

Gold-Nanoframework@CuSe Mediated Near-Infrared-II Laser-Triggered Chemodynamic Therapy and Photothermal Synergistic Antibacterial therapy

Jinfeng Cai¹, Ao Wu², Shuai Pan², Zhaoqin Zhu²✉, Fuguang Li¹✉

¹ Department of microbiology and Immunology, School of Basic Medical Sciences, Zhengzhou University, Zhengzhou 450001, China

² Department of Laboratory Medicine, Shanghai Public Health Clinical Center, Shanghai 201508, China

✉ Corresponding authors. E-mails: Zhaoqin Zhu, zhaoqinzhu@163.com; Fuguang Li, lifuguang@zzu.edu.cn

Received: Apr. 3, 2025; **Revised:** Apr. 28, 2025; **Accepted:** May 7, 2025

Citation: J.F. Cai, A. Wu, S. Pan, et al. Gold-nanoframework@CuSe mediated near-infrared-II laser-triggered chemodynamic therapy and photothermal synergistic antibacterial therapy. *Nano Biomedicine and Engineering*, 2025, 17(2): 304–314.

<http://doi.org/10.26599/NBE.2025.9290127>

Abstract

The treatment of multidrug-resistant bacteria is a difficult problem in clinical practice. These drug-resistant bacteria have reduced sensitivity to antibiotics, which contributes to the high incidence and mortality of bacterial infections worldwide. Photothermal therapy and chemodynamic therapy are advanced nanomaterial-based medical treatment methods and are widely used in tumor and antibacterial therapies due to their precision and rapidity. To overcome the limitations of conventional treatment for bacterial resistance, this study developed a near-infrared-II (NIR-II) responsive nanomaterial, a gold nanoframework coated with copper selenide (GNF@CS). This nanomaterial simultaneously triggers both chemodynamic therapy and photothermal therapy, while further promoting the chemodynamic process through *in-situ* heat generation. Ultimately, it shows significant antibacterial effects against both *Staphylococcus aureus* and *Escherichia coli*, providing a new non-antibiotic-dependent treatment strategy for clinically relevant bacterial infections.

Keywords: antibacterial; photothermal therapy; chemodynamic therapy; nanomaterials

Introduction

The development speed of antibiotics has lagged far behind the speed of bacterial antibiotic resistance, and bacteria have gradually changed from single antibiotic resistance to multi-drug resistance, pan-drug resistance, and even the emergence of superbacteria [1, 2]. Due to the continuous development, modification and overuse of antibiotics, the sensitivity of bacteria to antibiotics gradually began to decline and develop into antibiotic resistance. In the absence of new and highly effective

antibiotics or antimicrobial strategies, we have to increase the dose and duration of existing antibiotics [3–5]. In the long run, it is more likely to lead to bacterial resistance and drug-resistant strains, and the toxic side effects of antibiotics such as organ damage, rash, and vertigo will also increase [6,7].

In recent years, the problem of drug-resistant bacteria and multi-drug resistant bacteria has become increasingly prominent, which has attracted widespread attention [8]. These drug-resistant bacteria are less sensitive to antibiotics, or even completely insensitive. The infections caused by

these bacteria not only increase the difficulty of clinical treatment, but also impose a significant medical burden to countries worldwide [9]. It is also an important reason for the high morbidity and mortality of bacterial infections worldwide, which will undoubtedly bring great health threats and heavy economic burdens to people across the globe [10].

With the rapid development of nanotechnology, nano-antibacterial materials have been widely used in chemical, pharmaceutical, material, communication and energy fields [11–18]. Most nano-antibacterial materials induce bacterial death mainly through oxidative stress, electrolyte imbalance, and enzyme inhibition [19]. In addition, the characteristics of nanomaterials will also affect their contact and mode of action with bacteria, enabling them to kill bacteria through a variety of ways, thereby reducing the tolerance of bacteria to the antibacterial methods of nanomaterials [11].

Among them, photothermal therapy is the use of excessive heat energy to directly kill pathogens to treat bacterial infection [20–22], which is not limited to any drug-resistance bacteria, thus will not cause the generation and mutation of bacterial resistance. In addition, the near-infrared light used in photothermal therapy has excellent tissue penetration ability, which is suitable for *in vivo* antibacterial treatment. Chemodynamic therapy (CDT) is a kind of therapeutic regimen based on the conversion of endogenous chemicals to eliminate the source of infection within the lesion [23, 24], which uses the microenvironment of the lesion to activate the Fenton reaction or Fenton-like reaction to produce highly cytotoxic $\cdot\text{OH}$ for the specific treatment of pathogenic bacteria.

Herein, we have synthesized a hollow gold nanoframework coated with copper selenide (GNF@CS) with excellent photothermal therapy (PTT) properties in the near-infrared-II (NIR-II) region. GNF@CS has the advantages of high

stability, uniform shape and consistent size, high biological safety, and its excellent photothermal and chemical dynamic properties ensure its broad clinical application. Therefore, the synergistic CDT and PTT therapy mediated by GNF@CS is a new antimicrobial therapy without drug resistance and has potential application prospects (Scheme 1).

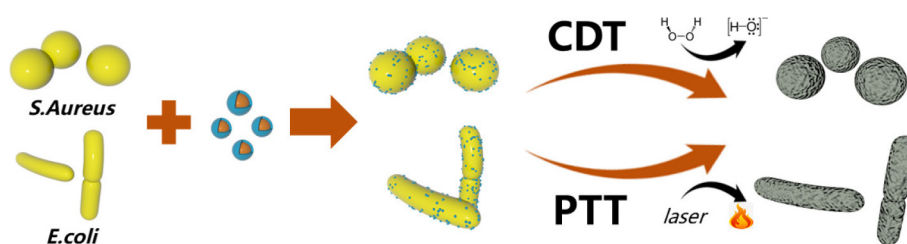
Experimental

Materials

Ascorbic acid, chloroform, granulesten, cholesterol, ethanol, CuCl_2 and selenous acid (H_2SeO_3) were purchased from Aladdin Chemistry Co., Ltd.. Chloroauric acid (HAuCl_4) was purchased from Meryer Biochemical Technology Co., Ltd.. Concentrated hydrochloric acid (HCl , 12.1 mol/L), and hydrogen peroxide (H_2O_2 , $\geq 30\%$) were purchased from Sinopharm Chemical Reagent Co., Ltd. CCK-8 staining kit was provided by the Beyotime Institute of Biotechnology.

Synthesis of GNF@CS

AA-loaded liposomes were synthesized by thin film hydration. Firstly, chloroform and ethanol were mixed in a 1:1 volume ratio to a total volume of 10 mL, and 60 mg soybean phospholipid and cholesterol were added to the above mixed solution according to the molar ratio of 7:3. After rotary evaporation to form a film, 5 mL of 1 mg/mL AA aqueous solution was added dropwise, and after ultrasonic treatment, chloroauric acid was added dropwise into the mixed system, and then the precipitated GNF was obtained by stirring for 10 min and centrifugation. After that, 1 mL of 0.5 mg/mL copper chloride and 1 mL of 0.3 mg/mL selenite were added dropwise to the GNF suspension and stirred for 10 min, then 1 mL of AA aqueous solution was added, and the product GNF@CS was obtained by centrifugation after gentle stirring for 10 min.



Scheme 1 Schematic illustration of the GNF@CS- mediated chemodynamic and photothermal synergistic therapy against *S. aureus* and *E. coli*.

