Light-responsive Au/TiO_2/PDA coating enhances the antibacterial performance of titanium implants

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Experimental

1 Materials

High-purity (99.99%) titanium (Ti) foils were supplied by Baoji Ti Industry Co. Ltd. Titanium-(IV) n-butoxide (TBT; 99%) were purchased from Alfa Aesar. Oleylamine (OM; 80−90%) and dopamine hydrochloride (98%) were acquired from Aladdin Industrial Inc. Cyclohexane (C_6H_{12}) and oleic acid (OA; 95%) were purchased from Sinopharm Chemical Reagent Co., Ltd. All the chemical reagents were at AR level.

2 Synthesis of Au NPs

Initially, CTAB-capped gold seed nanoparticles were prepared via the chemical reduction of chloroauric acid ($HAuCl_4$) using sodium borohydride ($NaBH_4$). Specifically, 7.5 mL of 0.1 M CTAB aqueous solution was mixed with 100 μL of 24 mM $HAuCl_4$, and the mixture was diluted to a total volume of 9.4 mL with deionized water. Subsequently, 0.6 mL of ice-precooled 0.01 M $NaBH_4$ was added to the above mixture under magnetic stirring conditions. Stirring was terminated after 3 min, and the resulting seed solution was statically stored at 30 °C for subsequent experimental use. It should be noted that the prepared gold seed solution is valid for use within 2–5 h after synthesis.

Gold nanoparticles with a particle size of 9 nm (9 nm-Au NPs) were fabricated by a seed-mediated growth approach. The growth solution was first configured with the following components: 500 mL of 0.1 M CTAC, 5.25 mL of 23.8 mM $HAuCl_4$ and 75 mL of 0.1 M ascorbic acid (AA). Thereafter, 6.7 mL of the aforementioned Au seed solution was introduced into the prepared growth solution to trigger the growth of gold nanoparticles, and the mixed system was incubated at 30 °C for 0.5 h. The obtained gold nanoparticles were further purified by centrifugation at 16000 rpm for 23 min; the centrifugal precipitates were collected and then redispersed in 500 mL of deionized water to form a uniform dispersion.

3 Qualitative characterization

The crystalline phases of the samples were characterized using X-ray diffraction (XRD, Rigaku SmartLab (3 kW), Japan). The microscopic morphology of the coatings was examined using transmission electron microscopy (TEM, JEM-F200, JEOL, Japan). The chemical states of the constituent elements were analyzed by X-ray photoelectron spectroscopy (XPS, Scientific K-Alpha, Thermo, USA). Electron paramagnetic resonance (ESR, EMXplus, Bruker, Germany) spectra were recorded using an ESR spectrometer. The surface wettability of all samples was evaluated by measuring the static water contact

angle with a contact angle meter (DSA30S, KRUSS, Germany) using ultrapure water droplets of 1 μL in volume.

4 Photothermal and photodynamic performance

A 300 W Xe lamp (CEL-PF300-T6, Beijing Zhongjiao Jinyuan Co., Ltd., China) was employed as a simulated solar light source. The emitted light passed through an integrated optical filter to conform to the AM 1.5 standard solar spectrum. This light source exhibited stable output performance over prolonged operation, thereby ensuring reproducibility and consistency of the experimental data. The UV-Vis-NIR absorption was investigated using an ultraviolet-visible-near-infrared spectrophotometer in the wavelength range of 300-900 nm.

To assess the PDT performance of the samples, sodium terephthalate was prepared by reacting terephthalic acid (TA, 0.5 mM) with sodium hydroxide (NaOH, 2 mM) solution. The samples were immersed in 3 mL of the resulting sodium terephthalate solution and irradiated after AM 1.5 irradiation for 10 min. The fluorescence spectra corresponding to the emission wavelength of 315 nm were recorded using a fluorescence spectroelectrochemical analyzer. The ESR was utilized to detect the generation of ROS, specifically ·OH, from Au/TiO_2/PDA@Ti under both AM 1.5 and non-right conditions. ·OH was captured using 5,5-dimethyl-1-pyrroline N-oxide (DMPO).

To evaluate the PTT performance, the samples were immersed in 1 mL of phosphate-buffered saline (PBS) and irradiated with a AM 1.5 light for 10 min. The temperature of each sample was recorded at 1-min intervals using an infrared thermal imaging camera, and the collected data were used to plot the heating profiles. Following irradiation, the samples were allowed to cool naturally to room temperature. This procedure was repeated for four consecutive cycles to assess the thermal stability and repeatability of the coating under multiple irradiation conditions.

