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COVID-19-inspired “artificial virus” to combat drug-resistant bacteria by membrane-intercalation- photothermal-photodynamic multistage effects

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A B S T R A C T

COVID-19 threatens human life because of the super destructiveness produced from its coronal morphology and strong transmembrane infection based on spike glycoprotein. Inspired by the coronal morphology of COVID-19 and its means of infecting, we designed an “artificial virus” with coronal morphology based on the concept of “defeating superbacteria with superviruses” by self-assembling a transacting activator of transduction peptide with triple-shell porous graphitic carbon nitride (g-C\textsubscript{3}N\textsubscript{4}) embedded with cobalt nanoparticles to forcefully infect methicillin-resistant \textit{Staphylococcus aureus} (MRSA). The results confirmed that this “artificial virus” had unique properties of crossing the bacterial cell membrane barrier, heating the internal bacterial microenvironment and triggering ROS outbreak, based on its coronal morphology, membrane penetration, temperature-rising and heat insulation, oxidase-like activity and excellent visible-light harvesting properties. It had a high sterilization efficiency of 99.99\% at 20 min, which was 18.6 times that of g-C\textsubscript{3}N\textsubscript{4}, and the efficiency remained at 99.99\% after 3 rounds of recycling and reuse. Additionally, it can rapidly inactivate bacteria in river water and accelerate wound healing.

1. Introduction

Antibiotics commonly used in humans make bacteria resistant and promote the production of superbugs, which poses a great threat to the survival of mankind. Traditional antibacterial agents usually are low efficacy, time-consuming and has side effects [1]. Although efforts have been made to find new antibacterial agents, the development of new drugs still lags far behind the evolution of antibiotic resistance [2,3]. The current challenges in the field of antibacterial and phototherapy include that strong cell membranes provide shelter to protect bacteria from foreign substances, and single bactericidal functions is difficult to destroy the strong metabolic homeostasis of drug-resistant bacteria [4,11,12]. Photodynamic therapy (PDT) can quickly produce ROS to destroy the DNA, proteins, and membranes of bacteria without producing drug-resistant bacteria [5]. Photothermal therapy (PTT) will not cause invasive secondary injury and can effectively eradicate biofilm [6]. Scientists have explored multifunctional materials based on PTT and PDT antibacterial models, such as metal materials (Cd, Cr, Cu, Fe, Mn, Ni, Pd, Rh, and Ru) [7,8] carbon nanomaterials (carbon nanotubes, graphene, carbon dots and nanodiamonds) [9,10], aggregation-induced emission materials (metallacycle-based supramolecular) [11–13] and halo-fluorescein [14]. The antimicrobial models of PTT and PDT show a good application prospect in the field of sterilization and phototherapy.

Currently, the coronavirus disease (COVID-19) has become a severe crisis worldwide for human health and economic development [15]. It is good at camouflaging its alien identity in the human body, and it has an incubation period of 14 days. It enters the cells through an external protein that can deceive the body’s defenses. The virus rapidly and massively proliferates, which causes fever, endocrine disorder and the outbreak of reactive oxygen species (ROS) [16]. Under normal circumstances, an appropriate amount of ROS can promote immunity, repair and survival, which is beneficial for human health. However, a large amount of uncontrolled ROS will attack various organs of the human body and eventually cause human death. It is precisely because of the...
destructive power of COVID-19, which is based on its passage through cell defenses that cause metabolic disorders and the outbreak of ROS, that it is prone to cause untold damage and suffering. Therefore, if we can construct an “artificial virus” that can sterilize on demand as an antibiotic, it would be a good strategy to combat superbugs. As one of the most important biomacromolecules in living organisms, the trans-acting activator of transduction peptide (TAT) has a good ability to penetrate the bacterial cell membrane based on its rich positively charged amino acids and stable secondary structures [17,18]. Graphitic carbon nitride (g-C$_3$N$_4$) has aroused wide interest from researchers because it has fewer disinfection byproducts and is effective against antibiotic-resistant strains [19]. Cobalt (Co) nanoparticles can enhance photothermal conversion ability with an additional magnetically targeted function [20].

