxysilane (APTES) in methanol were injected into the AuNRs solution three times at 30-minute intervals. The mixed solution was stirred for 24 h at 25 °C to obtain AuNR@SiO₂. Finally, the synthesized AuNR@SiO₂ was collected by centrifugation at 8,000 rpm for 5 min, and washed three times with ultra-pure water to remove CTAB. The product was dispersed in 30 mL of ultra-pure water for further use. The concentrations of gold in AuNRs and AuNR@SiO₂ was determined by using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) element analysis.

To prepare Tet-SiPc@AuNR@SiO₂, Tet-SiPc solution (50 μL, 1 mM) with DMSO as solvent was added to AuNR@SiO₂ solution (10 mL) whose gold concentration was 200 μg/ml as determined by ICP-OES. The mixture was stirred at room temperature for 48 h. The obtained product solution was centrifuged at 8,000 rpm for 5 min followed by consecutive washing three times with ultra-pure water, and the Tet-SiPc@AuNR@SiO₂ was then dispersed into 10 mL of ultrapure water for use.

2.4. Fluorescence quantum yields of Tet-SiPc

Fluorescence quantum yields (Φ₁) of Tet-SiPc in DMSO were determined by using the comparative method of Eq. 1.

\[ \Phi_1 = \frac{F_{\text{Std}} \cdot A_{\text{Std}} \cdot n^2}{F_{\text{Std}} \cdot A_{\text{Std}} \cdot n_{\text{Std}}^2} \]  

Where the integral areas of the emission curves of Tet-SiPc and unsubstituted ZnPc (n-ZnPc) are denoted by F and F Std respectively. The absorbance of Tet-SiPc and standard n-ZnPc at the excitation wavelength are represented by A and A Std respectively. The refractive indices of Tet-SiPc and standard n-ZnPc solvents are represented by n² and n Std², respectively. n-ZnPc in DMSO (Φ₁Std = 0.20) was employed as the standard.

2.5. Singlet oxygen quantum yields of Tet-SiPc

Singlet oxygen quantum yields (Φ₂) of Tet-SiPc were measured by the chemical trapping method based on the singlet oxygen quencher 1,3-Diphenylisobenzofuran (DPBF). Using DMSO as a solvent, Tet-SiPc (3 mL, 3×10⁻⁶ M) and DPBF (6×10⁻⁵ M) were mixed, and the mixture was continuously irradiated with a laser (671 nm, 100 mW/cm²). The decrease of DPBF absorbance at 417 nm was detected by UV-Vis spectra. Using n-ZnPc as a reference (Φ₂ = 0.67 for n-ZnPc in DMSO), the Φ₂ value of Tet-SiPc was calculated by Eq. 2:

\[ \Phi_2 = \frac{F_{\text{Std}} \cdot R_{\text{Std}} \cdot I_{\text{Std}}^2}{F_{\text{Std}} \cdot R_{\text{Std}} \cdot I_{\text{Std}}^2} \]  

2.6. Single oxygen quantum yield of AuNR, AuNR@SiO₂ and Tet-SiPc@AuNR@SiO₂

Singlet oxygen was monitored by chemical oxidation of 9,10-anthracenediylbis (methylen) dimalonic acid (ABDA) (3×10⁻⁴ M) in the presence of AuNR, AuNR@SiO₂ or Tet-SiPc@AuNR@SiO₂ whose gold concentration were found to be 100μg/mL by ICP-OES. The decrease in ABDA absorbance at 378 nm was monitored upon irradiation laser (671 nm, 100 mW/cm²), irradiation was stopped very 3 mins and UV-Vis absorption was recorded.