Characterization of GNF@CS NPs

Transmission electron microscopes (Tallos F200X G2 and Tecnai G2 spirit Biotwin) were used to characterize the morphology and size of the sample. Ultraviolet (UV)–visible (vis)–NIR spectrophotometer (Lamda 950) was used to measure the absorption spectrum of samples. Confocal laser scanning microscope (Leica STELLARIS 5) was used to observe the cells and capture fluorescent images. Real-time thermal imager (Testo 875-1) was used to observe temperature changes.

Chemodynamic properties of GNF@CS

Molecular beacon (MB) as a typical molecular probe was used to detect hydroxyl radical ($\cdot\text{OH}$) production. 8 $\mu\text{g/mL}$ MB, 10 mmol/L H_2O_2 , and 200 $\mu\text{g/mL}$ GNF@CS solution were allowed to stand at different temperature under different conditions. The degradation curve was also recorded by UV–vis spectrophotometer.

Photothermal properties of GNF@CS

The photothermal properties of the GNF@CS nanoparticles were tested using the 980 nm laser, and the power density was maintained at 1.5 W/cm² unless otherwise specified [25]. To test the time-dependent photothermal performance, the relative temperature infrared thermography was taken under 980 nm laser (1.5 W/cm²) irradiation with 0.4 mg/mL GNF@CS for different time conditions. GNF@CS with the concentration of 400 $\mu\text{g/mL}$ were laser irradiated to reach the maximum temperature (laser on) and naturally cooled to room temperature (laser off) using the laser on/off cycle to evaluate their photothermal stability. The on/off cycle was repeated 4 times. The photothermal conversion efficiency was calculated using Eq. (1) as previously reported [26, 27]:

$$\eta = \frac{hA(\Delta T_{\max} - \Delta T_{\max, \text{PBS}})}{I(1 - 10^{-A_\lambda})} \quad (1)$$

where η is the photothermal conversion efficiency, h is the heat-transfer coefficient, A is the surface area of the container, $T_{\max}$ is the maximum temperature of the GNF@CS, $T_{\max}$ of PBS is the maximum temperature of phosphate-buffered saline (PBS), I is the laser power intensity, and A_λ is the absorbance of the GNF@CS at 980 nm.

Bacteria

Staphylococcus aureus ATCC 25923 and *Escherichia*

coli ATCC 25922 used in this study were obtained from the laboratory of Shanghai Public Health Clinical Center.

CCK-8 assay of GNF@CS

CCK-8 assay was used to determine the antibacterial activity of GNF@CS against *E. coli* and *S. aureus* under different conditions. Taking *S. aureus* as an example, we diluted GNF@CS using serial multiple dilution method to obtain bacterial medium with concentration gradients of 1.6 mg/mL, 800 $\mu\text{g/mL}$, 400 $\mu\text{g/mL}$, 200 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, and 0 $\mu\text{g/mL}$. The medium containing various concentrations of GNF@CS and the bacterial suspension containing approximately 1×10^6 colony forming units per milliliter (CFU/mL) were then added to the 96-well plate at an equal ratio of 1:1. The GNF@CS concentrations in the final system were 800 $\mu\text{g/mL}$, 400 $\mu\text{g/mL}$, 200 $\mu\text{g/mL}$, 100 $\mu\text{g/mL}$, 50 $\mu\text{g/mL}$, 25 $\mu\text{g/mL}$, and 0 $\mu\text{g/mL}$, respectively. After incubation at 37 °C for 20 min, the survival of bacteria was detected to reflect the biological safety of the material. After that, 10 $\mu\text{mol/L}$ H_2O_2 treatment and laser treatment were applied respectively, and the survival of bacteria was detected to illustrate the chemodynamic effect and photothermal effect of GNF@CS.

Bacteria uptake of GNF@CS

Taking *S. aureus* as the example, culture medium containing 1 mg/mL GNF@CS and bacterial suspension containing about 1×10^8 CFU/mL were added into 6-well plates at a ratio of 1:1.5. The final concentration of GNF@CS in the system was 400 $\mu\text{g/mL}$. After incubation at 37 °C for 30 min, the cells were centrifuged at 3 000 r/min for 10 min, and the supernatant was discarded. The precipitate was resuspended and mixed with culture medium, and the color of the solution was observed.

Antibacterial Effects of GNF@CS Synergistic Therapy

Taking *S. aureus* as an example, the bacterial suspensions of each group that had taken or not taken GNF@CS were treated with a 980 nm laser for 5 min as required. 100 μL of the treated bacterial suspension was added to 2 mL of bacterial culture medium, and the OD value representing the turbidity of the bacterial suspension at this time was recorded. After incubation at 37 °C for 24 h, the turbidity of the

solution was recorded again for comparative analysis. In addition, 100 μL of the 10^6 -fold diluted laser-treated bacterial suspension was added to 10-cm culture dishes respectively. After incubation at 37 °C for 24 h, the number of colonies in each group was observed.

SEM observation of bacterial with different treatment

Taking *S. aureus* as an example, the bacterial suspensions of each group that had taken or not taken GNF@CS were treated with a 980 nm laser for 5 min as per the experimental requirements. After being held at 37 °C for 30 min, the precipitates were collected by centrifugation at 3000 r/min for 10 min, and then resuspended in 1 mL glutaraldehyde and fixed at 4 °C for 12 h. The bacteria were washed twice with PBS and then dehydrated with 30%, 50%, 80%, and 100% ethanol (twice each) for 10 min. Finally, the solution was dropped onto a silicon wafer and the bacterial morphology was observed by scanning electron microscopy.

Statistical analyses

Data were expressed as mean $\pm$ standard deviation (SD) ($n \geq 3$). Prism software was used for statistical significance analysis. The statistical signal indicated a significant difference (* $P < 0.01$, ** $P < 0.005$, *** $P < 0.001$).

Results and Discussion

Synthesis and characterization of GNF@CS

The GNFs were prepared by the liposome reduction method. On this basis, an in situ CuSe layer was grown on the surface of GNFs using a Se source. As shown in the transmission electron microscopy (TEM) results of Fig. 1(a), the prepared GNF@CS has good dispersion, uniform morphology and consistent particle size. The TEM results in the magnified view of Fig. 1(b) show that GNF@CS has a loose porous structure, which explains its strong absorption peak in the NIR II region from the structural perspective. The Au (1 1 1) lattice in Fig. 1(c) indicates that the core structure of GNF@CS is Au, and the CuSe (1 0 1) lattice shows that the shell layer of GNF@CS is CuSe [28, 29]. The elemental mapping results in Fig. 1(d) further confirm this point, from which it can be seen that GNF@CS is

composed of Au, Cu, and Se elements. The prominent white dots in the high-angle annular dark field (HADDF) results indicate heavy elements, and it can be seen that the inner core gold framework of GNF is a hollow structure. From the UV-vis absorption spectrum (Fig. 1(e)), it can be found that GNF itself has an absorption peak in the NIR-II region, but after coating a thin layer of CuSe on its surface, the intensity of the absorption peak in the NIR-II region has been significantly enhanced. It has strong absorption characteristics and a wide spectral range from 900 to 1100 nm in the NIR-II region. This provides a basis for its broad-spectrum characteristics in mediating photothermal therapy under 980 nm laser irradiation. The X-ray photoelectron spectra (XPS) test results (Fig. 1(f)) further confirm that the constituent elements of GNF@CS are Au, Cu, and Se. From the dynamic light scattering (DLS) experiments in Figs. 1(g) and 1(h), it was found that the hydrated particle size of GNF was approximately 58 nm, and that of GNF@CS was approximately 64 nm. The zeta potential variation in Fig. 1(i) can also prove the successful coating of the CuSe layer.

