

New concepts:

Photothermal therapy (PTT) and photodynamic therapy (PDT) have garnered significant attention for cancer therapy. The effective delivery of photothermal conversion agents and photosensitizers is critical for synergistic PTT and PDT. We propose a novel implantable *in situ* therapeutic composite scaffold platform for synergistic PTT and PDT. The composite porous scaffold is implantable after surgical resection to effectively deliver photothermal conversion agents and photosensitizers to the desired sites. The composite porous scaffold has an excellent photothermal conversion ability and thermosensitive release of photosensitizers. The composite scaffold can effectively eliminate breast cancer cells through the synergistic effects of PTT and PDT and will provide a new treatment method for breast cancer.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author.

Thermosensitive Liposomal Nanomedicine-Functionalized Photothermal Composite Scaffolds for Light-Guided Cancer Therapy

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Abstract: In breast cancer treatment, the elimination of residual cancer cells in a sustainable and controllable manner to prevent recurrence remains a critical challenge in postoperative adjuvant therapy. In this study, a novel implantable in situ therapeutic composite scaffold platform (ALA@lipo/Au/Gel/PGA) was developed based on a porous scaffold composed of biocompatible gelatin and polyglutamic acid (PGA). This platform incorporated the photothermal agent Au nanorods (AuNRs) and thermosensitive liposomes encapsulated with the photosensitizer precursor 5-aminolevulinic acid (ALA). Due to the satisfactory photothermal conversion effect of the ALA@lipo/Au/Gel/PGA composite scaffold, the use of near-infrared (NIR) light not only ablated the cancer cells in the scaffold through photothermal therapy (PTT) but also induced the accelerated release of encapsulated ALA from the thermosensitive liposomes. After uptake, ALA could generate cytotoxic reactive oxygen species to increase tumour cell elimination efficiency *via* photodynamic therapy (PDT). Both *in vitro* and *in vivo* experiments demonstrated the synergistic anticancer effects of the composite scaffold. These results highlight the potential of this phototherapy-induced composite scaffold as a new synergistic treatment method for breast cancer.

Keywords: Liposome; Composite scaffold; Photothermal therapy; Photodynamic therapy

Introduction

Cancer is one of the most prevalent diseases worldwide and is characterized by high morbidity and mortality rates ^{1,2}. Traditional cancer treatment strategies primarily include surgical resection, chemotherapy and radiotherapy ³. However, chemotherapy and radiotherapy often induce drug or radiation resistance and compromise the immune system, leading to various acute and chronic adverse effects ⁴⁻⁶. Surgical intervention remains an effective method for the precise removal of tumour lesions; unfortunately, achieving complete eradication remains challenging due to the complexity of the anatomical structures surrounding tumours. Incomplete removal can result in residual cancer cells, subsequently increasing the risk of cancer recurrence ^{7,8}.

The limitations of conventional cancer treatments highlight the need for novel therapeutic approaches that minimize adverse effects while increasing efficacy. Integrating light-based strategies into cancer therapy offers precise positioning of the treatment area with a reduced operating risk ⁹. Currently, nanoparticle-based phototherapy, including photothermal therapy (PTT) and photodynamic therapy (PDT), has garnered significant attention as a promising strategy for tumour treatment ^{10,11}. PTT utilizes an excitation light source, primarily near-infrared (NIR) light, to irradiate photothermal conversion agents at tumour sites. The absorbed optical energy is then converted into thermal energy, leading to a localized temperature increase in the tumour tissue and inducing irreversible cellular damage through hyperthermia ^{12,13}. On the other hand, PDT relies on a light-activated photosensitizer that undergoes energy transfer or electron exchange to molecular oxygen or surrounding substrates upon irradiation. This photochemical reaction generates cytotoxic reactive oxygen species (ROS), which induce oxidative damage to proteins, lipids and DNA within tumour cells, ultimately triggering cell death ^{14,15}. These external light-driven mechanisms have garnered significant attention because of their minimally invasive nature, high selectivity, and cost-effectiveness ^{16,17}.

