Full length article

**Controlled-temperature photothermal and oxidative bacteria killing and acceleration of wound healing by polydopamine-assisted Au-hydroxyapatite nanorods**

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**Abstract**

Since skin wounds are subject to bacterial infection and tissue regeneration may be impeded, there is demand for biomaterials that possess rapid bactericidal and tissue repair capability. Herein we report in situ promotion of wound healing by a photothermal therapy (PTT) assisted nanocatalytic antibacterial system utilizing a polydopamine (PDA) coating on hydroxyapatite (HAp) incorporated with gold nanoparticles (Au-HAp). The PDA@Au-HAp NPs produce hydroxyl radicals (\(\cdot\)OH) via catalysis of a small concentration of H\(_2\)O\(_2\) to render bacteria more vulnerable to the temperature change. The antibacterial efficacy against Escherichia coli and Staphylococcus aureus is 96.8% and 95.2%, respectively, at a controlled photo-induced temperature of 45°C which causes no damage to normal tissues. By combining catalysis with near-infrared (NIR) photothermal therapy, the PDA@Au-HAp NPs provide safe, rapid, and effective antibacterial activity compared to \(\cdot\)OH or PTT alone. In addition, this system stimulates the tissue repairing-related gene expression to facilitate the formation of granulation tissues and collagen synthesis and thus accelerate wound healing. After the 10-day treatment of skin wounds in vivo, PDA@Au-HAp group exhibits quicker recovery than the control group and both sterilization and healing are completed after the 10-day treatment.

**Statement of significance**

This study presents in situ promotion of wound healing by a low-temperature photothermal therapy (PTT) assisted nanocatalytic antibacterial system utilizing a polydopamine (PDA) coating on hydroxyapatite (HAp) incorporated with gold nanoparticles (Au-HAp). The PDA@Au-HAp NPs produce hydroxyl radicals (\(\cdot\)OH) via catalysis of a small concentration of H\(_2\)O\(_2\) to render bacteria more vulnerable to temperature change. After irradiation by 808 nm laser, the antibacterial efficacy against Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) is 96.8% and 95.2%, respectively, at a low photo-induced temperature of 45°C which causes no damage to normal tissues. In addition, this system stimulates the tissue repairing-related gene expression to facilitate the formation of granulation tissues and collagen synthesis and accelerate wound healing.

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**1. Introduction**

Microbial infection which can cause serious complications after surgery and during wound healing usually occurs at damaged tissues [1–4], and it is important to accelerate tissue regeneration in order to minimize the possibility of bacterial infection [5]. The
most widely used method to combat bacterial infection is antibiotics therapy [6–8], but overuse and abuse of antibiotics can generate bacterial resistance inevitably reducing the treatment effectiveness and even spurring the development of super bacteria [9,10]. Therefore, it is urgent to develop a new wound therapy with antibacterial effects.

Photothermal therapy (PTT) is based on the ability to convert light into heat to destroy microorganisms such as bacteria thermally [11–13]. PTT triggered by near-infrared (NIR) light usually requires a temperature of 50 °C or higher to denature proteins and kill microbes because cell damage such as apoptosis at a lower temperature (e.g., 45 °C) can be repaired, but the high temperature may cause inflammation and thermal damage to nearby tissues [14,15]. Therefore, a bactericidal strategy at a lower temperature is more desirable. Polydopamine (PDA) is well known for self-polymerization of dopamine to form surface-adherent nanocoatings on a wide range of materials [16]. In addition, PDA which has latent photothermal effects and excellent biocompatibility is a photothermal therapeutic agent that can reach 58 °C during cancer treatment in vivo and the chemical functional groups can be exploited [17,18]. Nevertheless, the high bactericidal temperature damages normal tissues and in fact, a simple antibacterial mechanism based on nanomaterials is not effective in eradicating bacteria totally [19,20].

As one of the most lethal reactive oxygen species (ROS), the hydroxyl radical (•OH) has high antibacterial activity and no bacterial resistance, because there is no detoxifying cell enzyme [21]. •OH can induce initial oxidative damage to cell wall and membrane and in combination with PTT, the damaged membrane has improved permeability and sensitivity to heat and the treatment time and side effects of PTT can be reduced [22,23]. The supporting role of H2O2 in dermal wound healing is subject to redox control and H2O2 is a relatively poorly reactive ROS, which allows it to migrate further from its site of generation to serve as a signaling molecule or second messenger [24,25]. Although H2O2 is a common antibacterial agent, the dismutant concentration of H2O2 of 0.5–3% may hinder wound healing and even damage normal tissues [26]. The use of V2O5, iron oxide, and graphene quantum dots (GQD) to catalyze the formation of ROS from H2O2 to enhance the antibacterial performance has been reported [27–29]. Au nanoparticles (NPs), widely used nanomaterials with attractive optical and electronic properties as well as excellent biocompatibility [30,31], have unique catalytic properties [32,33]. Hence, it is possible that AuNPs-based hybrid materials can catalyze the decomposition of small concentrations of H2O2 to generate hydroxyl radicals (•OH). Hydroxyapatite (HAp) is a widely accepted bioactive material, which can not only affect the cell behaviors but also form a phosphate layer to adsorb protein molecules to benefit cell adhesion, proliferation, and differentiation [34,35].