2.7. The loading rate of Tet-SiPc on AuNR@SiO₂

The loading rate of Tet-SiPc on AuNR@SiO₂ was measured according to the method described by Chen et al. The loading rate of Tet-SiPc on AuNR@SiO₂ was calculated according to Eq. 3:

\[ \phi = \frac{M_{\text{Tet-SiPc}} - M_{\text{Tet-SiPc@AuNR@SiO₂}}}{M_{\text{Tet-SiPc@AuNR@SiO₂}}} \times 100\% \]  

Where \( \phi \) is the loading rate of Tet-SiPc on AuNR@SiO₂, \( M_{\text{Tet-SiPc}} \) is the initial amount of Tet-SiPc, \( M_{\text{Tet-SiPc@AuNR@SiO₂}} \) is the amount of Tet-SiPc in solution and \( M_{\text{Tet-SiPc@AuNR@SiO₂}} \) is the amount of Tet-SiPc@AuNR@SiO₂ added.

2.8. The photo-induced release of Tet-SiPc from Tet-SiPc@AuNR@SiO₂

The photo-induced release of Tet-SiPc from Tet-SiPc@AuNR@SiO₂ was studied by fluorescence spectra. In briefly, 3 mL of Tet-SiPc@AuNR@SiO₂ solution with 100 μg/mL gold concentration was added to seven centrifuge tubes, respectively. Each centrifuge tube was irradiated with infrared laser (808 nm, 0.5 W/cm²) and a thermocouple thermometer was used to record the temperature of Tet-SiPc@AuNR@SiO₂ solution every one minute. The AuNR@SiO₂, AuNR and ultrapure water were used as controls. At the same time, the Tet-SiPc released from Tet-SiPc@AuNR@SiO₂ was monitored by the fluorescence spectroscopy. Tet-SiPc@AuNR@SiO₂ solution was irradiated to release the Tet-SiPc which was insoluble in solution under 808 nm (0.5 W/cm²). After the solutions were irradiated for 0, 5, 10, 15, 20 and 25 mins, the participation was centrifuged and re-dissolved in DMSO. The fluorescence of the above obtained Tet-SiPc solution was measured and the concentration of Tet-SiPc was calculated.
2.9. Construction of antibiotic-resistant *E. coli* DH5α strains and expression of β-galactosidase in *E. coli* DH5α cells

*E. coli* DH5α cells are not resistant to Ampicillin, Kanamycin and Zeocin. The Ampicillin, Kanamycin or Zeocin-resistant *E. coli* DH5α strain could be created by transforming a plasmid containing a corresponding antibiotic-resistant gene. In this study, pUC18, pET28a and pPICZa plasmids were used to transform *E. coli* DH5α. pUC18, pET28a and pPICZa contain an Ampicillin, Kanamycin and Zeocin-resistant gene, respectively. After successful transformation, *E. coli* DH5α will gain the ability to resist Ampicillin, Kanamycin or Zeocin. The LB plates containing Ampicillin (100 μg/mL), Kanamycin (50 μg/mL) or Zeocin (25 μg/mL) were used to confirm the ability of the transformed *E. coli* DH5α to resist the corresponding antibiotics.

The β-galactosidase is an important enzyme to metabolize the lactose in *E. coli*, but the LacZ gene encoding β-galactosidase is mutated in *E. coli* DH5α, which causes the loss of β-galactosidase activity. To regain the activity of β-galactosidase in *E. coli* DH5α, a recombinant plasmid expressing the functional β-galactosidase was constructed by using pAO815 plasmid as a backbone. The pAO815 was linearized by digestion with EcoRI restriction enzyme. LacZ gene with full length was amplified using primers 5′CCGGAATTCACCATGATAGATCCCGTCG 3′, reverse primer: 5′CCGGAATTCTATTTTTGACACCAGACCAACTG 3′ flanked by an EcoRI site at both 5′ and 3′ end by PCR. The PCR product was purified by using Premega Wizard SV Gel and PCR Clean-up System (Promega (Beijing) Biotech Co., Ltd. Beijing, China). The purified PCR product was digested using EcoRI and then ligated with the linearized pAO815 plasmid using T4 DNA ligase. The recombinant plasmid was confirmed by DNA sequencing and transformed into *E. coli* DH5α. The expression of β-galactosidase was verified on the LB plate containing 5-Bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal), a chromogenic substrate for β-galactosidase.