GNF@CS-mediated chemodynamic and photothermal properties

In Fig. 2(a), it can be observed that the generation of hydroxyl radicals by 200 $\mu\text{g/mL}$ GNF@CS at room temperature shows a clear temperature dependence with MB as the hydroxyl radical indicator, which is a significant feature of chemodynamic therapy. Further, in a hydrogen peroxide environment at the site of bacterial infection, GNF@CS dissociates to release Cu^{2+} ions that react with hydrogen peroxide in a Fenton-like reaction to produce hydroxyl radicals. In Fig. 2(b), it can be seen that the generation of hydroxyl radicals by GNF@CS also shows a temperature dependence. When the system temperature rises from room temperature to 42 °C, the generation of hydroxyl radicals significantly increases. As shown in Fig. 2(c), the photothermal performance of the GNF@CS solution in the treatment group is significantly better than that of the PBS solution, and this photothermal advantage becomes more pronounced with the increase in GNF@CS concentration.

With 1.5 W/cm^2 980 nm laser irradiation, the PBS solution in the control group reaches the highest temperature of 60 °C around 100 s. By contrast, the GNF@CS solution can reach the temperature above

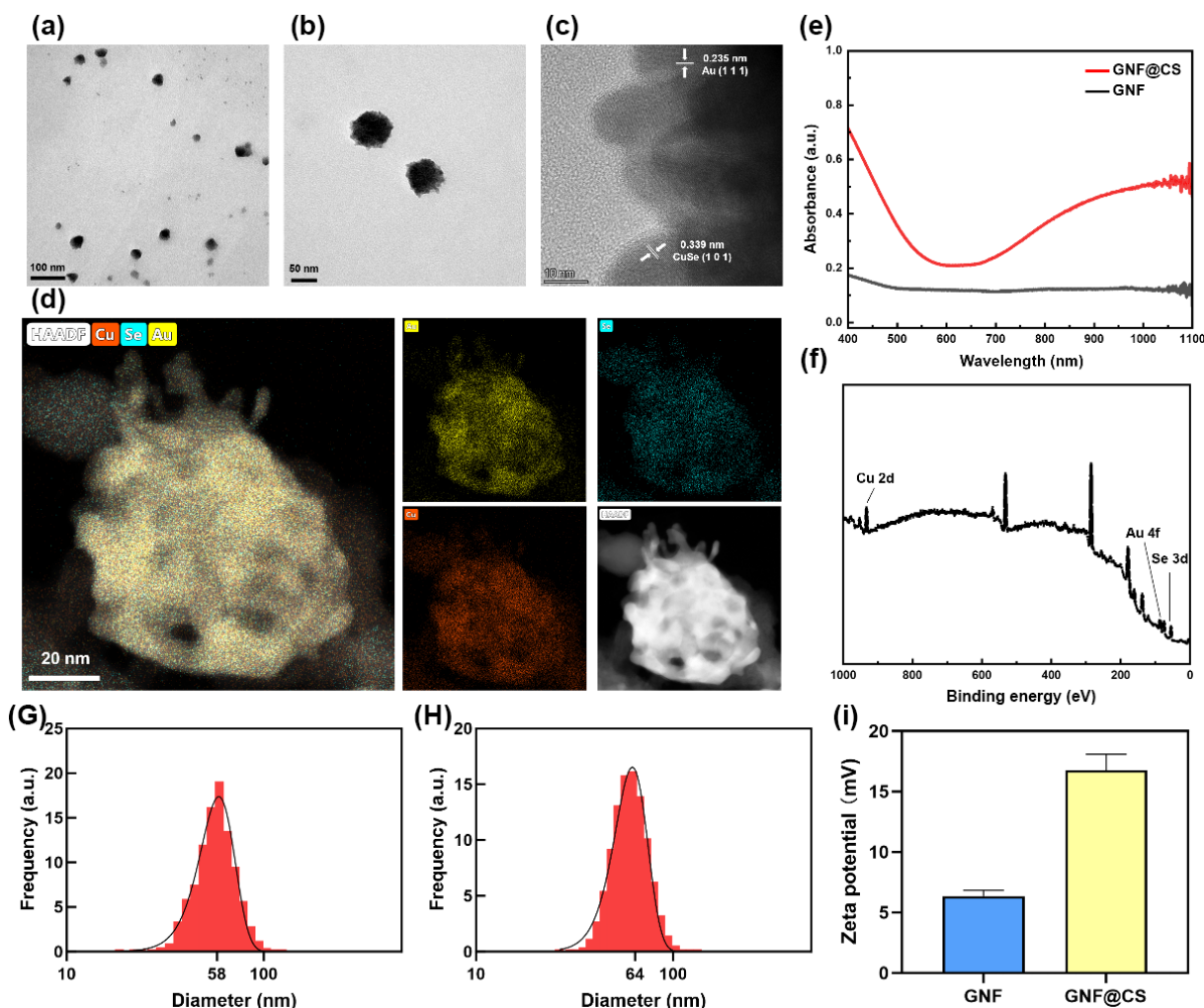


Fig. 1 Characterization of GNF@CS. **(a)** A large-field-of-view of the transmission electron microscopy (TEM) images of GNF@CS. **(b)** A magnified TEM results of an individual GNF@CS. **(c)** HRTEM image of GNF@CS and the lattice fringes of Au and CuSe. **(d)** Element mapping and HADDF results for GNF@CS in STEM mode. **(e)** UV-vis spectra of GNF and GNF@CS in the range of 400–1100 nm. **(f)** Full X-ray photoelectron spectra of GNF@CS. **(g)** and **(h)** DLS results of GNF and GNF@CS. **(i)** Zeta potential results of GNF and GNF@CS.

90 °C, for example, the 100 µg/mL GNF@CS solution can reach a temperature above 90 °C around 150 s. Meanwhile, as the GNF@CS concentration increases to 200 µg/mL, the solution can reach 80 °C around 50 s. When the GNF@CS concentration increases to 400 µg/mL, the solution can reach 70 °C within 25 s. Therefore, the photothermal process of GNF@CS shows a significant concentration dependence. The thermal imaging results in Fig. 2(d) further confirm this. As shown in Fig. 2(e), the natural cooling process of GNF@CS at different concentrations and the PBS control group after laser irradiation is similar, and both can return to room temperature after 600 s. The fitting analysis results of the photothermal conversion efficiency of GNF@CS (Fig. 2(f)) show that the photothermal conversion time constant of GNF@CS is 205.70 s, and the

photothermal conversion efficiency is 31.66%, which makes the photothermal conversion performance of GNF@CS outstanding among many traditional photothermal conversion materials, such as inorganic gold materials, carbon materials and copper sulfide materials [30–32]. The photothermal stability of a 400 µg/mL GNF@CS solution was tested (Fig. 2(g)). First, the solution was irradiated with a 980 nm laser until the highest temperature of 90 °C was reached, then the laser was turned off and the solution was allowed to cool naturally to room temperature. The experiment was repeated four times, and the photothermal stability of GNF@CS was observed by recording the highest temperature reached by the GNF@CS solution and the time required. It can be found that the heating time of GNF@CS is approximately the same in the four