In this work, a bifunctional hybrid system composed of Au NPs in conjunction with PDA-assisted HAp (PDA@Au-HAp) is designed and prepared to accelerate tissue regeneration and disinfect wounds at the same time. The PDA@Au-HAp NPs not only promote the expression of related genes in NIH3T3 cells, but also facilitate the formation of granulation tissue and collagen synthesis to accelerate wound healing. Production of •OH via catalytic decomposition of H2O2 at a low concentration enhances the photothermal effects to achieve high antibacterial efficacy at a controlled temperature of 45 °C in a short treatment time so as to avoid damage to normal tissues. The mechanism is schematically illustrated in Fig. 1.

2. Materials and methods

2.1. Synthesis of hydroxyapatite nanorods

The hydroxyapatite nanorods (HAp) were synthesized by a hydrothermal method. Typically, the 0.5 M aqueous solution of Ca(NO3)2·4H2O and 0.3 M aqueous solution of (NH4)2HPO4 were mixed with a molar ratio of Ca:P = 1:1.67 and adjusted to a pH of 8.7 with 25% ammonia. The mixture was put in a Teflon-lined stainless steel autoclave and heated to 130 °C for 12 h. After the reaction, the precipitate was separated by centrifugation, then washed with deionized (DI) water three times until neutral, freeze-dried, and calcined at 400 °C for 5 h.

2.2. Synthesis of Au-HAp

The Au nanoparticles (NPs) were deposited on HAp by a deposition–precipitation method. 0.06 g of HAuCl4·3H2O were dissolved in 45 mL of DI water at a pH of 9 by adding 0.1 M NaOH under vigorous stirring. 0.75 g of HAp were added to the HAuCl4 aqueous solution and the pH was adjusted to 9 with NaOH. After vigorous stirring for 1 h, the product was heated to 65 °C and stirred for another 1 h. The precipitate was filtered and washed with DI water until it was free of chloride ions, dried at 60 °C overnight, and calcined at 500 °C for 3 h for further use.

2.3. Synthesis of PDA@Au-HAp

0.5 g of the as-prepared Au-HAp were stirred in dopamine containing 1 mg/mL tris-buffer solution (100 mL, 10 mM; pH 8.5) for 24 h. The sample was separated by centrifugation, washed with DI water three times, and dried at 40 °C under vacuum.

2.4. Measurement and characterization

A transmission electron microscope (TEM; Tecnai G20, FEI, USA) was used to examine the size and morphology of the materials. The crystallinity and purity were determined by X-ray diffraction (XRD, D8A25, Bruker, Germany) using Cu Kα radiation (λ = 1.54051 and 1.54433 Å) over the 2θ range of 10–80°. The chemical composition was determined by Fourier-transform infrared spectroscopy (FTIR, Nicolet IS10, USA) and UV–Vis absorption spectra were recorded on the JASCO V550 UV/visible spectrophotometer (JASCO International Co., LTD., Tokyo, Japan). X-ray photoelectron spectroscopy (XPS, Thermo Fisher Scientific 250Xi) was performed to determine the chemical states.

2.5. Peroxidase-like activity measurement

The peroxidase-like activity of Au-HAp and PDA@Au-HAp was investigated by catalytic oxidation of the peroxidase substrate 3, 5, 5’-tetramethylbenzidine (TMB) in the presence of H2O2. Oxidation of TMB by Au-HAp/PDA@Au-HAp in 25 mM phosphate buffer (pH 4.0) produced a blue color with major absorbance peaks at 370 nm and 652 nm. The experiments were carried out with 200 μg/mL Au-HAp and PDA@Au-HAp, and 0.8 mM TMB was added into the buffer solution (400 μL, pH 4.0, 37 °C) in the presence of H2O2 (50 mM).

2.6. Detection of hydroxyl radicals (•OH)

The •OH radicals generated from H2O2 catalysis with Au-HAp and PDA@Au-HAp were evaluated by monitoring the fluorescence (FL) of 2-hydroxy terephthalic acid (TAOH) arising from oxidation of terephthalic acid (TA) in the aqueous solution. TA was reduced to TAOH by •OH with the maximum FL peak at 435 nm. Eight solutions (TA, H2O2, Au-HAp, PDA@Au-HAp, TA+Au-HAp, TA+PDA@Au-HAp, TA+Au-HAp+H2O2, and TA+PDA@Au-HAp+H2O2) were investigated. 0.3 mL of the 200 μg/mL Au-HAp and PDA@Au-HAp aqueous solution, 0.3 mL of 1 mM H2O2 aqueous solution, and 0.3 mL of 5 mM TA aqueous solution were mixed with 0.3 mL of 1.0 M acetate buffer (pH = 4.0) or 0.01 M PBS buffer.
(pH = 7.0) and diluted with DI water to 3 mL. The final concentrations were 200 μg/mL, 100 μM, 500 μM, and 100 μM for the Au-HAp or PDA@Au-HAp, H2O2, TA, and acetate buffer, respectively. The mixture was gently shaken and stored at 37 °C for 12 h in the dark. Changes in the fluorescence emission at 435 nm were recorded.