2.10. Antibacterial activity of Tet-SiPc@AuNR@SiO₂ against *E. coli* DH5α and antibiotic-resistant *E. coli* DH5α strains

*E. coli* DH5α or antibiotic-resistant *E. coli* DH5α strains were cultured in LB (Luria-Bertani) medium at 37 °C overnight until OD₆₀₀nm reached about 0.5. The culture was then diluted 1:10 in LB medium. 40 μL of Tet-SiPc@AuNR@SiO₂ (C₉₀=100 μg/mL) in sterile distilled water was added to 20 μL of bacterial dilution. The suspension was illuminated by 808 nm laser light at 0.5 W/cm² for 5 min and then irradiated by 671 nm laser for 10 min with a power density of 100 mW/cm². After the combined irradiation under 808nm and 671nm, the bacteria were diluted in a series of 10 folds with LB medium, 5 μL of each dilution was dropped on LB agar plates and incubated at 37 °C for 12-16 h until the colonies appeared. For colony forming units (CFU) assay or relative survive rate analysis, an aliquot (200 μL) of the above diluted bacterial suspension was plated on a 10 cm LB agar plate. The plates were incubated at 37 °C for 12-16 h until the colonies appeared, and then the total number of colonies on each plate was counted. The number of CFU/mL was calculated as number of colonies counted on a plate/0.2 mL and dilution factor. The cell survival rate was calculated by normalization with respect to the CFU value of the control group performed without the treatment of Tet-SiPc@AuNR@SiO₂. Each experiment was repeated three times.

2.11. Effect of Tet-SiPc@AuNR@SiO₂ on genomic DNA damage of *E. coli* DH5α

The genomic DNA of *E. coli* DH5α with or without the treatment of Tet-SiPc@AuNR@SiO₂ under a combined laser irradiation of 808 nm and 671 nm as mentioned above was extracted by using genomic DNA extraction kit (Tiangen (Beijing) Biotech Co., Ltd. Beijing, China) and quantified by a Nanodrop 2000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). 500 μg of genomic DNA was loaded into wells of an 1% agarose gel, and then the electrophoresis was performed at 100 V for 30 min. The gel was stained with ethidium bromide and photographed.

2.12. Effect of Tet-SiPc@AuNR@SiO₂ on enzyme activity of β-galactosidase

The engineered *E. coli* DH5α cells expressing the functional β-galactosidase were treated with or without Tet-SiPc@AuNR@SiO₂ under a combined laser irradiation of 808 nm and 671 nm as mentioned above. The bacterial cells were collected by centrifugation at 10,000 rpm for 5 min. After discarding the supernatant, the pellets were resuspended with Z-buffer (Na₂HPO₄ 60 mM, NaH₂PO₄ 40 mM, KCl 10 mM, MgSO₄ 1 mM, pH=7.0) with β-mercaptoethanol (adding 0.27 mL β-mercaptoethanol to 100 mL of Z-buffer), and then added 3 drops of chloroform and 2 drops of 0.1% sodium dodecyl sulfonate (SDS), vortexed at 3,000 rpm for 10 s, and this procedure was repeated three times. After incubating the bacterial solution at 28 °C for 5 min, 200 μL of 2-nitrophenyl-β-D-galactopyranoside (ONPG) (4 mg/mL in Z-buffer) was added and incubated at 30 °C until the yellow colour developed. The elapsed time was recorded in minutes. After the yellow colour developed, 500 μL of 1% Na₂CO₃ solution was added. The reaction tubes were centrifuged for 10 min at 14,000 rpm to pellet cell debris, and the supernatants were carefully transferred into a 96-well plate. Finally, the yellow colour developed was measured at 420 nm on a spectrophotometer. The enzyme activity of β-galactosidase was calculated according to Eq. 4 and the enzyme activity of control (no treatment with Tet-SiPc@AuNR@SiO₂) was normalized to 100%:

\[
\text{Enzyme activity} = \frac{OD_{420nm} - OD_{600nm} \times V \times I}{OD_{420nm}}
\] (Eq. 4)
Where $V$ is the volume of bacterial solution (mL), $t$ is the reaction time (min).