5 In vitro antibacterial activity

5.1 Plate counting assay

Gram-negative(*E. coli*) and Gram-positive (*S. aureus*) were employed to evaluate the antibacterial activity of the samples. The antibacterial performance of the coatings was assessed using the plate count method. Specifically, 1 mL of bacterial suspension (10^5 CFU/mL) was added to each well of a 24-well plate containing the prepared sample. Samples assigned to the light-irradiated group were exposed to AM 1.5 light illumination for 10 min, with the temperature maintained below 50 °C throughout the process. Subsequently, each bacterial suspension was diluted 100-fold, and 100 μL of the diluted solution

was spread evenly onto agar plates. After incubation at 37 °C for 24 h, the number of bacterial colonies was counted, and the antibacterial efficiency was calculated using the following formula:

$$Antibacterial\ ratio = [(Ncontrol\ -\ Nsample)/Ncontrol] \times 100\%$$

5.2 Bacteria morphology

The morphological characteristics of bacteria attached to the sample surfaces were examined using scanning electron microscopy (SEM). The adhered bacterial cells were first fixed in 2.5% glutaraldehyde solution for 4 h at 4 °C. Subsequently, the samples were dehydrated stepwise in ethanol solutions of increasing concentrations (20%, 40%, 60%, 80%, 90%, and 100%), with each dehydration step lasting 15 min. After the dehydration process, the samples were dried and subjected to SEM observation to analyze bacterial morphology.

5.3 Fluorescent staining of bacteria

Bacterial biofilms were formed by continuous cultivation of *E. coli* and *S. aureus* for 48 h. Following biofilm formation, the sample surfaces were stained with 4′,6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI). In this staining scheme, DAPI marks both live and dead bacteria with blue fluorescence, whereas PI specifically stains dead bacteria with red fluorescence. After 15 min of incubation in the dark, the samples were rinsed thoroughly with PBS to remove residual dye and subsequently observed using a confocal laser scanning microscope (CLSM, Nikon, Japan).

6 In vitro biological assessment

6.1 Cell compatibility

MC3T3-E1 preosteoblastic cells were employed to assess the in vitro cytocompatibility of the samples. The culture medium consisted of α-modified minimum essential medium (α-MEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin solution. During the experiment, the samples were placed in 24-well plates, and 1 mL of cell suspension was added to each well for co-culture. The light-irradiated group was exposed to AM 1.5 light illumination for 10 min. The seeded samples were cultured in an incubator for 1, 3, and 5 days, with periodic replacement of fresh medium. Cellular proliferation and viability were evaluated using a Cell Counting Kit-8 (CCK-8) assay. Briefly, under dark conditions, the culture medium was removed, and 10% CCK-8 working solution was added to each well. After 2 h of incubation, 100 μL of the reaction solution was transferred to a 96-well plate, and the absorbance at 450 nm was measured using a microplate reader.

6.2 Hematologic compatibility

The hemocompatibility of the samples was evaluated using fresh rabbit blood. Whole blood was centrifuged at 1000 rpm for 15 min to separate erythrocytes, which were subsequently resuspended in PBS to prepare a diluted erythrocyte suspension. Each sample was placed in a 24-well plate, and 2 mL of the diluted erythrocyte suspension was added to each well. The positive control consisted of erythrocytes treated with sterile water to induce complete hemolysis, while the negative control comprised 2 mL of the diluted erythrocyte suspension without sample exposure. All samples were incubated at 37 °C for 2 h. After incubation, the suspensions were centrifuged at 1500 rpm for 10 min, and 100 μL of the supernatant was collected to measure the absorbance at 540 nm.

6.3 Coating stability

To evaluate the stability of the coating in a simulated physiological environment, the Au/TiO_2/PDA@Ti samples were immersed in simulated body fluid and placed in a 37 °C shaker for 14 days. The surface morphology of the samples before and after immersion was observed by SEM. Meanwhile, the simulated body fluid was collected on the 7th and 14th days, and the concentrations of Ti and Au elements in it were quantitatively analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) to further assess the chemical stability of the coating.

7 Statistical analysis

All the experimental data were from triplicate independent experiments and presented as mean ± standard deviation (SD). The statistical analysis was performed through SPSS by oneway ANOVA and levels greater than 95% were considered significant.