2.7. Photothermal conversion efficiency (η) of PDA@Au-HAp

The photothermal conversion efficiency (η) of PDA@Au-HAp was calculated as follows. 500 μL of the PDA@Au-HAp aqueous dispersion with different concentrations (0, 50, 100, 200, 500, and 1000 μg mL⁻¹) were exposed to the 808 nm NIR laser for 10 min and the laser was turned off to let the solution cool to room temperature. The heating and cooling temperature patterns of one sample (200 μg mL⁻¹) were recorded on a thermal camera (FLIR, E50) and η was calculated according to Eq. (1):

$$\eta = \frac{hS(T_{\text{max}} - T_{\text{sur}}) - Q_0}{T_{\text{sur}}(1 - 10^{-\frac{s\tau_s}{S}})}$$

where h is the heat transfer coefficient, S is the sample container surface area, $T_{\text{max}}$ is the steady state maximum temperature, $T_{\text{sur}}$ is the ambient room temperature, $Q_0$ is the baseline energy input by the solvent and sample container without the PDA@Au-HAp, I is the laser power, and $A_{\text{808}}$ is the absorbance of PDA@Au-HAp at 808 nm. The value of $hS$ was calculated by Eq. (2):

$$\tau_s = \frac{m_cC_d}{hS}$$

where $\tau_s$ is the characteristic thermal time constant, $m_c$ is the mass of the PDA@Au-HAp solution (m_d) in g, and its heat capacity ($C_d$) is approximated to be 4.2 J g⁻¹ K⁻¹ (heat capacity of water). The heat energy ($Q_0$) of the sample container and solvent without PDA@Au-HAp were measured independently by Eq. (3):
\[ Q_0 = hS(T_{\text{max}} - T_{\text{surf}}) \]  

The time constant was \( \tau = 176.22 \) s based on the linear fit of the cooling period after 600 s vs. \(-\ln \theta\). Accordingly, the photothermal conversion efficiency calculated by Eqs. (3) and (1) was \( \eta = 13.28\% \).

### 2.8. Antibacterial experiments

Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) on the solid Luria-Bertani (LB) agar plate were transferred to 20 mL of liquid LB culture medium and grown at 37°C for 12 h under 180 rpm rotation. 500 µL of the diluted bacterial suspension (10^6 CFU/mL) were incubated with the LB culture medium containing PBS (control), H2O2, Au-HAp, PDA@Au-HAp, Au-HAp + H2O2, PDA@Au-HAp + H2O2. All the groups were processed under the same conditions with NIR irradiation (808 nm, 1.0 W cm\(^{-2}\)) for 10 min. The final concentrations of Au-HAp or PDA@Au-HAp, H2O2, and bacteria were 200 µg/mL, 100 mM, and 1.0 x 10^5 CFU mL\(^{-1}\). After incubation for 10 min, the solution was placed on a solid medium by the spread plate method and cultured for 24 h before counting the number of the bacterial colonies. The control experiments were performed in parallel.

**Antibacterial rate (%):**

\[
\text{Antibacterial rate} = \left(1 - \frac{\text{CFU}_{\text{experimental group}}}{\text{CFU}_{\text{control group}}}\right) \times 100\%
\]

After treatment with Au-HAp, PDA@Au-HAp and H2O2 for 8 h, the S. aureus and E. coli bacteria were harvested by centrifugation and washed with PBS. The bacteria were stained with PI (Propidium Iodide) and SYTO9 (LIVE/DEAD Backlight Bacterial Viability Kit) for 15 min and washed twice with PBS. The live and dead bacteria were then observed by fluorescence microscopy (IFM, Olympus, IX73).

### 2.9. Bacteria morphology

After the antibacterial tests, the bacterial suspension was drained completely and the adherent S. aureus and E. coli on the bottom of the 48-well plate were fixed with 2.5% glutaraldehyde for 2 h, dehydrated with alcohol of different concentrations of 30%, 50%, 70%, 90%, and 100% successively for 15 min, and dried in air prior to SEM observation.

### 2.10. Cell culture

The NIH3T3 cells were used to evaluate the cytotoxicity. The cells were cultured in 1% penicillin–streptomycin solution (HyClone) and MEM/EBSS (HyClone) supplemented with 10% fetal cells were cultured in 1% penicillin–streptomycin solution for 2 h, dehydrated with alcohol of different concentrations of 30%, 50%, 70%, 90%, and 100% successively for 15 min, and dried in air prior to SEM observation.