3. Results and discussion

3.1. Synthesis and characterization of Tet-SiPc

Tet-SiPc was obtained by a nucleophilic substitution reaction between 1-(4-hydroxyphenyl)-5-mercapto-tetrazole and dichloro-silicon phthalocyanine (SiPcCl₂) using anhydrous K₂CO₃ as a catalyst in a yield of 18 % (Scheme 1). The chemical structure of Tet-SiPc was characterized by various methods including $^1$H NMR, ESI-MS, FT-IR and Raman spectroscopy (Fig. S1-S4, ESI†). The UV/Vis spectra of Tet-SiPc in dimethyl sulfoxide (DMSO) showed typical spectra of Pc with a B band at 355 nm and a Q band at 683 nm (Fig. 1a). With the increase of the concentration of Tet-SiPc, the intensity of Q band enhanced while the sharp and position of Q band did not change, indicating that the introduction of mercapto-tetrazole functional groups could reduce the aggregation of Pcs. Upon excitation at 615 nm, Tet-SiPc showed emission at 677 nm (Fig. 1a). The fluorescence lifetime of Tet-SiPc was found to be 5.23 ns, and the fluorescence quantum yield ($\Phi_f$) was calculated to be 0.0522 (Table S1 and Fig. S5, ESI†). The quantum yield of singlet oxygen ($\Phi_O^1$) ($\Phi_f$) was found to be 0.355 (Table S1 and Fig. S6, ESI†).

3.2. Preparation and characterization of AuNR, AuNR@SiO₂ and Tet-SiPc@AuNR@SiO₂

In order to enhance the antibacterial efficiency of Tet-SiPc and reduce its phototoxicity, AuNR@SiO₂ was used as a nanocarrier to load Tet-SiPc to form a novel light control nanoswitch (Tet-SiPc@AuNR@SiO₂). The Tet-SiPc@AuNR@SiO₂ was prepared through adsorption of Tet-SiPc on AuNR@SiO₂. Briefly, AuNRs were synthesized using a seed-mediated growth method.⁴³ AuNR@SiO₂, a mesoporous silica layer wrapped on the surface of AuNR, was prepared by an improved method.⁴⁵ Tet-SiPc (1 mM) was added to the aqueous solution of AuNR@SiO₂ (200 μg/mL) and stirred for 48 h. After being centrifuged and dialysis, Tet-SiPc@AuNR@SiO₂ was obtained (Scheme 1). The TEM images showed that AuNR, AuNR@SiO₂ and Tet-SiPc@AuNR@SiO₂ were of uniform size and good dispersion in solution (Fig. 2a-c). The average length and width of AuNRs were about 59 nm and 16 nm, respectively, and the aspect ratio (AR) was calculated to be about 3.70. The thickness of the initial silica coating was measured to be about 22 nm for AuNR@SiO₂. After the Tet-SiPc was successfully loaded into the mesoporous silica of AuNR@SiO₂, the colour of the silica coating layer became darker due to the contribution of the high electron density of Tet-SiPc. The dynamic light scattering (DLS) size of AuNR was found to be 31 nm. After the mesoporous silica was coated on AuNR, the DLS size of AuNR@SiO₂ increased from 31 nm to 67 nm. Tet-SiPc was further loaded on AuNR@SiO₂, the DLS size of Tet-SiPc@AuNR@SiO₂ was increased to 106 nm (Fig. 2d-f). The average particle sizes measured by DLS were larger than that measured by TEM, which could be related to the swelling of nanoparticles in solution.⁴⁴ Energy dispersive X-ray spectroscopy (EDX) was used to demonstrate the successful coating of SiO₂ on AuNR. The content of N element in AuNR@SiO₂ was significantly higher than that in AuNR, and the element of Si appeared at AuNR@SiO₂, indicating the successful coating of SiO₂ on AuNR (Fig. S7-S8, Table S2-S3, ESI†). The presence of S element in Tet-SiPc@AuNR@SiO₂ proved the successful loading of Tet-SiPc on Tet-SiPc@AuNR@SiO₂ (Fig. S9, Table S4, ESI†). The successful preparation of Tet-SiPc@AuNR@SiO₂ was also confirmed by the UV/Vis absorption spectra (Fig. 2g). AuNR had two surface plasmon resonance absorption peaks. The lateral surface plasmon resonance absorption peak was located at 514 nm and the longitudinal surface plasmon resonance absorption peak was found to be at 801 nm. After the AuNR was coated with mesoporous silica, the shape and position of the lateral characteristic absorption peak at 514 nm unchanged, while the longitudinal surface plasmon resonance was red-shifted from 801 nm to 824 nm. In the absorption spectrum of Tet-SiPc@AuNR@SiO₂ a characteristic absorption peak of Tet-SiPc at 683 nm and the longitudinal surface plasmon resonance absorption peak of AuNR continued.
Fig. 1 (a) UV-visible spectra of Tet-SiPc in DMSO; (b) Fluorescence spectra of Tet-SiPc in DMSO. (λ_{ex} = 615 nm)