cycles, and the same highest temperature can be reached, indicating that GNF@CS has excellent photothermal stability. At the same time, to verify that the GNF@CS-mediated heating process is achieved through the photothermal conversion effect of laser irradiation, the laser power dependence of this heating process was verified. As shown in the experimental results (Fig. 2(h)), when the laser power is reduced from 1.5 W/cm² to 1 W/cm², the temperature of the GNF@CS solution drops from above 90 °C to around 70 °C at 120 s. Additionally, from a single photothermal cycle (Fig. 2(i)), it can be found that when the laser power is 1.0 W/cm², the GNF@CS solution takes about 200 s to reach a maximum temperature of about 80 °C, and when the laser power is 0.8 W/cm², the GNF@CS solution

takes about 250 s to reach a maximum temperature of about 70 °C. The decrease in the maximum temperature that the GNF@CS solution can reach and the increase in the required time indicate the dependence of the heating process on laser power, which further confirms that the heat generation process of GNF@CS is indeed the conversion of 980 nm laser energy into thermal energy.

Bacterial killing by GNF@CS-mediated synergistic therapy

The CCK-8 assay was used to detect the cytotoxicity of GNF@CS to human normal skin cells (HSF and HaCat cells). As shown in Figs. 3(a) and 3(b), at the concentration of 400 µg/mL, the survival rates of both cells were higher than 90%, indicating that GNF@CS

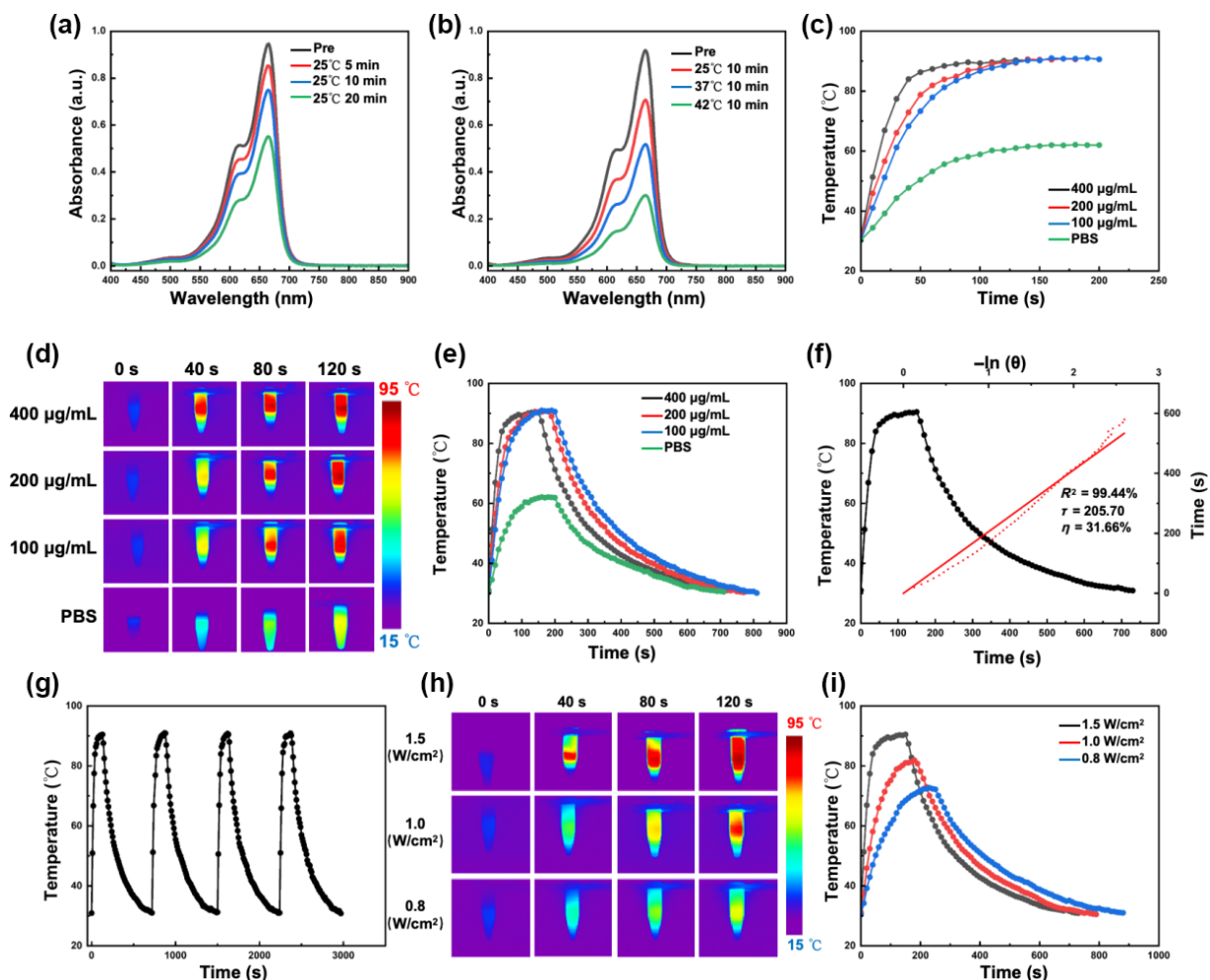


Fig. 2 Properties of GNF@CS. (a) Time-dependent chemodynamic performance of GNF@CS at 25 °C. (b) Temperature-dependent chemodynamic performance of GNF@CS at 10 min. (c) Temperature changes of GNF@CS solution at different concentrations and PBS solution under 980 nm laser irradiation (1.5 W/cm²) over time. (d) Infrared thermal images of GNF@CS at different concentrations and PBS at different times under 980 nm laser irradiation (1.5 W/cm²). (e) Temperature changes over time in a single cycle of GNF@CS with different concentrations and PBS solution irradiated to maximum temperature and then naturally cooled. (f) Photothermal conversion efficiency of GNF@CS over multiple cycles. (g) Photothermal stability of GNF@CS over multiple cycles. (h) Infrared thermal images of 400 µg/mL GNF@CS under 980 nm laser irradiation at different powers and durations. (i) Heating and cooling curves of 400 µg/mL GNF@CS during one photothermal cycle at different powers.

has excellent biocompatibility. Meanwhile, the CCK-8 was used to evaluate the bactericidal effect of GNF@CS-mediated synergistic therapy on *S. aureus* and *E. coli*, and to further determine the minimum inhibitory concentration (MIC) of GNF@CS for synergistic antibacterial therapy. As shown in Fig. 3(c), when only GNF@CS was incubated, there was almost no obvious bactericidal effect observed at concentrations below 200 $\mu\text{g/mL}$. When the concentration increased to 400 $\mu\text{g/mL}$ and 800 $\mu\text{g/mL}$, the survival rate of bacteria slightly decreased, but still remained above 80%. However, when GNF@CS was co-incubated with hydrogen peroxide (Fig. 3(d)), the survival rate of bacteria

decreased significantly when the GNF@CS concentration reached 25 $\mu\text{g/mL}$ due to the CDT effect. As the concentration of GNF@CS increased, the survival rate of bacteria decreased further, and when the concentration reached 400 $\mu\text{g/mL}$, the survival rate of bacteria dropped to about 10%. In Fig. 3(e), it can be found that when laser irradiation was applied along with GNF@CS-mediated synergistic therapy was performed, the survival rate of bacteria was dropped below 1% at a concentration of 400 $\mu\text{g/mL}$. For *E. coli* (Figs. 3(f)–3(h)), the chemodynamic therapy and synergistic therapy mediated by GNF@CS showed results similar to those observed for *S. aureus*. When the concentration