### 2.11. Cell viability

The in vitro cytotoxicity was measured using a 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyloxazolium bromide (MTT) assay of cellular activity on the NIH3T3 cells. The NIH3T3 cells (200 µL total volume per well) were seeded on 96-well plates and incubated for 24 h, the samples were added and incubated for 24 h. To determine the toxicity, the MTT assay with a concentration of 5 µg/mL was added into each well and incubated for 4 h at 37°C. The medium was discarded, and a dimethyl sulfoxide (DMSO) solution (200 µL) was added to each well. The plate was shaken for 10 min and the supernatant fluid was taken out to measure the optical density (OD) on a SpectraMax i3 Platform (Molecular Devices, California, USA) at a wavelength of 490 nm. The results were expressed as the percentage of cell viability and all the measurements were carried out in triplicate.

### 2.12. In vitro cell morphology and migration assay

The cells with a density of 1 x 10^4 cell/mL were cultured on 48-well plates for 24 h and rinsed three times with PBS (pH = 7.4). 4% formaldehyde was fixed to fix the cells at room temperature for 10 min. The cells were stained with FITC (YiSen, Shanghai) at room temperature for 30 min after rinsing with PBS (pH = 7.4) three times. Subsequently, the cells were stained with DAPI (YiSen, Shanghai) for 30 s. An inverted fluorescence microscope (IFM, Olympus, IX73) was used to observe the cell morphology.

In the cell migration assay, an in vitro scratch assay was conducted as previously reported. The cells were seeded on 6-well plates and cultured in the cell culture medium for 24 h to produce a confluent monolayer. A vertical scratch was created with a P1000 pipette tip. Each well was washed with 1 mL of the medium to remove all debris and smooth the scratch edge. 2 mL of the medium were added to each well and the concentration of HAp, Au-HAp, and PDA@Au-HAp was 200 µg/mL. After 24 h, the cells were examined for the morphology and photographed by a fluorescence microscope (IFM, Olympus, IX73). The relative wound area (%) = A/A0 × 100%, where the initial wound area (A0) and wound area after 24 h incubation (A) were determined using the ImageJ software.

### 2.13. Quantitative real-time polymerase chain reaction (qRT-PCR)

The total RNA was extracted using Total RNA Kit I (OMEGA) and quantified on the Nanodrop 2000 spectrophotometer (Thermo Scientific). The RNA samples (500 ng each) were reverse transcribed to cDNA using a PrimeScript RT Master Mix (TaKaRa, Japan) according to the manufacturers’ instructions. qRT-PCR was performed on a BIO-RAD CFX96 touch q-PCR system (CFX96, BIO-RAD) using SYBR Premix Ex Taq™ II (TliRNaseH Plus) (Takara). The gene expression of bFGF, COL I, and COL III was analyzed and the gene expression level of GAPDH was used for normalization. Quantification of the gene expressions was based on the comparative cycle-threshold method.

### 2.14. In vivo mice wound model and healing process

All the experimental protocols were approved by Hubei Provincial Centers for Disease Prevention & Control and the male Sprague-Dawley rats (2 months old; 250 ± 12 g) were obtained from Wuhan Centers for Disease Prevention & Control. The rats were individually raised in cages at a standardized temperature for 2 days and evenly divided into four groups with three rats in each group: PBS + NIR (control group, group I), H2O2 + NIR (group II), PDA@Au-HAp + NIR (group III), PDA@Au-HAp + H2O2 + NIR (group IV). Prior to surgery, anesthesia was administered intraperitoneally with 3% pentobarbital sodium solution (Sigma) at a dosage of 1.0 mL/kg. A wound of d = 6 mm (~28 mm^2) was made surgically on the right and left sides of the backbone. The wounds were infected with the bacterial suspension of 10 µL S. aureus with 1 x 10^6 CFU mL\(^{-1}\) and then 5 µL of 200 µg mL\(^{-1}\) of PDA@Au-HAp and 100 µM H2O2 solutions were dropped onto the wound area of the corresponding groups. After 10 min (for NIR treated groups), the wound area was irradiated with an 808 nm NIR laser (power density: 1 W cm\(^{-2}\)) for 10 min. The other four groups including PBS (control group), HAp, Au-HAp, PDA@Au-HAp were treated under the same conditions without NIR irradiation and bacterial suspension. A homemade Band-Aid consisting of sterile cotton covering the wound was changed at 24 h intervals. The wounds were photographed after 2, 5, and 10 days. To monitor the antibacterial activity in vivo, the samples in each group were collected from the wound area using a sterile swab and they were placed in 5 mL of the LB broth and shaken for 8 h at 37°C. 20 µL of the bacterial suspension of the culture
solution were collected and spread on the LB agar plate and incubated at 37°C for 24 h to form viable colony units. Finally, 1 mL of whole blood was collected from the rat for routine analysis on a veterinary automatic blood cell analyzer (Mindray BC-2800 Vet). The skin tissues were fixed with 10% formaldehyde before the histology analysis. Hematoxylin and eosin (H&E) staining was performed in the histological analysis and Masson’s trichrome staining was employed for collagen formation assessment.