Fig. 2 TEM images of (a) AuNR, (b) AuNR@SiO_2 and (c) Tet-SiPc@AuNR@SiO_2; Particle size distribution of (d) AuNR, (e) AuNR@SiO_2 and (f) Tet-SiPc@AuNR@SiO_2 in water; (g) UV-Vis absorption spectra of AuNR, AuNR@SiO_2 and Tet-SiPc@AuNR@SiO_2 (C_{Au}=6.5 μg/mL); (h) Curves of temperature change of AuNR, AuNR@SiO_2 and Tet-SiPc@AuNR@SiO_2 solutions under laser irradiation (808 nm laser with power density of 0.5 W/cm^2, PBS solution as a control; The gold concentration in AuNR, AuNR@SiO_2 and Tet-SiPc@AuNR@SiO_2 was 100 μg/mL); (i) Curves of absorption change of ABDA mixed
with AuNR, AuNR@SiO\(_2\) or Tet-SiPc@AuNR@SiO\(_2\) for different irradiated times (671nm laser with power density of 100 mW/cm\(^2\)). The gold concentration of AuNRs, AuNR@SiO\(_2\) and Tet-SiPc@AuNR@SiO\(_2\) was 100 μg/mL.

to redshift to 845 nm were observed, indicating that Tet-SiPc was successfully loaded in AuNR@SiO\(_2\). The loading ratio of Tet-SiPc in Tet-SiPc@AuNR@SiO\(_2\) was calculated to be 48% by UV-Vis absorption spectra. The photothermal effect of AuNR, AuNR@SiO\(_2\) and Tet-SiPc@AuNR@SiO\(_2\) irradiated by 808 nm laser with 0.5 W/cm\(^2\) for 15 min was shown in Fig. 2h. AuNR, AuNR@SiO\(_2\) and Tet-SiPc@AuNR@SiO\(_2\) exhibited excellent photothermal properties. The temperature of AuNR, AuNR@SiO\(_2\) and Tet-SiPc@AuNR@SiO\(_2\) increased to 49.9, 51.7 and 56.2 °C after a 15-minute illumination, respectively. Tet-SiPc@AuNR@SiO\(_2\) possessed the highest photothermal conversion efficacy, which is probably due to the theranostic photothermal effect of Tet-SiPc loaded on the mesoporous silica and AuNR@SiO\(_2\).\(^{45}\)

The \(^{1}\text{O}_2\) generation ability of AuNR, AuNR@SiO\(_2\) and Tet-SiPc@AuNR@SiO\(_2\) was evaluated by using 9,10-anthracenediyl-bis-(methylene) dimalonic acid (ABDA) as a probe.\(^{20}\) The decrease of the ABDA absorption at 378 nm as a function of irradiation time was observed upon irradiation at 671 nm (Fig. S10-S12, ESI†). The Tet-SiPc@AuNR@SiO\(_2\) exhibited the highest ability to produce \(^{1}\text{O}_2\), followed by AuNR@SiO\(_2\) and AuNR (Fig. 2i). The best \(^{1}\text{O}_2\) generation ability of Tet-SiPc@AuNR@SiO\(_2\) may be related to the synergistic generation of \(^{1}\text{O}_2\) by both Tet-SiPc and AuNR@SiO\(_2\) in the nanosystem.\(^{46}\)