Herein, inspired by COVID-19, we synthesized an “artificial virus” (TCNCoT) composed of triple-shell porous graphitic carbon nitride loaded with Co nanoparticles (TCNCo) by template and in situ growth methods and coated with TAT through electrostatic self-assembly. As illustrated in Scheme 1, by mimicking the coronal morphology and the means of infecting of COVID-19 cells, TCNCoT displayed tentacle-like structures on its surface, successfully penetrated the bacterial cell membrane by overcoming the bacterial membrane bottleneck, and then released TCNCo with photothermal and photodynamic effects into the bacteria. A responsive battle was launched to realize efficient sterilization. Furthermore, due to its outstanding sterilization performance, this “artificial virus” was also successfully applied to treat actual river water disinfection and drug-resistant bacteria infected wounds in a mouse model.

2. Experimental

2.1. Chemicals and reagents

1,4-benzenedicarboxylic acid, cyanamide (98%), tetraethyl orthosilicate, triethylene diamine, cetyltrimethylammonium bromide, 1,2-bis (triethoxysilyl)ethane and cobalt nitrate hexahydrate were purchased from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). N-N-dimethyformamide (DMF) was purchased from Kelong Chemical Reagent Co., Ltd. (Chengdu, China). All chemical reagents were of analytical grade.

![Scheme 1](image-url)

Scheme 1. a) Schematic diagram of COVID-19 with coronal morphology infecting cells. b) Schematic diagram of “artificial virus” with coronal morphology infecting bacteria to achieve MRSA killing.

2.2. Synthesis of the “artificial virus” (triple-shelled graphitic carbon nitride@cobalt nanoparticles@transacting activator of transduction peptide, TCNCoT)

Triple-shelled SiO$_2$ nanoparticles were prepared by the method of Lu et al [21]. They were then annealed at 800 ºC for 4 h before being further used. First, triple-shelled graphitic carbon nitride nanoparticles (TCN) were synthesized according to the following steps: 2 g of cyanamide were added to 5 mL of deionized water. After stirring for 1 h, 0.1 g of triple-shelled SiO$_2$ was added to the solution that was stirred for another 48 h. The corresponding powder obtained by centrifuging and drying was calcined at 600 ºC for 4 h under argon gas atmosphere. Second, triple-shelled graphitic carbon nitride@cobalt nanoparticles (TCNGCo) were synthesized according to the following steps: 302 mg of 1,4-benzenedicarboxylic acid and 216 mg of triethylene diamine were added to 50 mL of dimethylformamide, followed by 60 min of stirring. Cobalt nitrate hexahydrate (260 mg) was added to the mixed solution. After vigorous stirring for 60 min, 2 g of triple-shelled graphitic carbon nitride was added to the mixed DMF solution under 24 h of magnetic stirring. After the solvent was removed, the solid powder was transferred to a quartz boat, heated for 8 h to 800 ºC under argon atmosphere and maintained at this temperature for 1 h. Finally, TCNCoT was obtained according to the following steps: TCNCo was obtained by removing the triple-shelled SiO$_2$ template by Na$_2$CO$_3$ solution (0.2 mol/L) at 60 ºC for 24 h. Transducing peptide (0.8 mg/mL) and TCNCo (0.8 mg/mL) were mixed, stirred at 25 ºC for 24 h, centrifuged and freeze-dried to obtain TCNCoT. The stability of TCNCoT was measured according to following steps: The TCNCoT were placed in PBS and water, respectively, and shaken at 180 rpm for 48 h. Then, the TCNCoT was recovered by centrifugation and freeze-drying. Repeated the above steps for 3 cycles.

The ultraviolet–visible absorption value and bactericidal efficacy of the corresponding TCNCoT were measured, respectively.