2.15. Statistical analysis

All the data are evaluated as mean ± standard deviation based on at least three tests and contrasted with Kruskal–Wallis one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Synthesis and characterization of polydopamine-assisted Au-hydroxyapatite nanoparticles

The schematic of the synthesis of PDA@Au-HAp is shown in Fig. 2a. As shown in the TEM images (Fig. 2b), the average length of the HAp nanorods varies from 80 to 100 nm and the transverse dimensions of the nanorods are 20–30 nm. After hydrothermal treatment with HAuCl₄ (precursor of Au NPs), the Au NPs are homogeneously distributed in the HAp nanorods and the average size is 4.9 ± 0.9 nm (Fig. 2c) according to the statistical analysis of 200 AuNPs by TEM. Further covering of PDA does not change the shape of the Au-HAp nanorods significantly (Fig. 2d), but the transverse dimension increases slightly. The high-resolution image shows that the thickness of the polymer coating is about 15 nm. The interplanar spacing between 0.28 nm and 0.34 nm represents the (2 1 1) and (0 0 2) planes of hydroxyapatite and the Au NPs are loaded along the direction of (2 2 0) with an interplanar distance of 0.14 nm (Fig. 2e) [36,37]. The EDS maps disclose homogeneous distributions of Ca, P, and Au in the PDA@Au-HAp NPs (Fig. S1). The XRD patterns are shown in Fig. 2f. The diffraction peaks from the (0 0 2), (2 1 1), (1 1 2), and (3 0 0) planes correspond to the expected peaks of HAp (JCPDS: 09–0432) [38]. As shown in Fig. 2g, the absorption peaks at 563 cm⁻¹ and 602 cm⁻¹ in the FTIR spectra of the precipitated samples can be assigned to P-O bending of the phosphate group resulting from -PO₄ in HAp, and those at 1620 cm⁻¹ and 1550 cm⁻¹ stem from amide I and amide II, indicating strong adhesion between HAp and PDA coating.

Fig. 2. (a) Schematic illustration of the synthesis of PDA@Au-HAp by loading Au NPs and the modification of PDA on hydroxyapatite synthesized by hydrothermal method. TEM images of (b) HAp, (c) Au-HAp, (d) PDA@Au-HAp and corresponding size distribution of Au NPs of PDA@Au-HAp (scale bar = 100 nm); (e) HR-TEM image of PDA@Au-HAp (scale bar = 5 nm); (f) XRD, (g) FT-IR and (h) XPS analysis of HAp, Au-HAp and PDA@Au-HAp.
As shown in Fig. 2h, the N 1s signal is observed from PDA@Au-HAp and the intensity of the O 1s peak increases, indicating successful coating of PDA. Au-HAp shows the Au 4f signal, but it is almost not detected due to the covering by PDA. Fig. S2a shows the C 1s XPS spectra of PDA@Au-HAp. The C–C peak at a binding energy of 284.8 eV and C–N peak at a binding energy of about 285.6 eV confirm the presence of the PDA coating [41]. The fitted peaks at 399.1, 400, and 400.6 eV correspond to C–N, C–N and C–NH₃ in PDA, respectively (Fig. S2b) [42]. The peaks at 531.5 and 532.8 eV represent O–C and C–OH in PDA (Fig. S2c), respectively, further corroborating successful grafting of PDA on the surface of Au-HAp [43].

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.actbio.2018.07.030.