3.3. Light-controlled Tet-SiPc release from the nano-switch Tet-SiPc@AuNR@SiO\(_2\)

Most of theranostic agents were “always-on” models for therapeutic intervention, leading to low signal-to-noise ratio and microbial drug resistance. The nanomaterials for controlled release can effectively overcome these deficiencies.

In order to confirm that the Tet-SiPc@AuNR@SiO\(_2\) was an excellent light-controlled nano-switch, a series of experiments were carried out. Fluorescence of Tet-SiPc in Tet-SiPc@AuNR@SiO\(_2\) was quenched by the AuNR@SiO\(_2\) without irradiation. Upon the irradiation by NIR light, Tet-SiPc was released from Tet-SiPc@AuNR@SiO\(_2\) (Fig. 3a). The amount of Tet-SiPc released from Tet-SiPc@AuNR@SiO\(_2\) was quantified by fluorescence spectra (Fig. 3b-c). The photothermal effect of Tet-SiPc@AuNR@SiO\(_2\) could change the exothermic adsorption equilibrium and then promoted Tet-SiPc released. But the irrad-
Fig. 4 Antibacterial activity of Tet-SiPc, AuNR, AuNR@SiO$_2$ and Tet-SiPc@AuNR@SiO$_2$ against *E. coli* DH5α strain. (a) Colony assay was performed on *E. coli* DH5α treated with Tet-SiPc, AuNR, AuNR@SiO$_2$ and Tet-SiPc@AuNR@SiO$_2$ dissolved in water or 10% DMSO aqueous solution. The concentration of Tet-SiPc was 50 μM, the Tet-SiPc concentration in Tet-SiPc@AuNR@SiO$_2$ was 50 μM and the gold concentration in AuNRs, AuNR@SiO$_2$ and Tet-SiPc@AuNR@SiO$_2$ was 100 μg/mL. After laser irradiation (808 nm laser light at 0.5 W/cm$^2$ for 5 min followed by 671 nm laser for 10 min with a power density of 100 mW/cm$^2$), *E. coli* DH5α solutions were diluted in 10-fold serial dilutions and 5 μL of diluted solution was dropped into the LB plate; (b) Colony forming units (CFU) of *E. coli* DH5α treated with Tet-SiPc, AuNR, AuNR@SiO$_2$ and Tet-SiPc@AuNR@SiO$_2$ dissolved in water or 10% DMSO aqueous solution. The concentration of Tet-SiPc was 50 μM, the Tet-SiPc concentration in Tet-SiPc@AuNR@SiO$_2$ was 50 μM and the gold concentration in AuNR, AuNR@SiO$_2$ and Tet-SiPc@AuNR@SiO$_2$ was 100 μg/mL. The data is expressed as means ± SD of three experiments. NS means no statistical significance, ***P<0.001.
Fig. 5 Antibacterial activity of Tet-SiPc@AuNR@SiO₂ against three antibiotic-resistant *E. coli* DH5α strains under laser irradiation (808 nm laser light at 0.5 W/cm² for 5 min followed by 671 nm laser for 10 min with a power density of 100 mW/cm²). (a) Verification of Ampicillin, Kanamycin and Zeocin-resistant *E. coli* DH5α strains on plates with the corresponding antibiotics; (b) Analysis of antibacterial activity of Tet-SiPc@AuNR@SiO₂ against three antibiotic-resistant *E. coli* DH5α strains by Colony assay. The Tet-SiPc concentration in Tet-SiPc@AuNR@SiO₂ was 50 μM and the gold concentration in Tet-SiPc@AuNR@SiO₂ was 100 μg/mL; (c) Analysis of survival rate of three antibiotic-resistant *E. coli* DH5α strains after the treatment with Tet-SiPc@AuNR@SiO₂ under laser irradiation. The Tet-SiPc concentration in Tet-SiPc@AuNR@SiO₂ was 50 μM and the gold concentration in Tet-SiPc@AuNR@SiO₂ was 100 μg/mL. The data is expressed as means ± SD of three experiments.