2.3. Characterisation

A transmission electron microscope (TEM, JEM-1230, Japan) and high-resolution transmission (HRTEM, Hitachi, Tokyo, Japan) were used to investigate the morphology of TCNCoT. X-ray photoelectron spectroscopy (XPS) and high-resolution XPS spectra were collected by an Axis Ultra DLD X-ray photoelectron spectrometer. X-ray diffraction (XRD) patterns were obtained by a Bruker D8 diffractometer with high-intensity Cu-K$_\alpha$ radiation. The zeta potentials were measured using a Zetasizer Nano-ZS instrument (Malvern ZEN3600, UK). Electron spin resonance (ESR) spectra were recorded using a JEOL JES-FA200 electron spin resonance spectrometer. PL emission spectra were measured using an FLS 980 series of fluorescence spectrometers.
2.4. Bactericidal activity measurement

The sterilization tests of TCNCoT were evaluated according to a previous method with slight modifications [22]. All the glassware and culture medium solution were autoclaved at 121°C for 30 min before the microbiological experiments [23]. First, bacterial cells were incubated in a nutrient solution at 37°C for 18 h and then centrifuged to remove metabolites. A cell count of approximately 10⁸ colony-forming units per milliliter (CFU/mL) was obtained by diluting the bacterial solution with phosphate buffer solution (PBS, 10 mM, pH 7.4). For each bactericidal experiment, 1 mg of catalyst powder and 5 mL of MRSA suspension were pipetted into a container. Then, the bactericidal experiment was irradiated by a commercial 300 W xenon lamp PLS-SXE300UV (Beijing Perfectlight Technology Ltd, China) equipped with an optical cut-off filter (λ > 420 nm), and the average power energy density of the irradiation was approximately 0.1 W·cm⁻² measured by a PL-MW2000 spectroradiometer (Beijing Perfectlight Technology Ltd, China). At a given irradiation time interval (0, 5, 10, 15, 20, 25, 30 min), 100 μL of tested suspension samples were spread on fresh LB agar plates, and incubated at 37°C for 24 h. The corresponding bactericidal activity of TCNCoT was calculated.

2.5. Bactericidal mechanism

Bacterial coagulate experiments were carried out with freeze-dried rabbit plasma (purchased from Beijing Land Bridge Technology Co., Ltd (Beijing, China)). The fluorescent-based cell live/dead test was recorded with a fluorescence microscope (Olympus IX71, Tokyo, Japan). Scanning electron microscopy (SEM, Hitachi S-4800, Japan) was used to investigate the apparent morphology of bacteria [24]. TEM measurements of MRSA were carried out according to the following steps: First, MRSA was fixed overnight with 4% glutaraldehyde. The MRSA suspension was washed with KH₂PO₄-K₂HPO₄ buffer (0.1 M) 3 times and fixed with 1% OsO₄ + 1.5% CsFe₆N₆ for 1 h. Then, 1% uranyl acetate was added to the MRSA solution for negative staining at 4°C overnight. Finally, bacterial cells were embedded in Epon and hardened at 60°C for 5 days, and corresponding ultrathin sections were used for TEM. The real-time temperature was measured by an infrared thermal imager (FLIR systems, Sweden).

2.6. Recyclable and reusable water disinfection test

The Weihe River water in Yangling, Shaanxi, China was chosen as representative water for recyclable and reusable water disinfection experiments (sampling time: December 2021). TCNCoT was added to Weihe River water at a concentration of 0.2 mg/mL, and it was irradiated with visible light for 5 min. Then, the TCNCoT was recycled by the magnet, and the corresponding number of bacteria was counted by the plate counting method.

2.7. Safety evaluation

Haemolysis and cell experiments were used to evaluate the safety of TCNCoT. Fresh mouse blood was obtained and the damaged red blood cells were washed clean with PBS. TCNCoT was added to intact red blood cells and incubated at 37°C for 4 h. The corresponding supernatant was taken and the absorbance was measured at 492 nm. In addition, NIH 3 T3 cells were incubated to the logarithmic phase and interacted with TCNCoT. Cell viability was calculated according to MTT methods.

2.8. In vivo wound healing test

Kunming mice (5-week-old) were randomly and equivalently divided into three groups. A wound was cut on the backs of mice. After dropping 100 μL of MRSA solution (10⁸ CFU mL⁻¹) onto the wound, the different materials were added and illuminated for 5 min by visible light on Day 1. Representative wounds were recorded and sectioned for observation. All the experimental operations involved in animals were reviewed and approved by the Animal Ethics Committee of Northwest A&F University. All rats were treated following the Laboratory Animal Care and Use Guidelines strictly.