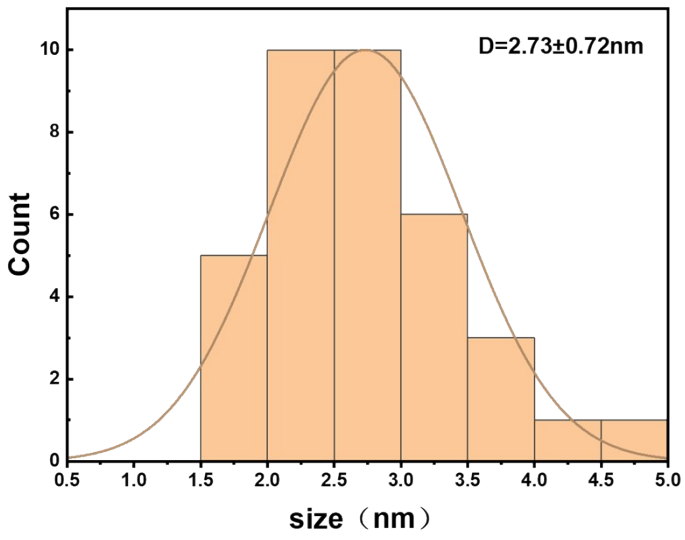

Figure S1 Size distribution histogram of TiO_2 QDs.

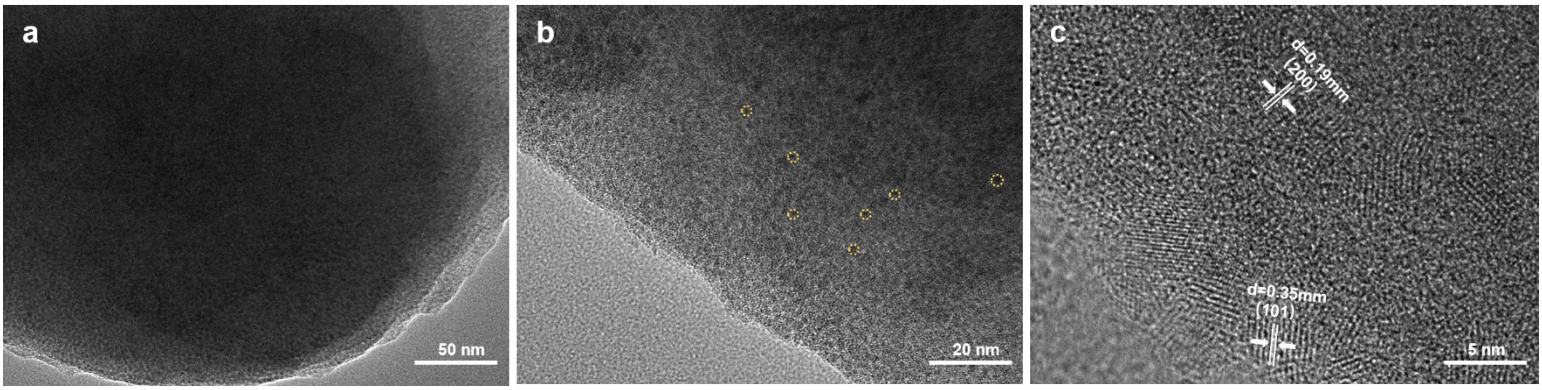

Figure S2 **(a-c)** HRTEM images of TiO_2 QDs.

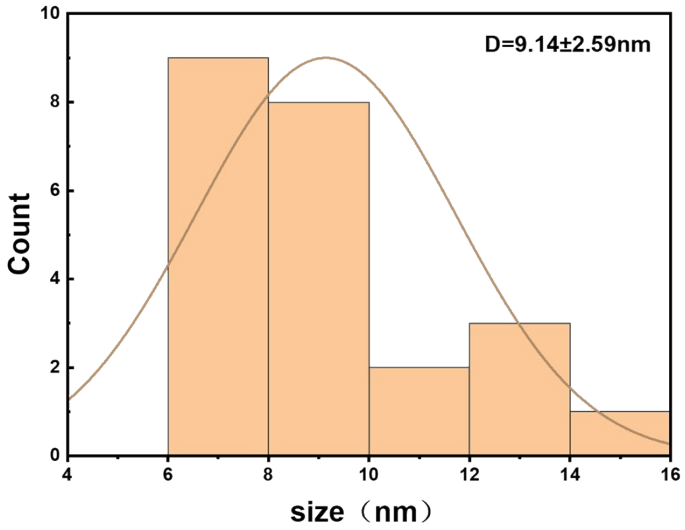

Figure S3 Size distribution histogram of Au NPs.