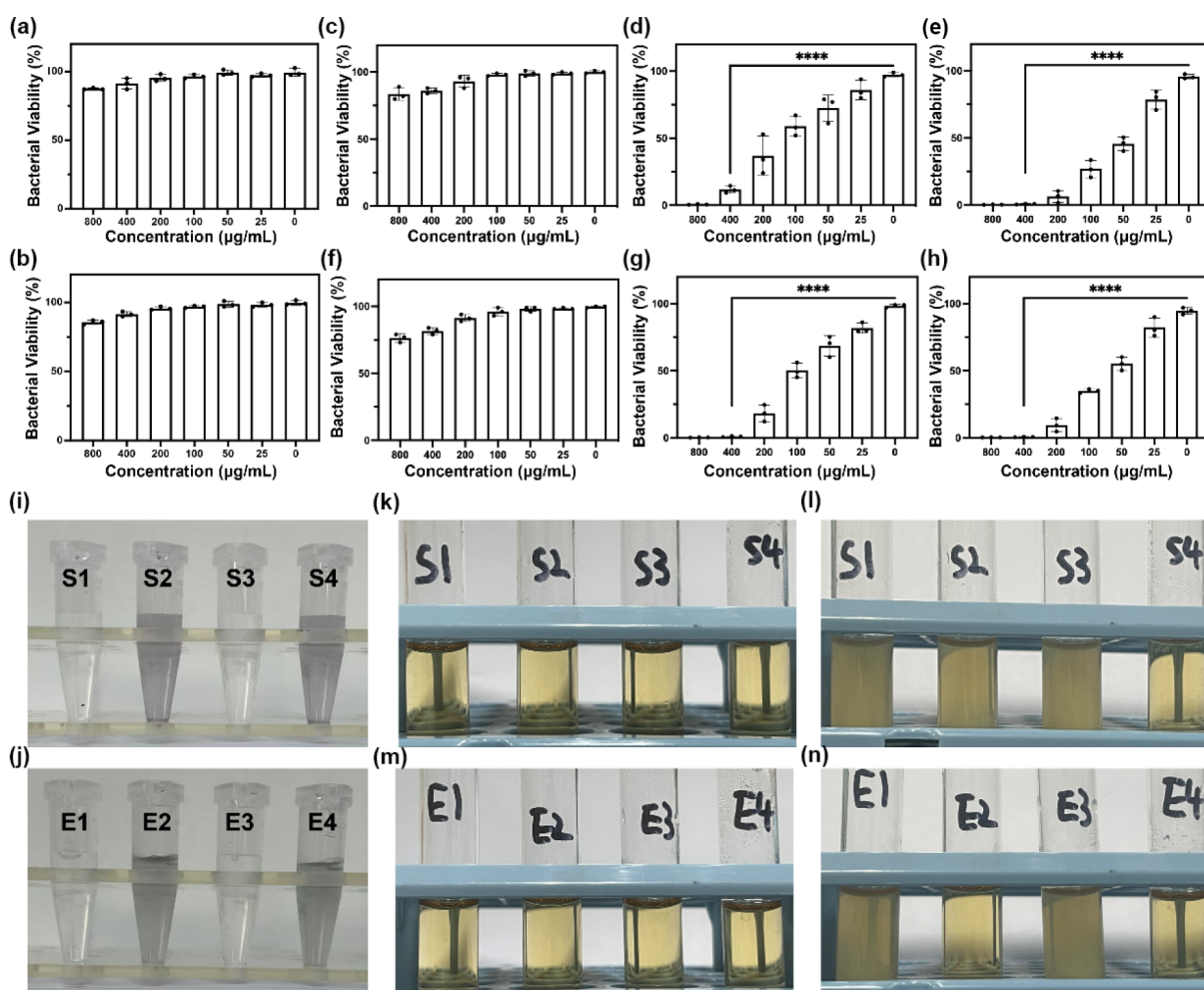


Fig. 3 Bacterial killing via synergistic therapy. Survival rate of (a) HaCat cells and (b) HSF cells after treatment with different concentrations of GNF@CS by CCK-8 assay. (c) *S. aureus* after treatment with different concentrations of GNF@CS, (d) *S. aureus* after co-incubation with different concentrations of GNF@CS and H_2O_2 , and (e) *S. aureus* after incubation with different concentrations of GNF@CS and irradiation of the laser (Laser, 980 nm, 1.5 W/cm^2 , 5 min). (f) *E. coli* after treatment with different concentrations of GNF@CS, (g) *E. coli* after co-incubation with different concentrations of GNF@CS and H_2O_2 , (h) *E. coli* after incubation with different concentrations of GNF@CS and irradiation of the laser. (Laser, 980 nm, 1.5 W/cm^2 , 5 min). (i) Color of the solution after co-incubation of different groups of *S. aureus* and (j) *E. coli* with GNF@CS (post-centrifugation and resuspension). (k) Visual appearance of freshly treated *S. aureus* in each group. (l) Visual appearance of *S. aureus* incubated for 24 h after treatment in each group. (m) Visual appearance of freshly treated *E. coli* in each group. (n) Visual appearance of *E. coli* incubated for 24 h after treatment in each group.

reached 400 $\mu\text{g/mL}$, the survival rate of *E. coli* dropped below 1%. In subsequent experiments, *S. aureus* and *E. coli* were divided into 4 groups: Group 1 (control: no GNF@CS, no laser irradiation), Group 2 (GNF@CS only, no laser irradiation), Group 3 (only laser irradiation for 5 min), and Group 4 (GNF@CS added and laser irradiation for 5 min). Additionally, the groups labeled “S” and “E” represent *S. aureus* and *E. coli*, respectively. Since GNF@CS appears light blue in aqueous solution, the bacteria collected after co-incubation with GNF@CS were resuspended and the solution color was observed to assess the uptake of GNF@CS by bacteria. As shown in Figs. 3(i) and 3(j), both *S. aureus* and *E. coli* successfully internalized GNF@CS. In addition, to visually evaluate the antibacterial efficacy of GNF@CS-mediated synergistic therapy against *S. aureus* and *E. coli*, the treated bacteria were diluted and resuspended in the culture medium. Fig. 3(k) shows that the diluted bacterial suspensions of all four groups were clear

and transparent immediately after treatment, indicating that *S. aureus* cells were present at this stage. After incubation at 37 °C for 24 h, it was found that the bacterial suspensions of Group S1 and S3 were highly turbid, while the bacterial suspension of Group S2 was turbid but significantly clearer than those of Group S1 and S3 (Fig. 3(l)), and the solution of the synergistic therapy group S4 remained relatively clear, indicating that nearly all *S. aureus* in this group had been killed. This phenomenon is consistent with the above CCK-8 experiment results. Similar to *S. aureus*, the ability of GNF@CS-mediated synergistic therapy to inhibit the proliferation of *E. coli* can also be seen in Figs. 3(m) and 3(n).

SEM of GNF@CS-mediated synergistic therapy

The therapeutic effects of GNF@CS-mediated synergistic therapy on *S. aureus* and *E. coli* were observed using scanning electron microscopy (SEM).