Nevertheless, the effective delivery of functional nanomaterials into the tumour site remains a major challenge. Common administration methods, such as the intravenous injection of nanoparticles, often result in rapid clearance by the immune system, imposing an additional metabolic burden^{18–20}. A substantial portion of these materials fail to reach the target tumour site, significantly reducing their therapeutic efficiency. Further research is needed to design drug delivery systems for the efficient delivery and utilization of photoreagents to development of more effective phototherapies.

Recently, the use of localized drug delivery systems, such as implantable scaffolds^{21–23} and injectable hydrogels^{24–26}, has garnered significant attention as a promising strategy for postoperative cancer treatment. By incorporating or conjugating functional therapeutic particles, these carriers can be directly implanted into the tumour cavity following surgical resection, enabling the precise, sustained delivery of therapeutic agents²⁷. This approach realized a streamlined "tumour resection–scaffold implantation–extracorporeal therapy" process, alleviating the need for additional invasive procedures and ultimately reducing patient suffering. Compared with systemic administration, localized drug delivery significantly reduces the required therapeutic dosage, allows for repeated *in situ* treatments and minimizes systemic drug exposure, thereby mitigating adverse effects^{28–30}.

In this study, an implantable light-activated localized drug delivery composite scaffold (ALA@lipo/Au/Gel/PGA) was designed to eliminate residual tumour cells. A controllable synergistic PTT-PDT approach was employed to maximize therapeutic efficacy while minimizing adverse effects (**Figure 1**). The composite scaffold was fabricated by hybridizing biocompatible polymers of gelatin and polyglutamic acid (PGA) with Au nanorods (AuNRs) and thermosensitive liposomes encapsulated with the photosensitizer prodrug 5-aminolevulinic acid (ALA). The AuNRs served as a photothermal agent to ensure efficient photothermal conversion for PTT and to control ALA release from the thermosensitive liposomes. ALA is a precursor of the photosensitizer PpIX, which is an endogenous metabolite produced *via* the Heme biosynthesis pathway³¹. The released ALA could enable sequential local photodynamic therapy to eradicate residual cancer cells. Upon irradiation with an 805 nm NIR laser,

the heat generated by the scaffold directly induced the thermal ablation of the tumour cells in the scaffold while simultaneously triggering the accelerated release of ALA from the thermosensitive liposomes. Subsequent light activation of the released photosensitizer facilitated photodynamic therapy, further enhancing the cytotoxic effects on distant cancer cells. Both *in vitro* cell culture and *in vivo* animal experiments revealed that the composite scaffold, upon exposure to specific light irradiation, could effectively eliminate residual cancer cells due to the synergistic effect of photothermal-photodynamic therapy.