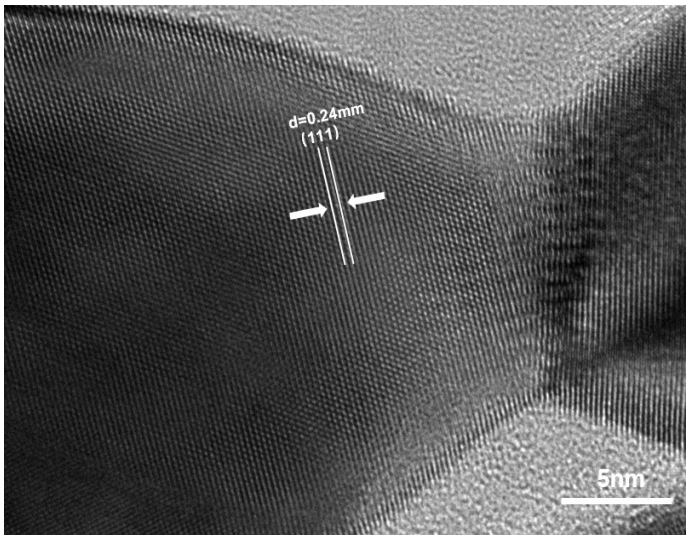

Figure S4 HRTEM images of Au NPs.

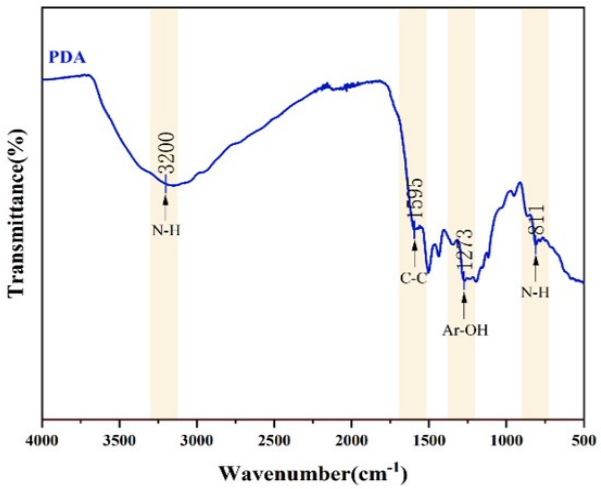

Figure S5 FTIR infrared spectra of PDA.

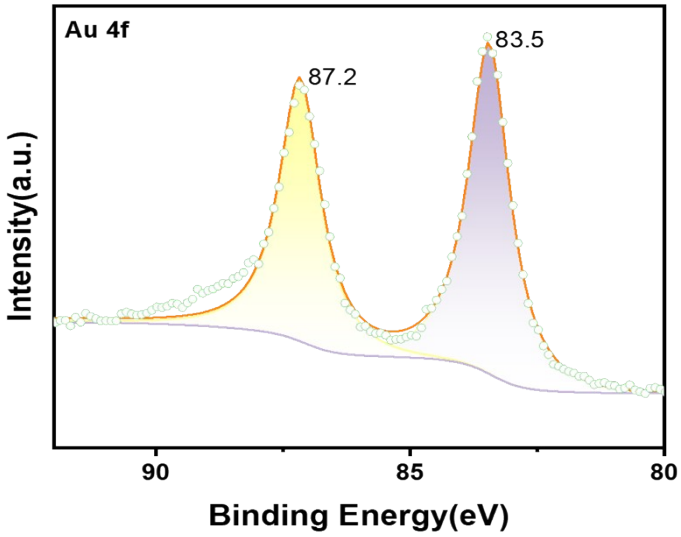

Figure S6 High-resolution XPS spectrum of Au 4f.