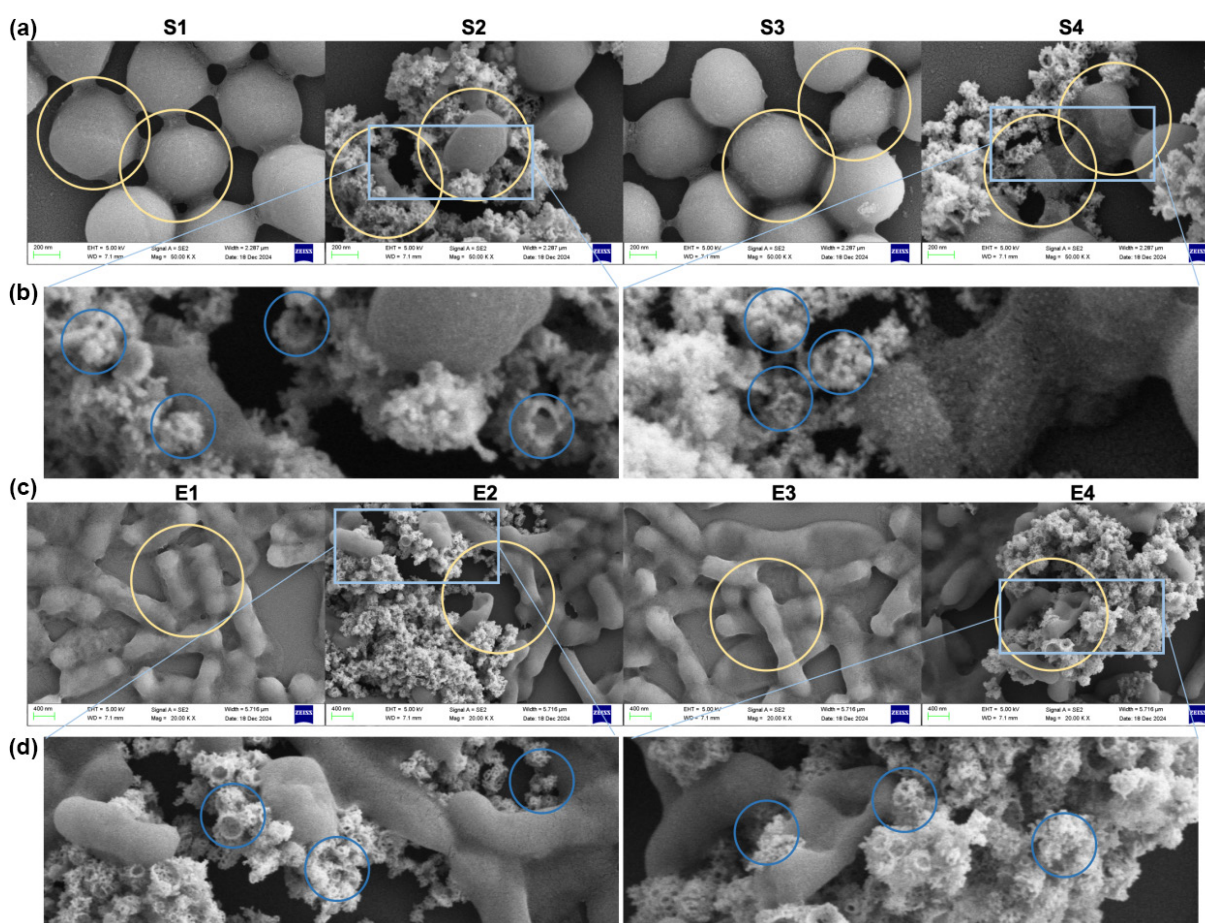


Fig. 4 SEM analysis of bacteria with different treatments. SEM images of (a) *S. aureus* and (b) its local magnification area, (c) *E. coli* and (d) its local magnification area with different treatments in each group. The blue rectangular area shows the enlarged region of the co-incubation group with GNF@CS, the yellow circle highlights bacterial morphology of different groups, and the blue circle indicates the GNF@CS. (GNF@CS concentration, 400 $\mu\text{g/mL}$; Laser, 980 nm, 1.5 W/cm², 5 min.)

As depicted in Fig. 4(a), the *S. aureus* in Groups S1 and S3 exhibited plump morphology, with intact surfaces and normal viability, showing no signs of rupture. In Group S2, the bacteria successfully captured and internalized GNf@CS on their cell surfaces (Fig. 4(b)), and due to the CDT effect, surface fractures, cell lysis, and leakage of cellular contents occurred though some *S. aureus* cells still maintained a normal morphology. Finally, after co-incubation with GNf@CS and irradiation with a 980 nm laser for 5 min, all the *S. aureus* in group S4 presented cracks, widespread leakage of contents, complete rupture of the bacterial surface, and loss of structural integrity. Similarly, the bactericidal effect of GNf@CS-mediated synergistic therapy on *E. coli* could be directly observed in Figs. 4(c) and 4(d). The *E. coli* in groups E1 and E3 were morphologically intact. In group E2, the uptake of GNf@CS on the bacterial surface could be observed, and some

bacteria exhibited obvious shrinkage and surface cracks, compromising their viability. In Group E4, after co-incubation with GNf@CS and 5-minute irradiation with a 980 nm laser, all the *E. coli* showed varying degrees of shrinkage, structural damage, and complete leakage of contents. The above SEM results demonstrate that the GNf@CS-mediated synergistic therapy effectively kills *S. aureus* and *E. coli* through the combined effects of CDT and PTT.

Antibacterial effect of GNf@CS-mediated synergistic therapy

To further evaluate the inhibitory effect of GNf@CS-mediated synergistic therapy on bacterial proliferation, the number of viable bacteria after treatment was quantified by the plate colony counting method. First, the bacterial suspension after various treatments was diluted 10⁶-fold, from which 100 μ L was evenly spread on the Petri dish and incubated for