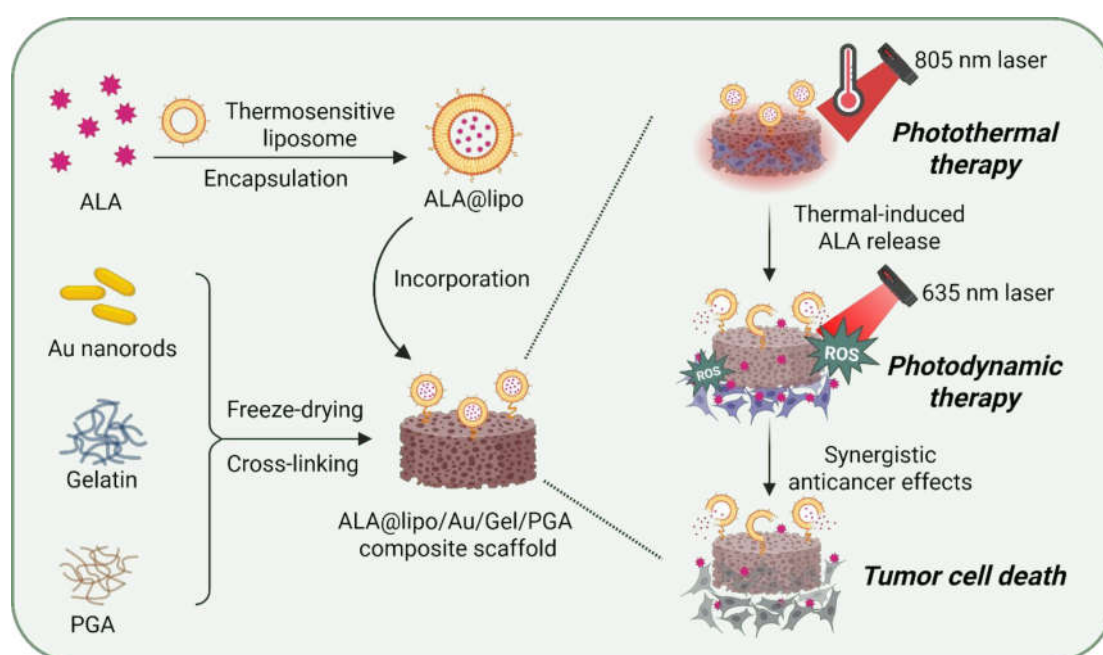


Figure 1 Schematic illustration of the preparation of the ALA@lipo/Au/Gel/PGA composite scaffold and its application in synergistic PTT-PDT for breast cancer treatment. The illustration was generated with BioRender (<https://app.biorender.com/>).

Results

Characterization of the AuNRs and liposomes

AuNRs were synthesized using a seed-mediated growth method. The morphology and optical properties of the AuNRs were characterized by transmission electron microscopy (TEM) and UV-Vis-NIR spectroscopy. As shown in **Figure 2A**, the synthesized AuNRs exhibited a uniform rod-shaped morphology. The size distribution

of AuNRs is shown in **Figure S1**. They had an average longitudinal length of 64.3 ± 4.2 nm and a transverse length of 15.6 ± 2.1 nm. The AuNR aqueous dispersion solution appeared dark red (inset in **Figure 2B**), the UV-Vis-NIR absorption spectrum displayed a distinct shoulder peak in the visible region (500-550 nm), corresponding to the transverse resonance along the short axis of the AuNRs (**Figure 2B**). As the wavelength increased, a strong and sharp absorption peak was observed in the NIR region, which was attributed to the longitudinal resonance along the long axis of the AuNRs³². This characteristic absorption peak indicated the potential application of the AuNRs in photothermal conversion.

The thermosensitive liposomes were prepared using a thin-film hydration method. The size distributions of liposomes without or with ALA encapsulation were analysed using DLS (**Figure 2C** and **2D**). The DPPC/Chol/DSPE-PEG-NH₂ liposomes and ALA-encapsulated liposomes exhibited a uniform size distribution, which was determined by the pore size of the polycarbonate membrane used during extrusion. They had average sizes of 207.2 ± 64.3 and 222.9 ± 71.9 nm, respectively. The results indicated that ALA encapsulation did not significantly alter the size of the liposomes, indicating the stability of the formulation. The ALA encapsulation efficiency within the liposomes calculate to be $35.7 \pm 6.2\%$.