Irradiation time was over 15 min, the decrease in the fluorescence intensity of Tet-SiPc was observed, which may be caused by the photo bleach of Tet-SiPc by irradiation or the aggregation behaviour of Tet-SiPc released into water. Light can manipulate very precisely to release the Tet-SiPc in the Tet-SiPc@AuNR@SiO₂, which provides a strategy to prevent the bacteria from developing drug resistant by long exposure to the Tet-SiPc@AuNR@SiO₂ in antibacterial application.

3.4. Antibacterial activity of the nano-switch Tet-SiPc@AuNR@SiO₂ against *E. coli* DH5α
E. coli DH5α was selected as a model to evaluate the antibacterial activity of Tet-SiPc@AuNR@SiO₂. Upon irradiation, both Tet-SiPc and AuNR@SiO₂ did not show obvious antibacterial efficacies against DH5α, indicating that the photodynamic efficacy of Tet-SiPc or the photothermal efficacy of AuNR@SiO₂ alone did not present enough antibacterial activity against DH5α. The Tet-SiPc@AuNR@SiO₂ exhibited a significant antibacterial efficacy with a killing rate of 99.83% (Fig. 4), which can be explained that AuNR@SiO₂ served as a photothermal agent to absorb the energy of NIR laser and convert it into heat energy, which triggered the release of Tet-SiPc from Tet-SiPc@AuNR@SiO₂ and promoted the released Tet-SiPc to produce \(^{1}\text{O}_2\) to kill the bacteria through the synergistic photodynamic and photothermal effect.\(^{47}\)

3.5. Antibacterial activity of the nano-switch Tet-SiPc@AuNR@SiO₂ against antibiotic-resistant E. coli DH5α strains

We wondered whether the Tet-SiPc@AuNR@SiO₂ exhibited the same effect in killing the antibiotic-resistant bacteria as the non-resistant bacteria. Three antibiotic-resistant DH5α strains, DH5α (Amp\(^r\)), DH5α (Kan\(^r\)) and DH5α (Zeo\(^r\)), were obtained through transformation of plasmid pUC18, pET28a and pPHCza into DH5α cells, respectively. Their ability to resist antibiotics was confirmed on the plates containing the corresponding antibiotics (Fig. 5a). The antibacterial activity of Tet-SiPc@AuNR@SiO₂ against the antibiotic-resistant DH5α was evaluated. The results showed that the Tet-SiPc@AuNR@SiO₂ also exhibited strong antibacterial activities against all DH5α (Amp\(^r\)), DH5α (Kan\(^r\)) and DH5α (Zeo\(^r\)) strains (Fig. 5b, c).

3.6. Antibacterial mechanism of the nano-switch Tet-SiPc@AuNR@SiO₂

DNA and enzymes are the most important biological macromolecules in bacterial cells. DNA stores the genetic information of bacteria and controls the metabolism of bacteria.\(^{48}\) Enzymes catalyse nearly all the chemical reactions in cells and are also essential for cell survival. We suspected that Tet-SiPc@AuNR@SiO₂ impaired DNA and enzymes in cells under laser light, which caused the death of cells. The genomic DNA of DH5α treated with or without Tet-SiPc@AuNR@SiO₂ was isolated and analysed by the agarose gel electrophoresis. The result was shown in Fig. 6a. The genomic DNA treated by Tet-SiPc@AuNR@SiO₂ upon irradiation was detected a smear band in agarose gel, suggesting that DNA was fragmented. The β-galactosidase encoded by lacZ gene is a favour reporter for the quantitative analysis of enzymatic activity in microorganism. In this study, β-galactosidase was used as a reporter to evaluate enzymic damage of DH5α treated by Tet-SiPc@AuNR@SiO₂.