2.9. Statistical analysis

All results were statistically evaluated by an SPSS software (SPSS 23.0 for windows, SPSS Inc., Chicago, IL). Data were expressed as mean ± standard deviation. Duncan’s test was used to evaluate the difference of results. The levels of significance were labeled with *p < 0.05, **p < 0.01, ***p < 0.001.

3. Results and discussion

3.1. Synthesis and morphology of TCNCoT

TCNCoT was successfully fabricated through three steps. First, triple-shell graphitic carbon nitride (TCN) was prepared with SiO₂ as the template and cyanamide as the precursor. Second, cobalt nanoparticles were grown on TCN in situ with cobalt nitrate hexahydrate as a precursor. Finally, TAT was assembled on the surface of TCNCo driven by electrostatic interactions to form TCNCoT (Fig. 1a). The morphology of TCNCoT was systematically investigated. TCN and TCNCo were similar in shape and appearance, both of which being triple-shell nanoparticles (Fig. 1b and c). Tentacle-like structures can be observed on the surface of TCNCoT, which were produced by membrane-intercalating peptides assembled on the surface of TCNCo (Fig. 1d). The lattice distance of 0.205 nm and the XRD peak of TCNCoT demonstrated that Co nanoparticles (d = 0.205 nm) were successfully grown on TCN (Fig. 1d, inset; Figure S1, Supporting Information). Element mappings and XPS spectra can capture N (corresponding to g-C₃N₄), Co (corresponding to Co nanoparticles), and O (corresponding to TAT) on the surface of TCNCoT (Fig. 1e; Figure S2, Supporting Information). The above results demonstrated that TCNCoT with coronal morphology was successfully synthesized. Ultraviolet–visible spectrophotometry was employed to further confirm the formation of TCNCoT. As shown in Fig. 2a, the absorption peak of the peptide occurred at 274 nm, and the absorption peak at 323 nm was the characteristic absorption peak of TCNCo. The characteristic peaks of TAT and TCNCo can be observed simultaneously in TCNCoT. Moreover, the zeta potential was examined to confirm the self-assembly mechanism and the composition of the assembly TCNCoT. As shown in Fig. 2b, TCNCo was negatively charged (-19.37 mV) and TAT was positively charged (22.28 mV). With TAT decoration, the assembled TCNCoT was positively charged (2.01 mV), demonstrating that the positively charged TAT was exposed outside the surface of TCNCo. This specific structure of TAT on the exterior surface will be beneficial for intercalation into the bacteria cell membrane. The high-resolution XPS spectra results were also consistent with the above findings (Fig. 2c and d; Figure S3, Supporting Information).

3.2. Bactericidal properties of TCNCoT

First, we tested the bactericidal performance of TCNCoT. Compared with g-C₃N₄ (5.36%, 20 min), TCNCoT showed the best bactericidal efficacy, and 99.99% of MRSA was killed within 20 min (Fig. 2e and g). We also compared TCNCoT with the reported photocatalysts, and found that the bactericidal efficacy of TCNCoT was better (Fig. 2e; Table S1, Supporting Information). To further confirm the bactericidal efficacy of TCNCoT, we employed SEM to characterize the morphology of MRSA. Fold changes and depressions appeared on the surface of bacteria with the extension of illumination time (Fig. 2b and i). Obvious folds and depressions were observed at 20 min, which was consistent with the above bactericidal performance. The coagulation test can be used to
detect the residue of live bacteria because live bacteria can produce coagulase to coagulate rabbit plasma [25]. The corresponding angle decreased from 33.3° (0 min) to 22.6° (20 min), indicating that there were no residual live bacteria after 20 min of visible-light irradiation (Fig. 2j). We further measured the fluorescent staining of MRSA at 20 min, and the results were consistent with the above phenomenon (Fig. 2k). In addition, the bactericidal efficacy of TCNCoT can be further enhanced by increasing the TCNCoT concentration (Fig. 2l). Interestingly, TCNCoT can also completely kill *E. coli* within 20 min, indicating that it has broad-spectrum bactericidal activity (Figure S4, Supporting Information). To prove the stability of TCNCoT, TCNCoT was placed into PBS and water for 48 h (shaking at 180 rpm), and was recovered by centrifugation and freeze-drying. This lyophilized TCNCoT was redisolved and followed the above steps for three consecutive cycles. The characteristic ultraviolet–visible absorption value and bactericidal efficacy of the corresponding materials were measured. The results showed that the characteristic absorption peaks (274 nm for TAT, 323 nm for TCNCo) and bactericidal efficacy were basically unchanged both in PBS and water (Figure S5, Supporting Information). Therefore, we could conclude that TCNCoT are stable.