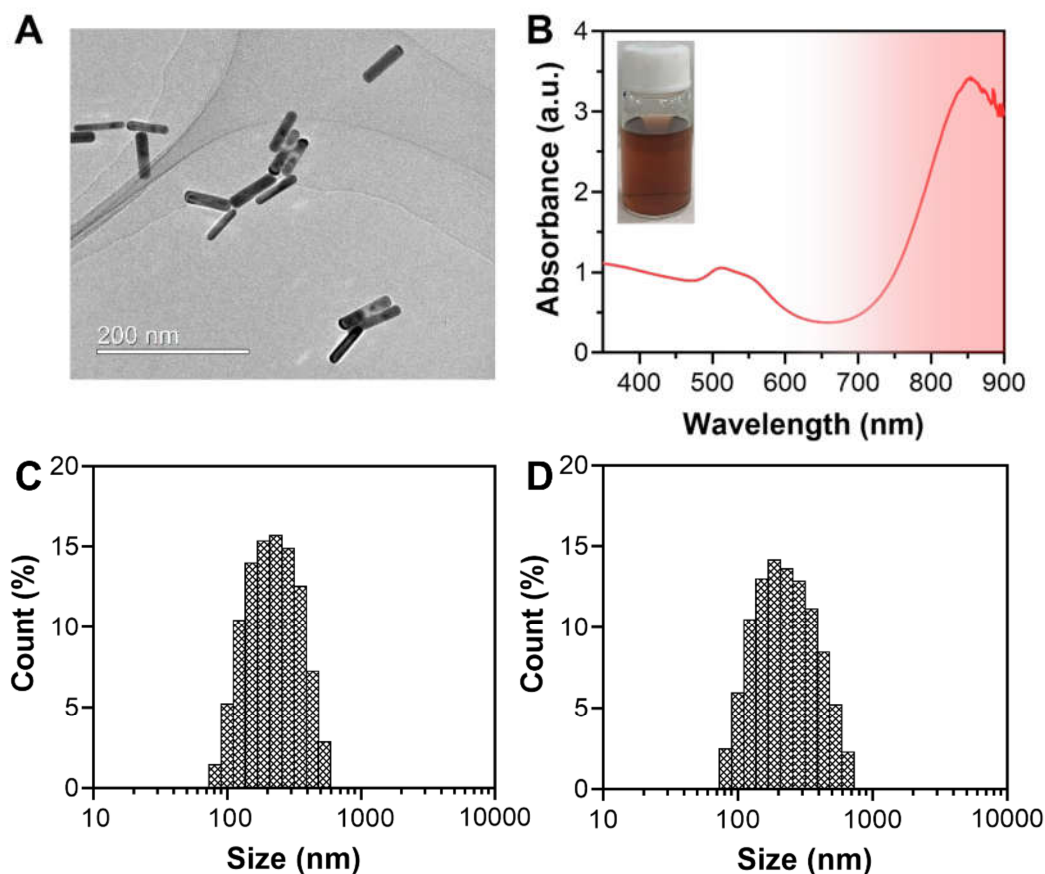


Figure 2 Characterization of the AuNRs and liposomes. (A) TEM image and (B) UV-Vis-NIR spectrum of the AuNRs. The inset shows the gross appearance of the aqueous dispersion of the AuNRs. Hydrodynamic size distributions of (C) DPPC/Chol/DSPE-PEG-NH₂ and (D) ALA-encapsulated DPPC/Chol/DSPE-PEG-NH₂ liposomes.

Characterization of the composite scaffolds

Scaffolds containing various therapeutic agents were prepared using ice particulates as porogen templates and gelatin/PGA as the base matrix. The AuNRs were incorporated during the initial fabrication process of the scaffolds, while ALA was encapsulated in DPPC/Chol/DSPE-PEG-NH₂ liposomes first and then the liposomes were immobilized in the scaffolds. The ALA-encapsulated liposomes were immobilized in the scaffolds *via* an EDC/NHS-mediated aqueous-phase coupling reaction, which formed amide bonds between the amino groups on the liposomes and the carboxyl groups in the scaffold matrix. This mild reaction was proceeded under an aqueous condition at room temperature, ensuring effective immobilization without compromising liposomal

integrity. The pore structures of the scaffolds were observed by SEM (**Figure 3A-D**). All the scaffolds exhibited similar spherical pores, which was attributed to the use of ice microparticles of a consistent size during scaffold fabrication. The large spherical pores were interconnected by numerous small pores (**Figure 3E-H**), which formed during the freezing processes and facilitated the slow growth of the fine ice crystals between the larger ice particulates. Upon freeze-drying to remove both the ice particulates and the fine ice crystals, this unique interconnected porous structure was achieved. The composite scaffolds without AuNRs appeared white, whereas the AuNR-embedded composite scaffolds presented a dark red colouration. High-magnification SEM images clearly revealed that the rod-shaped AuNR particles were uniformly distributed and adhered to the scaffold matrix (**Figure S2**), confirming the successful incorporation of the AuNRs into the Au/Gel/PGA composite scaffolds.