3.2. Peroxidase-like activity and photothermal properties

The peroxidase-like activity of Au-HAp and PDA@Au-HAp is evaluated in the catalysis of peroxidase substrates 3, 3'-5', 5'-tetramethylbenzidine (TMB) [44]. Both Au-HAp and PDA@Au-HAp catalyze oxidation of TMB in the presence of H₂O₂ (100 μM) and produce a blue color after reacting for 10 min (Fig. 3a). The inset picture shows the photographs of the reaction system H₂O₂ + TMB, Au-HAp + H₂O₂ + TMB and PDA@Au-HAp + H₂O₂ + TMB from left to right. Au-HAp + H₂O₂ + TMB shows a deeper color than PDA@Au-HAp + H₂O₂ + TMB, indicating that the former has better catalytic activity due to the hindrance of PDA between the Au NPs and catalytic system. As shown in Fig. 3b, the relative catalytic activity rises gradually with temperature and like other nanomaterial-based peroxidase mimics [45], the maximum temperature of Au-HAp under irradiation is 43 °C and so we cannot get data at over 45 °C. The activity of Au-HAp and PDA@Au-HAp depends on the concentration of H₂O₂ (Fig. S3a) and pH (Fig. S3b). Even at a pH of 3.0, the peroxidase-like activity of PDA@Au-HAp remains at about 30% compared to that at pH of 7.0, revealing high catalytic activity in the physiological pH range of 5.0–7.4. The catalytic activity of Au-HAp and PDA@Au-HAp in the presence of a small concentration of H₂O₂ (100 μM) is verified by oxidation of terephthalic acid (TA) which reacts with ·OH to form 2-hydroxyl terephthalic acid (TAOH) and exhibits fluorescence at 435 nm [46]. Fig. 3c shows the fluorescence change in the solution of Au-HAp, PDA@Au-HAp, TA, and H₂O₂. After reacting for 12 h, fluorescence at 435 nm increases indicating the presence of ·OH radicals. TA + Au-HAp + H₂O₂ and TA + PDA@Au-HAp + H₂O₂ exhibit obvious fluorescence at a pH of 7.4. As shown in Fig. 3d, the absorption peak at 548 nm observed from Au-HAp and PDA@Au-HAp indicates AuNPs. PDA@Au-HAp shows broad absorption compared to pure HAp and Au-HAp, especially in the near infrared (NIR) region. It has been reported that the π-π* transition of the polymeric backbone of the benzenoid ring contributes to the broad absorption, especially near the NIR region [47]. The inset picture shows color changes in the different samples. In comparison with the milky white solution, the color of Au-HAp suspension becomes light red and that of the PDA@Au-HAp suspension is dark brown due to modification of PDA. The photothermal behavior of these samples in PBS solutions is examined by excitation with the 808 nm NIR (1 W cm⁻²). As shown in Fig. 3e and f, at 200 μg/mL, the temperature of the PDA@Au-HAp PBS solution increases from 25.2 to 43.2 °C after irradiation for 10 min. In comparison, the temperature of pure PBS increases by only 0.5 °C and that of Au-HAp increases from 25.1 to 34.7 °C. As shown in Fig. S4, the photothermal conversion efficiency of PDA@Au-HAp is calculated to be 13.28% using the method developed by Roper et al. [48]. Meanwhile, the temperature change of PDA@Au-HAp at different laser power is shown in Fig. S5.

3.3. In vitro antibacterial evaluation

As shown in Fig. 4a and b, a reduced bacteria population is observed from PDA@Au-HAp + H₂O₂. The number of bacterial colo-

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**Fig. 3.** Relative catalytic activity of Au-HAp and PDA@Au-HAp after incubation for 10 min towards (a) different concentration and (b) different temperature under 808 nm irradiation. Inset shows the photograph of the reaction system H₂O₂ + TMB, Au-HAp + H₂O₂ + TMB, and PDA@Au-HAp + H₂O₂ + TMB from left to right (1 mM TMB, 200 μg/mL samples and 100 μM H₂O₂). (c) Formation of hydroxyl radical (·OH) determined at pH = 7.4 with TA as a fluorescent probe. Reaction conditions: 200 μg/mL samples, 100 μM H₂O₂, 0.5 mM TA, PBS (pH = 7.4) incubated for 12 h at 37 °C under dark conditions; (d) UV–vis-NIR absorption and digital images of HAp, Au-HAp, and PDA@Au-HAp dispersed in PBS. (e) Temperature changes and corresponding (f) Infrared thermography of HAp, PDA@HAp, Au-HAp, and PDA@Au-HAp irradiated by the 808 nm laser.
cies on PDA@Au-HAp is slightly less than that of the control group and that on Au-HAp is even less. A slightly decreased number of colonies is observed from the H2O2 group, and HAp shows poor antimicrobial characteristics. After exposure to 808 nm NIR for 10 min, the bacterial colonies decrease in large number on PDA@Au-HAp + H2O2, but those on PDA@Au-HAp decrease slightly. Because of the photothermal effect, the quantity of bacteria on Au-HAp also decreases and there are differences among the control, H2O2, and HAp.

As shown in Fig. 4c and d, after culturing without light for 20 min, the antibacterial ratios of PDA@Au-HAp against E. coli and S. aureus are 34.6% and 13.7%, respectively, compared to 45.8% and 25.1% on pure Au-HAp. It is probably due to covering by PDA which hinders contact with bacteria and reduces the toxicity of the Au NPs. However, in the presence of 100 μM H2O2, the ratios on PDA@Au-HAp + H2O2 group against E. coli and S. aureus are 84.2% and 50.2%, respectively, suggesting that PDA@Au-HAp catalyzes decomposition of H2O2 to produce OH to improve the bacterial resistance. Meanwhile, owing to the small H2O2 concentration, the H2O2 group only shows weak antibacterial characteristics and HAp itself has no obvious antibacterial activity. The concentration dependent antibacterial results are shown in Fig. S6. Fig. S6a and S6b show that a large concentration of H2O2 (above 10^{-2}) kills E. coli and S. aureus effectively because of OH, but PDA@Au-HAp exhibits negligible antibacterial effects towards E. coli and S. aureus (54.1% and 20.5%) even at the concentration of 1 mg/mL (Fig. S6c and S6d). The results show that peroxidase catalysis alone cannot produce the ideal bactericidal effects.