Fig. 6 Tet-SiPc@AuNR@SiO₂ impaired genomic DNA and enzymes of DH5α cells. (a) The genomic DNA of DH5α was degraded after the treatment with the Tet-SiPc@AuNR@SiO₂ (The Tet-SiPc concentration in Tet-SiPc@AuNR@SiO₂ was 50 \(\mu\)M and the gold concentration in Tet-SiPc@AuNR@SiO₂ was 100 \(\mu\)g/mL) under laser irradiation (808 nm laser light at 0.5 W/cm\(^{2}\) for 5 min followed by 671 nm laser for 10 min with a power density of 100 mW/cm\(^{2}\)); (b) The DH5α-LacZ strain successfully expressed the reporter β-galactosidase; (c) Analysis of β-galactosidase activity with or without the treatment of the Tet-SiPc@AuNR@SiO₂ (The Tet-SiPc concentration in Tet-SiPc@AuNR@SiO₂ was 50 \(\mu\)M and the gold concentration in Tet-SiPc@AuNR@SiO₂ was 100 \(\mu\)g/mL) under laser irradiation (808 nm laser light at 0.5 W/cm\(^{2}\) for 5 min followed by 671 nm laser for 10 min with a power density of 100 mW/cm\(^{2}\)).
SiPc@AuNR@SiO$_2$ under laser irradiation. β-galactosidase activity is deficient in DH5α because of the ΔM15 mutation of LacZ gene. In order to restore the β-galactosidase activity, we constructed a plasmid expressed the LacZ gene and transformed it into DH5α, named DH5α-LacZ. The DH5α-LacZ strain showed blue colonies in the plate containing X-Gal, indicating that the β-galactosidase activity was regained in DH5α-LacZ (Fig. 6b). The β-galactosidase activity of DH5α-LacZ strain treated with or without Tet-SiPc@AuNR@SiO$_2$ was analysed. The result showed that the β-galactosidase activity of DH5α-LacZ was totally undetectable after the treatment of Tet-SiPc@AuNR@SiO$_2$ under laser irradiation (Fig. 6c).

The possible antibacterial mechanism is that the Tet-SiPc@AuNR@SiO$_2$ generated more efficiently heat and ROS upon irradiation. The enzymes and DNA in cells are denatured at high temperatures, and these denatured macromolecules are more vulnerable to impair by ROS. The synergistic photothermal and photodynamic effect gives the Tet-SiPc@AuNR@SiO$_2$ more effective ability to kill E. coli cells.

4. Conclusions

In this work, we successfully constructed a near-infrared light-controlled nano-switch Tet-SiPc@AuNR@SiO$_2$. This nano-switch was assembled through adsorption of Tet-SiPc in the mesoporous silica layer of AuNR@SiO$_2$. The Tet-SiPc@AuNR@SiO$_2$ realized precisely controlled release of Tet-SiPc from AuNR@SiO$_2$ and the generation of ROS as well as excellent photothermal conversation efficacy through simple light irradiation, and exhibited a synergistic photothermal and photodynamic effect in killing both E. coli and antibiotic-resistant E. coli strains. The degradation of genomic DNA and the loss of enzyme activity in E. coli cells after the treatment with the Tet-SiPc@AuNR@SiO$_2$ under irradiation could be the main causes for killing the bacteria.

Author contributions

Qiuhao Ye: Methodology, Data curation, Formal Analysis, Writing-review & editing. Shuanghuang Xiao: Investigation, Writing-original draft. Ting Lin: Investigation. Yufeng Jiang: Investigation, Methodology, Formal Analysis. Yiru Peng: Conceptualization, Methodology, Project administration, Supervision, Writing - review & editing, Funding acquisition. Yide Huang: Methodology, Supervision, Writing - review & editing.

Conflicts of interest

There are no conflicts to declare.

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