### 3.3. Membrane-intercalating effect analysis

To explore the membrane-intercalating mechanism of TCNCoT, we assessed the representative substances and the morphology of the cell membrane in bacteria. Once the bacterial cell membrane was destroyed, the nucleic acid and total protein in the cytoplasm could be detected (Fig. 3a and b; Figure S5, Supporting Information). The intracellular ATP contents were quantified using ATP content test kit (Phosphomolybdic acid colorimetry) purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). In brief, adding standard solution, substrate solution, precipitant, chromogenic solution and terminator into corresponding bacterial suspension according to the instructions of the kit. The absorbance value was measured at 636 nm and the intracellular ATP concentration was calculated. The ATP concentration and membrane potential also decreased during the inactivation process as the bacteria died (Fig. 3c-d). TCNCoT made the bacteria leak more nucleic acid and protein than TCNCo and resulted in a rapid decline in ATP concentration and membrane potential, indicating that TAT could improve bacterial killing efficiency. To exclude the influence of illumination, TCNCoT was placed in PBS (0.01 M, pH 7.4) under dark conditions. A slight leakage of nucleic acid and total protein can be found over time, which can be attributed to the damaging effect of TAT on the bacterial membrane (Figure S7 and S8, Supporting Information). Meanwhile, TCNCoT also showed slight bactericidal activity under dark conditions (Figure S9, Supporting Information), which was consistent with ATP concentration and membrane potential measurements (Figure S10 and S11, Supporting Information). Therefore, it is inferred that TAT can cause the outflow of a small amount of cellular contents by destroying the cell membrane of MRSA.

To further confirm that TCNCoT successfully penetrated the bacterial cell membrane by overcoming the bacterial membrane bottleneck, the representative MRSA was fixed, sectioned and observed. We put TCNCoT into the neutral PBS containing MRSA and acted under dark conditions for 20 min together, in order to eliminate the influence of other effects on TCNCoT membrane penetration ability. Under the same conditions, MRSA without TCNCoT treatment was compared with the control group. As shown in Fig. 3e, f and i, the MRSA membrane of the control group was compact and had a complete and clear outline. The membrane of MRSA treated with TCNCoT showed obvious holes compared with that of the control group, which proved that TCNCo had the ability to cross the membrane barrier (Fig. 3g, h and j). However, the sterilization efficiency at 20 min was only 6.2% under dark conditions (Figure S9, Supporting Information), which was far less than that under visible-light irradiation (99.99%). This result demonstrated that the lethal death of MRSA required the cooperation of membrane-intercalation...
Fig. 2. Characterization and bactericidal performance of TCNCoT. a) Ultra-violet-visible absorption spectrum and b) zeta potential of TAT, TCNCo and TCNCoT. High-resolution XPS spectra of c) N 1 s and d) Co 2p. e) The statistical analysis of MRSA cell density at different irradiation time. f) Bactericidal performance comparison of TCNCoT with other reported photocatalysts. g) The growth of MASR colonies under irradiation from 0 min to 30 min. Evaluation of the bactericidal efficacy of TCNCoT: h) Schematic diagram of changes to bacterial morphology at different treatment time. i) Representative SEM images of MRSA and j) coagulase assay of MRSA treated with TCNCoT at different treatment time. k) Fluorescent images of live/dead MRSA, with PI (red) to show dead bacteria and DAPI (blue) to indicate viable bacteria. l) Comparison of the bactericidal efficacy of different concentrations of TCNCoT.
from TAT and other effects controlled by visible light.