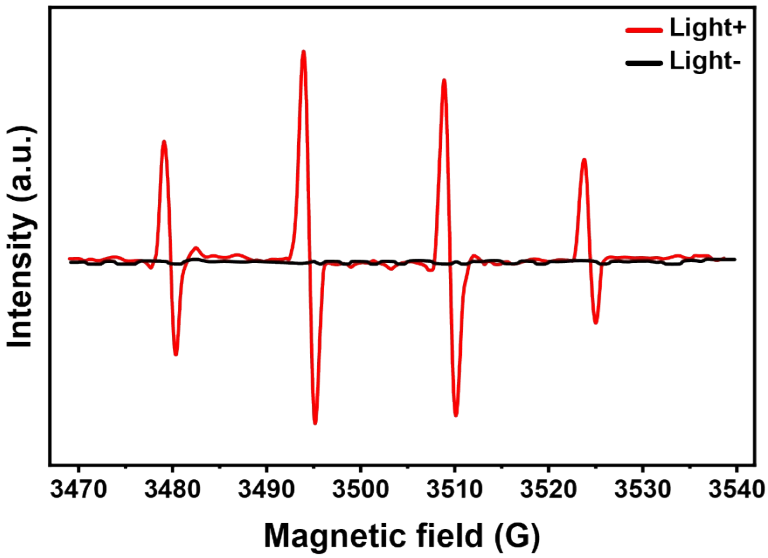

Figure S7 ESR spectra in the dark and under AM 1.5G light irradiation for Au/TiO_2/PDA@Ti samples.

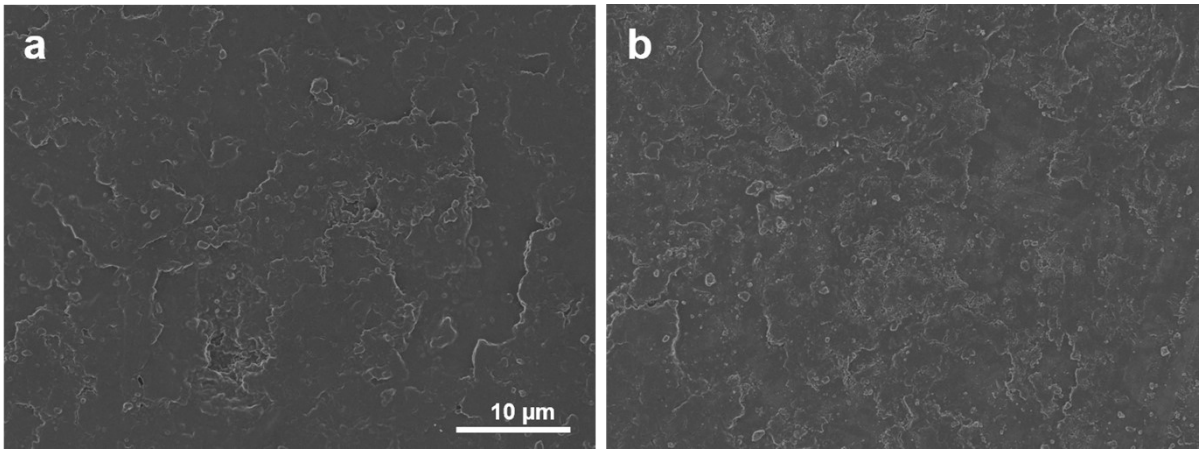

Figure S8 SEM images of the coating surface after 14 days of immersion.

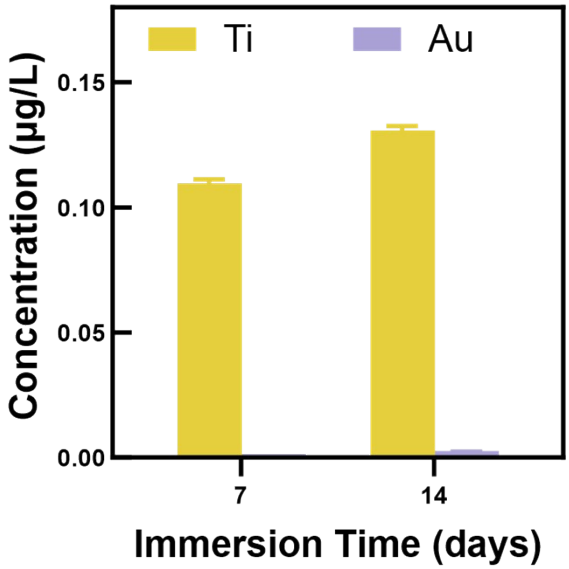

Figure S9 Ion release concentrations from the Au/TiO_2/PDA@Ti coating in simulated body fluid.