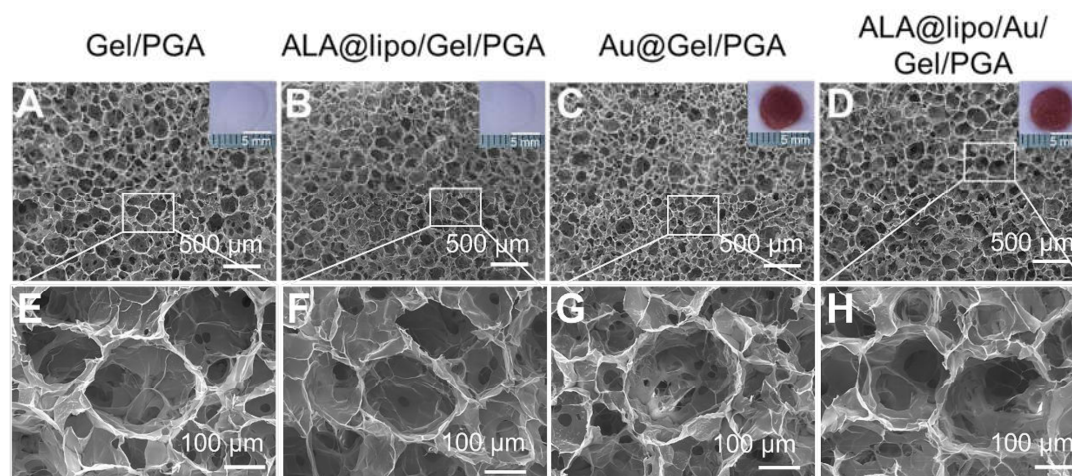


Figure 3 Characterization of the composite scaffolds. SEM images of (A, E) Gel/PGA, (B, F) ALA@lipo/Gel/PGA, (C, G) Au/Gel/PGA and (D, H) ALA@lipo/Au/Gel/PGA composite scaffolds at low (A-D) and high (E-H) magnification. The insets show macroscopic photographs of each composite scaffold.

Photothermal conversion and drug release performance of the composite scaffolds

An 805 nm laser was employed as the NIR light source to investigate the photothermal conversion properties of the composite scaffolds. The scaffolds were irradiated with an NIR laser at intensities of 1.5 or 2.0 W/cm² for 10 min. During NIR laser irradiation,

the Gel/PGA and ALA@lipo/Gel/PGA composite scaffolds exhibited only slow and slight temperature increases (**Figure 4A and S3A**). In contrast, the Au/Gel/PGA and ALA@lipo/Au/Gel/PGA composite scaffolds demonstrated rapid and significant temperature increases upon NIR laser exposure (**Figure S3B and 4B**). After 10 min of NIR laser irradiation at intensities of 1.5 and 2.0 W/cm², the temperatures of the ALA@lipo/Au/Gel/PGA composite scaffold reached to 59.0 °C and 64.1 °C, respectively. The temperature elevation was positively correlated with the laser intensity, indicating that a higher laser power resulted in more rapid and pronounced heating.

These results suggested that the Gel/PGA and ALA@lipo/Gel/PGA composite scaffolds lack photothermal conversion capabilities, rendering them ineffective for photothermal therapy. In contrast, the Au/Gel/PGA and ALA@lipo/Au/Gel/PGA composite scaffolds, owing to the incorporation of AuNRs as photothermal agents, exhibited excellent photothermal conversion efficiency. Therefore, the Au/Gel/PGA and ALA@lipo/Au/Gel/PGA composite scaffolds could effectively generate localized high-temperature environments upon NIR laser irradiation, thereby exhibiting great potential for photothermal therapy applications.