### 3.4. Photothermal effect analysis

Next, we explored the photothermal mechanism of TCNCoT by investigating the temperature-rise performance of TCNCoT under visible-light irradiation. As shown in Fig. 4b and e, the temperature of TCNCo and TCNCoT gradually rose while g-C$_3$N$_4$ and TCN remained at room temperature with the extension of irradiation time, demonstrating that the photothermal properties of the materials were due to the existence of Co nanoparticles. We compared the photothermal performance of TCNCo and TCNCoT, and found that the maximum temperature of TCNCoT ($55^\circ$C) was higher than that of TCNCo ($50^\circ$C), which indicated that TCNCoT had a better photothermal effect than TCNCo. TCNCoT can still maintain excellent thermal properties after 3 cycles (Fig. 4c). It took 8 min to reach the maximum temperature and 25 min to fall to room temperature. Those numbers for TCNCo were 12 min and 8 min, respectively (Fig. 4d). Meanwhile, we found that the single photothermal bactericidal efficacy of TCNCoT was 18.1% through inactivating TAT (TCNCoT was treated at 121°C for 30 min) and adding ROS scavengers. We also tested the single photothermal efficacy of TCNCo (only 9.1%) by adding ROS scavengers (Figure S12, Supporting Information). The above two comparisons show that although TAT is inactivated, it can still act as a thermal insulation layer to improve the photothermal effect.

Therefore, we can conclude that the photothermal effect of TCNCoT...
is derived from the cooperation between the photothermal effect given by Co nanoparticles and the thermal insulation effect given by TAT (Fig. 4a). In addition, we also found that TCNCoT had concentration-dependent temperature characteristics with a maximum temperature of 60.3 °C (300 ug/mL, Fig. 4f). This characteristic is beneficial for further improving the bactericidal performance by increasing the concentration of TCNCoT.

Fig. 4. Photothermal mechanism of TCNCoT. a) Schematic diagram of photothermal generation and thermal insulation of TCNCoT. b) Temperature curves and c) photothermal pictures of TCNCoT with various irradiation times. Heat insulation effect of d) TCNCoT and e) TCNCo irradiated for three on/off cycles. f) Comparison of the photothermal performance of different concentrations of TCNCoT.
The photodynamic inactivation process of TCNCoT by the triple-shell structure and oxidase-like activity in an acidic environment was explored (Fig. 5a). As shown in Fig. 5b, TCNCoT can catalyze the discoloration of 3,3′,5,5′-Tetramethylbenzidine (TMB) at pH = 4–6, but TCNCoT cannot discolor TMB at pH = 7–8. These phenomena indicate that TCNCoT has excellent oxidase-like activity only under acidic conditions, which is conducive to targeting the bacterial acidic microenvironment. We chose the condition of pH = 6 to compare the oxidase-like activity of different materials because the pH value of the bacterial microenvironment was approximately 6 [26]. The results show that g-C₃N₄ and TCN did not discolor TMB, while TCNCo and TCNCoT could discolor TMB, indicating that the oxidase-like activity was derived from Co nanoparticles. The oxidase-like activity of TCNCo and TCNCoT were similar, which demonstrated that the addition of TAT had little effect on the oxidase-like activity (Fig. 5c). We also studied the stability of the oxidase-like activity of TCNCoT and found that TCNCoT had excellent enzyme activity stability, as revealed by the dark colour of TMB and absorbance (652 nm) after 9 days (Fig. 5e; Figure S13, Supporting Information). The oxidase-like activity showed an upwards trend with increasing TCNCoT concentration (Fig. 5d). To prove the contribution of the triple-shell structure of g-C₃N₄, we compared the bactericidal activity of g-C₃N₄ and TCN and found that the bactericidal activity of TCN was significantly better than that of g-C₃N₄ (Fig. 2e). Meanwhile, TCN showed weak photoluminescence (PL) intensity compared with that of g-C₃N₄ (Figure S14, Supporting Information), indicating that the triple-shell structure could improve the photocatalytic activity by enhancing visible-light harvesting and inhibiting the recombination of electron-hole pairs [27]. To explore the types of ROS in the whole process, we carried out an ROS scavenging experiment. The results showed that the addition of 1,4-benzoquinone and isopropanol significantly decreased the bactericidal efficacy of TCNCoT, and that anhydrous sodium sulfate had no influence on the bactericidal efficacy (Fig. 5f). This result indicated that -OH and -O₂ were produced during the inactivation process. We further tested ESR and found that there were signal peaks of 1:2:2:1 and 1:1:1:1, suggesting that -OH and -O₂ together played a role in the sterilization process (Fig. 5g and h). Therefore, we can conclude that the photodynamic effect of TCNCoT comes from the oxidase-like activity and triple-shell structure, which leads to an outbreak of ROS.