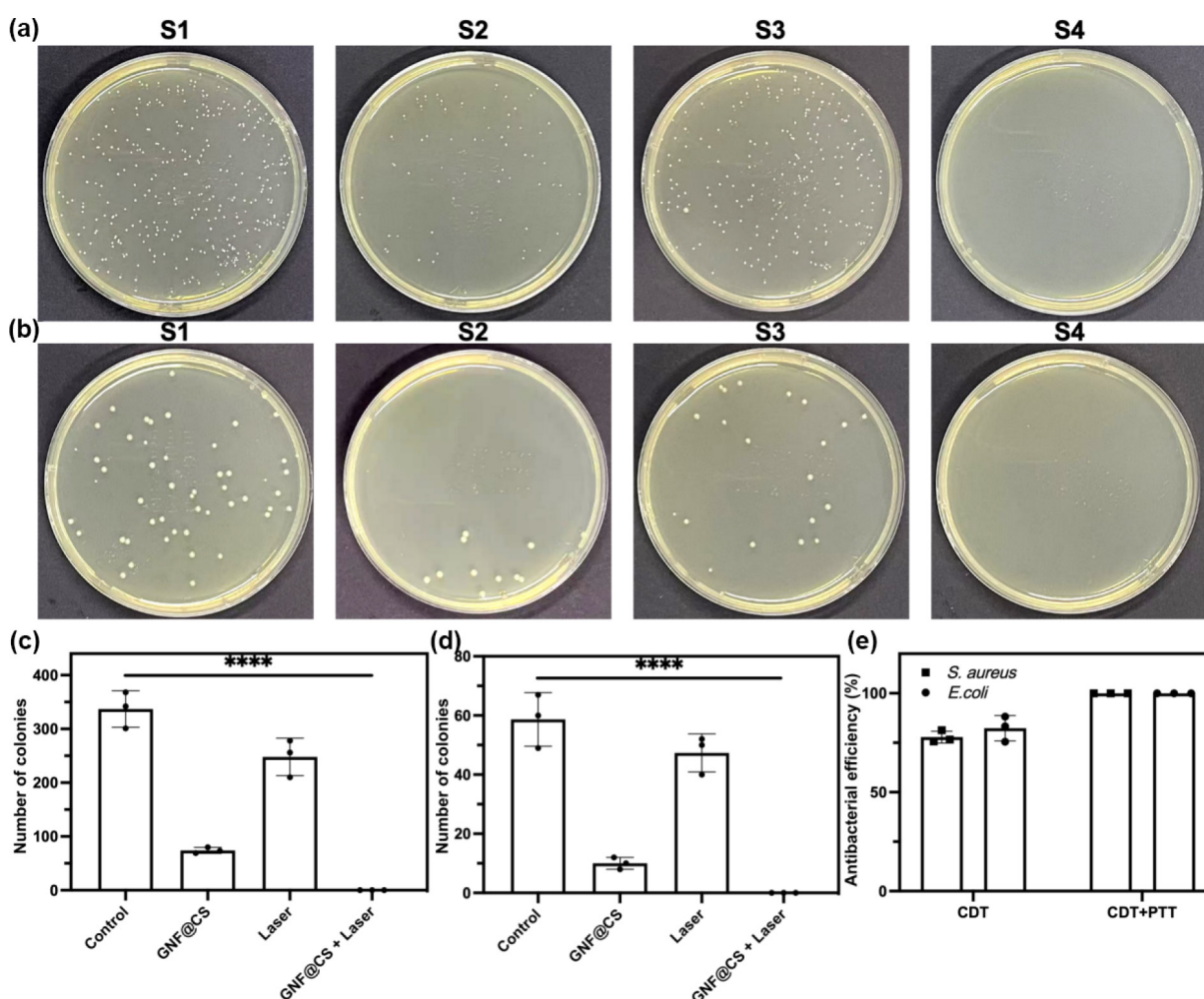


Fig. 5 Plate colony experiments of different treatment groups. (a) Photographs of *S. aureus* and (b) *E. coli* coated plates incubated for 24 h at 10⁶-fold dilutions after different treatments. (c) Statistics of the number of viable colonies of *S. aureus* and (d) *E. coli* on coated plates incubated for 24 h at 10⁶-fold dilutions after different treatments. (e) GNf@CS mediated bactericidal effect of CDT and CDT-PTT synergistic therapy.

24 h at 37 °C. From the results in Figs. 5(a) and 5(b), it can be found that the Group in S1 and E1 groups maintained high viability, while the bacteria in S3 and E3 groups showed some inhibition of viability after laser irradiation, but still retained considerable viability. However, the bacterial activity of Groups S2 and E2 was significantly inhibited due to the CDT effect, but there were still some viable bacteria remaining. With GNF@CS-mediated CDT and PTT co-treatment, neither Groups S4 nor E4 contained living bacteria, demonstrating that 400 µg/mL GNF@CS could kill more than 99% of the bacteria. The number of colonies was more visually displayed in Figs. 5(c) and 5(d). It was observed that the number of viable colonies in Group S1 was about 337, in Group S2 was about 74, that in Group S3 was about 248, that in E1 group was about 59, that in Group E2 was about 10, that in E3 group was about 48. However, the number of viable colonies in both S4 and E4 of the final synergistic treatment group was 0. Quantitative analysis showed that GNF@CS-mediated CDT achieved a bactericidal rate of about 80.1%, while the bactericidal effect reached more than 99% with the synergistic treatment of CDT and PTT (Fig. 5(e)). These results demonstrate that GNF@CS-mediated photothermal therapy can significantly inhibit the proliferation of *S. aureus* and *E. coli* and exhibits effective antibacterial activity.

Conclusions

In summary, we synthesized gold hollow nanoframeworks capable of responding in the NIR-II region and applied them to antibiotic-free phototherapy after coating them with a thin layer of CuSe. With 980 nm laser irradiation, GNF@CS exhibited significant bacterial and proliferation inhibition effect on *Staphylococcus aureus* and *Escherichia coli*. Compared with other traditional treatment methods, GNF@CS mediated synergistic therapy can not only trigger the CDT and PTT processes simultaneously, but also enhance the CDT process via the local hyperthermia generated by photothermal therapy. This combined approach demonstrates excellent antibacterial effect, providing a new strategy for the clinical antibiotic-dependent treatment of drug-resistant bacteria infections.

CRediT Author Statement

Jinfeng Cai: data curation, visualization, and writing

original draft. **Fuguang Li:** conceptualization, project administration, supervision, writing–review, and editing. **Zhaoqin Zhu:** review, editing, and visualization. **Ao Wu:** formal analysis, methodology, and investigation. **Shuai Pan:** data curation and investigation.

Conflict of interests

The authors declared no conflict of interests.

References

- [1] D.G. Joakim Larsson, C.F. Flach. Antibiotic resistance in the environment. *Nature Reviews Microbiology*, 2021, 20(5): 257–269. <https://doi.org/10.1038/s41579-021-00649-x>
- [2] J.M.A. Blair, M.A. Webber, A.J. Baylay, et al. Molecular mechanisms of antibiotic resistance. *Nature Reviews Microbiology*, 2014, 13(1): 42–51. <https://doi.org/10.1038/nrmicro3380>
- [3] D.M.P. De Oliveira, B.M. Forde, T.J. Kidd, et al. Antimicrobial resistance in ESKAPE pathogens. *Clinical Microbiology Reviews*, 2020, 33(3): e00181–19. <https://doi.org/10.1128/CMR.00181-19>
- [4] S.R. Partridge, S.M. Kwong, N. Firth, et al. Mobile genetic elements associated with antimicrobial resistance. *Clinical Microbiology Reviews*, 2018, 31(4): e00088–17. <https://doi.org/10.1128/CMR.00088-17>
- [5] S. Gardete, A. Tomasz. Mechanisms of vancomycin resistance in staphylococcus aureus. *The Journal of Clinical Investigation*, 2014, 124(7): 2836–2840. <https://doi.org/10.1172/JCI68834>
- [6] J.J. Zhang, W.R. Li, Z.H. Tao, et al. Endogenous glucose-driven cascade reaction of nano-drug delivery for boosting multidrug-resistant bacteria-infected diabetic wound healing. *Journal of Colloid and Interface Science*, 2024, 672: 63–74. <https://doi.org/10.1016/j.jcis.2024.05.204>
- [7] E.M. Darby, E. Trampari, P. Siasat, et al. Molecular mechanisms of antibiotic resistance revisited. *Nature Reviews Microbiology*, 2022, 21(5): 280–295. <https://doi.org/10.1038/s41579-022-00820-y>
- [8] C.L. Holmes, M.T. Anderson, H.L.T. Mobley, et al. Pathogenesis of gram-negative bacteremia. *Clinical Microbiology Reviews*, 2021, 34(2): e00234–20. <https://doi.org/10.1128/CMR.00234-20>
- [9] M.D. Johansen, J.L. Herrmann, L. Kremer. Non-tuberculous mycobacteria and the rise of Mycobacterium abscessus. *Nature Reviews Microbiology*, 2020, 18(7): 392–407. <https://doi.org/10.1038/s41579-020-0331-1>
- [10] S.J. Peacock, G.K. Paterson. Mechanisms of methicillin resistance in staphylococcus aureus. *Annual Review of Biochemistry*, 2015, 84: 577–601. <https://doi.org/10.1146/annurev-biochem-060614-034516>
- [11] C.K.O. Dzuvor, H.H. Shen, V.S. Haritos, et al. Coassembled multicomponent protein nanoparticles elicit enhanced antibacterial activity. *ACS Nano*, 2024, 18(5): 4478–4494. <https://doi.org/10.1021/acsnano.3c11179>
- [12] H. Zhang, P. Zong, M. Chen, et al. *In situ* synthesis of multilayer carbon matrix decorated with copper particles: Enhancing the performance of Si as anode for Li-ion batteries. *ACS Nano*, 2019, 13(3): 3054–3062.