When the bacteria are incubated with PDA@Au-HAp + H2O2 for 10 min and then irradiated with 808 nm NIR for 10 min (Fig. 4c–d), the antibacterial rates increase to 96.8% and 95.2% for E. coli and S. aureus thus suggesting some synergy of the photothermal and catalytic effects. Nevertheless, PDA@Au-HAp only shows 46.3% and 23.2% antibacterial effects against E. coli and S. aureus under NIR irradiation and so a temperature of 45.2°C is not enough to kill the majority of the bacteria. The antibacterial effects of Au-HAp improve slightly because of the weak photothermal effect. The results show that S. aureus is more tolerant to temperature than E. coli. It is possibly because the Gram-positive S. aureus possesses a thick peptidoglycan layer (20–80 nm) which consists of amino acids, surface proteins, teichoic acids, and lipoids, whereas the Gram-positive bacteria E. coli lacks the outer membrane thus becoming more prone to external interactions through a the peptidoglycan layer.

The morphology and membrane integrity of the bacteria before and after the different treatments are examined by SEM. As shown in Fig. 5a and b, clear edges and smooth and intact cell walls are observed from the control group. After culturing with H2O2 or PDA@Au-HAp for 20 min, a few disruptions occur on the cell walls indicating that H2O2 or PDA@Au-HAp alone has little toxicity against E. coli. PDA@HAp and Au-HAp show almost no antibacterial activity even with NIR irradiation (Fig. 5a). However, after light exposure of PDA@Au-HAp + H2O2 for 20 min, the red arrow in the figure shows that the bacterial surface becomes rough and wrinkled because the generated OH oxidizes lipids and damages the cell membrane. After treatment with PDA@Au-HAp + H2O2 for 10 min and irradiation for another 10 min, the E. coli cells lose the cellular integrity completely as manifested by outflow of the cell matrix indicative of stronger antibacterial activity. As shown in Fig. 5b, the results for S. aureus are similar to those of E. coli. In
the control group, the S. aureus cells are spherical and smooth but in the presence of PDA@Au-HAp or H$_2$O$_2$, the membranes are damaged. The cell walls are rough and damaged after light irradiation and incubation with PDA@Au-HAp + H$_2$O$_2$ for 10 min. The results are consistent with those observed by optical spectroscopy. The antibacterial ability is also assessed by LIVE (green)/DEAD (red) kit fluorescent microscopy. Fig. 5a and b show that almost all the E. coli and S. aureus cells are viable after treated with Au-HAp and PDA@Au-HAp even with irradiation. In the presence of H$_2$O$_2$, only small red fluorescent signals are detected but after mixing PDA@Au-HAp with H$_2$O$_2$, the red spots increase, indicating better bactericidal effects. The results demonstrate efficient controlled-temperature PTT in the presence of a small concentration of H$_2$O$_2$.

3.4. In vitro wound healing evaluation

An in vitro scratch assay is conducted to determine the effects of PDA@Au-HAp samples on skin cell migration [52]. The NIH3T3 cells have a similar wound width after scratching with a pipet tip (Fig. 6a). The cells move toward the wound area and the scratch...
almost disappears from PDA@Au-HAp after 24 h. The wound area (Fig. 6b) decreases to 20.1% for PDA@Au-HAp compared to the control group (68.4%). HAp and Au-HAp show decreases of 38.4% and 37.3%, respectively, possibly because PDA benefits cell growth and adhesion [53]. The scratch assay indicates that PDA@Au-HAp promotes migration of NIH3T3 cells. As shown in Fig. 7c, the control (PBS), HAp, Au-HAp, and PDA@Au-HAp show reduced cell viability compared to the control after incubation for 1 and 3 days. The cytotoxicity of PDA@Au-HAp depends on the concentration (Fig. S7a). At 1000 μg/mL, more than 70% of the cells still survive. In addition, Fig. S7b shows that H2O2 at a concentration below 10^{-4} M has less influence on the NIH3T3 cells. When H2O2 is mixed with PDA@Au-HAp, the cell viability decreases rapidly but recovers three days later (Fig. S8).

Fig. 6. (a) In vitro scratch assay of NIH3T3 cells cultured under different conditions. (b) Quantification of the relative wound area after 24 h. (c) Relative viabilities of NIH3T3 cells incubated with Control (PBS), HAp, Au-HAp and PDA@Au-HAp for 1 day and 3 days. Data were presented as the mean ± standard deviations: *P < 0.05, **P < 0.01 and ***P < 0.001, n = 3.