Pioneer researchers have systematically studied and discussed that photodynamic and photothermal synergistic sterilization can boost the sterilization efficiency [28–30]. They also explored some novel synergistic bactericidal strategies based on photothermal or photodynamic, and further revealed the synergy mechanism [31,32]. Inspired by these pioneering research work, we designed the bactericidal efficacy evaluation experiment under different treatments to verify the contribution of membrane intercalation, a photothermal effect and photodynamic effect. According to the above research results, we selected the photodynamic efficiency at 20 min for comparison. First, we tested the sterilization efficiency of TAT (11.3%) and TCNCo (40.7%) and found that this value (52%) was much lower than that of TCNCoT (99.9%) at the same concentration, demonstrating that TAT offered a synergistic bactericidal effect (Figure S15, Supporting Information). Second, we excluded the photothermal effect of TCNCoT by the flowing water bath, and the sterilization efficiency under this condition was 50.9%. Meanwhile, we measured the bactericidal efficiency (18.6%) of a single photothermal effect of TCNCoT by inactivating TCNCoT for 30 min at 121 °C and adding ROS scavengers to exclude photodynamic effects (Figure S16,
A synergistic sterilization with the photothermal effect that can be obtained from the sum of the above two efficiencies (69.5%) was <99.99%. Finally, we excluded the photodynamic effect of TCNCoT by adding an ROS scavenger, and the corresponding sterilization efficiency was 53.5%. TCNCoT stood at 121 °C for 30 min to inactivate TAT and then removed the photothermal effect of TCNCoT by flowing water. The sterilization efficiency was 15% (Figure S17, Supporting Information), which indicated synergistic sterilization of TCNCoT with a photodynamic effect, since the sum value (68.5%) was <99.99%. The above results fully demonstrate the synergistic sterilization of membrane intercalation, the photothermal effect and the photodynamic effect, which endows TCNCoT with super-bactericidal ability.

3.6. Recyclable and reusable water disinfection test

We evaluated the safety of TCNCoT by cell and hemolysis experiments. TCNCoT showed negligible toxicity even when the material concentration increased to 300 μg/mL (Fig. 6a; Figure S18, Supporting Information), which was revealed by the high cell viability (97.11%, 300 μg/mL) and low hemolysis ratio (1.39%, 300 μg/mL). It also displayed excellent magnetic recovery performance (Fig. 6b; Video 1, Supporting Information). The results of the circulating bactericidal test showed that the bactericidal activity of TCNCoT against MRSA could still reach 99.99% after three cycles (Fig. 6c). Considering the excellent bactericidal performance, magnetic recovery and stability of TCNCoT, we carried out a water sterilization experiment on Weihe River water. The process diagram of sterilizing river water and the subsequent magnetic recycling is shown in Fig. 6e and f. The concentration of bacteria in river water was approximately 10^5 CFU/mL (Fig. 6g), and TCNCoT can completely kill microorganisms in water within 5 min (Fig. 6h-i) because the actual number of bacteria in river water was far less than MRSA and E. coli (The original concentration were approximately 10^8 CFU/mL). In addition, TCNCoT also showed good circulation characteristics in the actual river water sterilization process, and sterilization efficiency still reached 99.19% after 3 rounds of recycling and reuse (Fig. 6j). Therefore, it has good applicability in recyclable and reusable environmental water sterilization based on its safety, excellent bactericidal activity and magnetic recovery characteristics.