- <https://doi.org/10.1021/acsnano.8b08088>
- [13] K. Wei, Y. Wu, X. Zheng, et al. A light-triggered J-aggregation-regulated therapy conversion: From photodynamic/photothermal therapy to long-lasting chemodynamic therapy for effective tumor ablation. *Angewandte Chemie International Edition*, 2024, 63(23): e202404395. <https://doi.org/10.1002/anie.202404395>
- [14] W. Li, Y. Xiao, G.H. Guo, et al. Cuprous oxide nanocomposites with photothermal (PTT) and chemical dynamics (CDT) effects induce cuproptosis in breast cancer using the strategy of increasing inflow and reducing outflow. *Nano Today*, 2024, 56: 102223. <https://doi.org/10.1016/j.nantod.2024.102223>
- [15] Y. Zhao, S. Zhao, Y. Du, et al. Inverse oxide/alloy-structured nanozymes with NIR-triggered enzymatic cascade regulation of ROS homeostasis for efficient wound healing. *Advanced Materials*, 2025, 37(14): e2418731. <https://doi.org/10.1002/adma.202418731>
- [16] H. Zhang, T. Wang, H. Liu, et al. Second near-infrared photodynamic therapy and chemotherapy of orthotopic malignant glioblastoma with ultra-small Cu_{2-x}Se nanoparticles. *Nanoscale*, 2019, 11(16): 7600–7608. <https://doi.org/10.1039/c9nr01789e>
- [17] S.Y. Xie, H.L. Liu, K. Yang, et al. Versatile copper-chalcogenide-based nanoparticles for the treatment of brain diseases. *Nano Biomedicine and Engineering*, 2025, 17(1): 91–110. <https://doi.org/10.26599/nbe.2025.9290117>
- [18] T. Wang, H. Zhang, Y. Han, et al. Light-enhanced O₂-evolving nanoparticles boost photodynamic therapy to elicit antitumor immunity. *ACS Appl Mater Interfaces*, 2019, 11(18): 16367–16379. <https://doi.org/10.1021/acsnano.9b03541>
- [19] J.M. An, S. Kang, C.W. Koh, et al. Scepterin-Au nano-aggregates (SANA) for overcoming drug-resistant Gram-negative bacteria. *Nanoscale Horizons*, 2022, 7(8): 873–882. <https://doi.org/10.1039/d2nh00279e>
- [20] W. Zhang, K. Cai, Z. Sun, et al. Elevating second near-infrared photothermal conversion efficiency of hollow gold nanorod for a precise theranostic of orthotopic bladder cancer. *ACS Nano*, 2023, 17(19): 18932–18941. <https://doi.org/10.1021/acsnano.3c04175>
- [21] M. Overchuk, R.A. Weersink, B.C. Wilson, et al. Photodynamic and photothermal therapies: Synergy opportunities for nanomedicine. *ACS Nano*, 2023, 17(9): 7979–8003. <https://doi.org/10.1021/acsnano.3c00891>
- [22] B. Li, W. Wang, L. Zhao, et al. Photothermal therapy of tuberculosis using targeting pre-activated macrophage membrane-coated nanoparticles. *Nature Nanotechnology*, 2024, 19(6): 834–845. <https://doi.org/10.1038/s41565-024-01618-0>
- [23] J.W. Mei, D.D. Xu, L.T. Wang, et al. Biofilm microenvironment-responsive self-assembly nanoreactors for all-stage biofilm associated infection through bacterial cuproptosis-like death and macrophage re-rousing. *Advanced Materials*, 2023, 35(36): 2303432. <https://doi.org/10.1002/adma.202303432>
- [24] X.J. He, Y. Lv, Y.L. Lin, et al. Platinum nanoparticles regulated V2C MXene nanoplateforms with NIR-II enhanced nanozyme effect for photothermal and chemodynamic anti-infective therapy. *Advanced Materials*, 2024, 36(25): 2400366. <https://doi.org/10.1002/adma.202400366>
- [25] Y. Liu, X. Zhu, Z. Wei, et al. Customized photothermal therapy of subcutaneous orthotopic cancer by multichannel luminescent nanocomposites. *Advanced Materials*, 2021, 33(30): e2008615. <https://doi.org/10.1002/adma.202008615>
- [26] J.Q. Yan, X.D. Ma, D.N. Liang, et al. An autocatalytic multicomponent DNAzyme nanomachine for tumor-specific photothermal therapy sensitization in pancreatic cancer. *Nature Communications*, 2023, 14: 6905. <https://doi.org/10.1038/s41467-023-42740-2>
- [27] M. Wu, H. Liu, D. Li, et al. Smart-responsive multifunctional therapeutic system for improved regenerative microenvironment and accelerated bone regeneration via mild photothermal therapy. *Advanced Science*, 2024, 11(2): e2304641. <https://doi.org/10.1002/adv.202304641>
- [28] C.Z. Yang, M. Wang, M.Y. Chang, et al. Heterostructural nanoadjuvant CuSe/CoSe₂ for potentiating ferroptosis and photoimmunotherapy through intratumoral blocked lactate efflux. *Journal of the American Chemical Society*, 2023, 145(13): 7205–7217. <https://doi.org/10.1021/jacs.2c12772>
- [29] M. Zhao, X. Wang, X. Yang, et al. Hollow metal nanocrystals with ultrathin, porous walls and well-controlled surface structures. *Advanced Materials*, 2018, 30(48): e1801956. <https://doi.org/10.1002/adma.201801956>
- [30] A. Sharma, P. Verwilt, M. Li, et al. Theranostic fluorescent probes. *Chemical Reviews*, 2024, 124(5): 2699–2804. <https://doi.org/10.1021/acs.chemrev.3c00778>
- [31] S.Y. Hao, H.C. Han, Z.Y. Yang, et al. Recent advancements on photothermal conversion and antibacterial applications over MXenes-based materials. *Nano-Micro Letters*, 2022, 14(1): 178. <https://doi.org/10.1007/s40820-022-00901-w>
- [32] J.J. Han, S. Zeng, Y.H. Chen, et al. Prospects of coupled iron-based nanostructures in preclinical antibacterial therapy. *Advanced Drug Delivery Reviews*, 2023, 193: 114672. <https://doi.org/10.1016/j.addr.2022.114672>

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