Fig. 7. Wound healing-related gene expressions of (a) bFGF, (b) COL I and, (c) COL III with treatment of Control (PBS), HAp, Au-HAp, and PDA@Au-HAp. The data are generated by real-time PCR and presented as relative to control cells cultured in PBS at day 2 by using normalization against a GAPDH reference. The data are presented as the mean ± standard deviations: *P < 0.05, **P < 0.01 and ***P < 0.001, n = 3. (Concentration: HAp, Au-HAp, and PDA@Au-HAp: 200 μg/mL).
To further investigate the process of wound healing, the gene expression of the basic fibroblast growth factor (bFGF), type I collagen (Col I), and type III collagen (Col III) for NIH3T3 fibroblasts are analyzed by the quantitative reverse transcription polymerase chain reaction (qRT-PCR). Fig. 7a shows that for HAp, Au-HAp, and PDA@Au-HAp, the mRNA expressions of bFGF are higher than that of the control on the 5th and 10th day and reduced bFGF mRNA expression is observed on the 10th day. This provides evidence about extracellular matrix (ECM) reorganization and release of growth factors during this period [54]. PDA@Au-HAp shows the highest mRNA level because PDA promotes cell adhesion and migration. The cells cultured with HAp, Au-HAp, and PDA@Au-HAp show higher mRNA levels of COL I and COL III throughout the observation period than the control group (Fig. 7b–c), further suggesting that the hydroxyapatite-based materials enhance production of collagen fibers during healing. The enhanced gene expressions of COL I and COL III reveal enhanced recombination of the collagen network of PDA@Au-HAp.

Bacterial infection often occurs during wound healing. To evaluate the in vivo photothermal therapeutic effects, the real-time disinfection and wound healing process are investigated with an animal model under NIR irradiation. The experiments are divided into four groups: (I) PBS + NIR (control), (II) H₂O₂ + NIR, (III) PDA@Au-HAp + NIR, and (IV) PDA@Au-HAp + H₂O₂ + NIR. The traumas are photographed on days 2, 5, and 10. The results are shown in Fig. 10. All the groups show severe bacterial infection with ichors after the 2-day treatment. As shown in Fig. 10a, recovery of group (III) and group (IV) is better than that of the control after the 5-day treatment, especially PDA@Au-HAp + H₂O₂ + NIR. Moreover, the wounds in group (I) and group (II) show exudation of tissue fluids, whereas group (III) begins to scab and group (IV) has no purulent fluids after the 5-day treatment. At the end of the 10-day treatment, the wounds of the control and H₂O₂ + NIR groups do not recover well, but those of PDA@Au-HAp and H₂O₂ exhibit productive healing. The size of the wounds also shows the healing trend (Fig. 10b).

Fig. 8. (a) Representative skin wound photographs on days 0, 2, 5, and 10 (scale bar = 5 mm) and (b) Relative wound area of control (PBS), HAp, Au-HAp, and PDA@Au-HAp groups. The data are presented as the mean ± standard deviations: *P < 0.05, **P < 0.01 and ***P < 0.001, n = 3.
Fig. 9. H&E and Masson staining of wound tissues treated with control (PBS), HAp, Au-HAp, and PDA@Au-HAp on days 2, 5, and 10 (scale bar = 50 μm). Black rectangles, green arrows, blue arrows, cyan arrows, blue rectangles, and red rectangles represent disorder in the collagen fibers, dermal fibroblasts, newborn blood vessels, red blood cells, infected necrotic foci, and connective tissues, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Fig. 10. Assessment of wound healing accompanied with bacterial infection (scale bar = 5 mm). (a) Representative skin wound photographs induced by S. aureus on days 0, 2, 5, and 10. (b) Relative wound area of control (PBS), H2O2 + NIR, PDA@Au-HAp + NIR, and PDA@Au-HAp + H2O2 + NIR groups. The data are presented as the mean ± standard deviations: *P < 0.05, **P < 0.01 and ***P < 0.001, n = 3.
4. Conclusion

A controlled-temperature photothermal antibacterial therapeutic platform comprising PDA-assistance and Au NPs loaded HAp nanorods is designed and demonstrated. The Au-loaded HAp nanorods show excellent peroxidase catalytic activity and PDA has remarkable photothermal conversion efficiency. By taking advantage of the peroxidase catalytic and photothermal capability, the materials can kill both *E. coli* (Gram-negative) and *S. aureus* (Gram-positive) with higher efficacy in a short time at a low photothermal temperature of 45°C compared to the peroxidase catalytic process or separate photothermal therapy alone. The treatment thus avoids possible damage of normal tissues at a higher temperature. The PDA@Au-HAp promotes tissue reconstruction by improving the formation of granulation tissues and synthesis of collagens. The results reveal a safe, simple, effective, and rapid way to disinfect wound and expedite tissue regeneration and healing.

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