3.7. In vivo wound healing

We further evaluated the in vivo wound healing performance of TCNCoT. As shown in Fig. 7a, we established a model of MRSA infection of the wound, and monitored the number of residual bacteria and the healing degree of the wound at different time points. We found that the wound area showed a trend of gradual reduction over time, and TCNCoT showed a faster wound healing effect compared with that of the control group (Fig. 7b and d). The residual area of the TCNCoT group was 15.8%, while the PBS and g-C3N4 groups were 70.55% and 48.65% on Day 8, respectively (Fig. 7g). We also monitored the changes in the bodyweight of mice during the healing process. The weight of the mice decreased in the first 2 days, which can be attributed to MRSA infection. The mice gradually gained weight after 3 days, with the TCNCoT group recovering the fastest among all groups (Fig. 7d). We further isolated the residual bacteria at the wounds and found that the bacteria in the TCNCoT group were the least abundant (Fig. 7h; Figure S19, Supporting Information), indicating that TCNCoT had an excellent bactericidal effect. To further evaluate the wound healing effect of TCNCoT, wounds on Day 3, 6 and 8 were taken as representatives for HE and Masson’s trichrome staining assays. The results suggest that thick new tissue, obvious hair follicles and an intact upper epidermis appeared in the TCNCoT group (Fig. 7e, i and j). Meanwhile, Masson staining revealed that the wounds in the TCNCoT groups produced a large amount of...
collagen (Fig. 7f). In addition, we also compared the five organs of mice among different groups to further verify the safety of TCNCoT. The results showed that there were no significant changes in the heart, liver, spleen, lung and kidney of mice treated with TCNCoT (Fig. 7c). Therefore, the above results fully proved that TCNCoT with excellent biocompatibility not only had excellent bactericidal properties but could also significantly accelerate wound tissue healing.

4. Conclusions

In summary, inspired by COVID-19, an “artificial virus” was fabricated for rapid circulating sterilization of river water and wound healing. This “artificial virus” had a strong infection effect produced by the membrane-intercalation ability of TAT, photothermal effect produced by the photothermal ability of cobalt nanoparticles and heat insulation and a photodynamic effect produced by triple-shell porous graphitic carbon nitride and the oxidase-like activity of cobalt nanoparticles. First, the photodynamic and photothermal effects of this “artificial virus” are controlled by visible-light irradiation. Second, the oxidase-like activity of this “artificial virus” is controlled by the acidic bacterial environment. Finally, this “artificial virus” is magnetic and can be enriched, targeted and recycled under the action of magnetic force. This “artificial virus” has demonstrated its feasibility in environmental and biomaterial fields. This work mainly provides a unique idea and attempt of “fighting poison with poison”.

Author contributions

Yongsheng Ni and Jianlong Wang conceived and designed the experiments. Yongsheng Ni, Jingyao Wang, Mengyi Wang and Qiaoling Wang performed the experiments. Hongqing Nie provided the assistance in software and methodology. Yongsheng Ni analyzed and arranged the

Fig. 7. Wound healing ability of TCNCoT. a) Schematic diagram illustrating the operation process. b) Representative wound photographs of the mice during the process of wound healing. Scale bar: 0.5 cm. c) Tissue section analysis of the heart, liver, spleen, lung and kidney. d) Traces of wound healing and body change of mice from 0 days to 8 days. e) HE staining images (red arrow: blood vessels; black arrow: hair follicles; blue double arrow: granulation tissue; and black docking arrow: epidermis). f) Masson staining images of the wounds. Scale bar: 200 μm. g) Statistics of wound area in different groups. h) Residual bacteria at wounds at different time points. i) Statistical graph of granulation tissue thickness and j) epidermis thickness of different groups on the 3rd, 6th, and 8th days.
data, wrote and reviewed the paper. Lizhi Liu, Jing Sun, Tianli Yue and Ming-Qiang Zhu gave guidance and assistance on experiments and editing. Jianlong Wang funded and supervised the experiment.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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