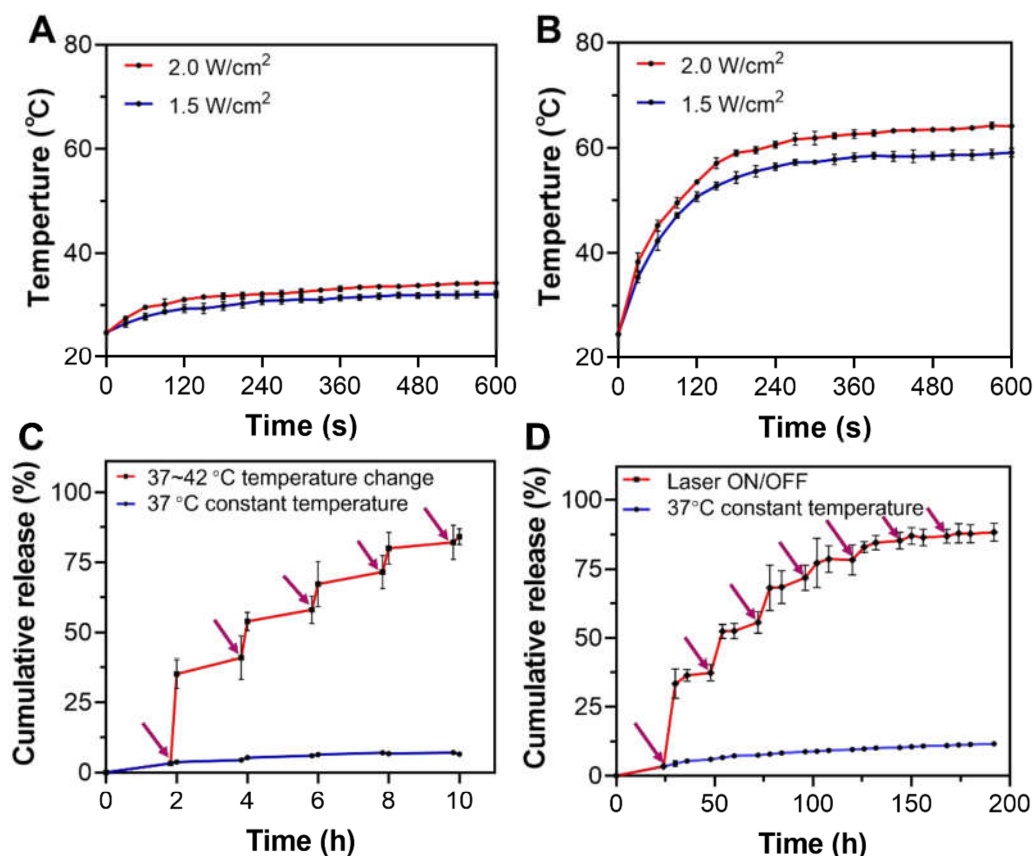


Figure 4 Curves showing the temperature change of the (A) Gel/PGA and (B) ALA@lipo/Au/Gel/PGA composite scaffolds after 10 min of 805 nm NIR laser irradiation at intensities of 1.5 and 2.0 W/cm². (C) Profiles of ALA release from the ALA-encapsulated liposomes with or without periodic temperature changes between 37 °C and 42 °C. (D) ALA release profiles from the ALA@lipo/Au/Gel/PGA composite scaffolds with or without periodic on/off cycles of 1.5 W/cm² 805 nm NIR laser irradiation. The downwards arrows indicate the time points at which the temperature increase or 805 nm NIR laser irradiation started. The data are presented as the means $\pm$ S.D.s (n = 3).

The profile of ALA release from the ALA-encapsulated DPPC/Chol/DSPE-PEG-NH₂ liposomes was evaluated at a constant-temperature of 37 °C in PBS or periodically alternating-temperature in PBS (37 °C for 110 min, 42 °C for 10 min in continuous cycles) (**Figure 4C**). The results indicated that ALA exhibited a very slow-release profile at the constant temperature of 37 °C in PBS, with only $6.5 \pm 0.7\%$ of the ALA

released after 10 h. Interestingly, when the release environment temperature alternated between 37 °C and 42 °C, the ALA release remained slow at 37 °C but increased significantly at 42 °C. During the first temperature change cycle, only $3.3 \pm 0.3\%$ of ALA was released after incubation at 37 °C for 110 minutes. However, upon heating at 42 °C for 10 minutes, $31.8\% \pm 5.1\%$ of the encapsulated ALA was released. During the subsequent temperature cycles, ALA release remained slow at 37 °C but accelerated at 42 °C. After 5 temperature cycles over 10 h, the cumulative ALA release reached $84.2 \pm 2.8\%$, which was significantly greater than the release observed in PBS at the constant temperature of 37 °C. The increased release upon heating could be attributed to the elevated temperature, which could induce the phase transition of the thermosensitive liposome membrane, thereby increasing its permeability and promoting the rapid leakage of the encapsulated ALA ³³.

ALA@lipo was loaded into the Au/Gel/PGA scaffold to prepare the ALA@lipo/Au/Gel/PGA composite scaffold. The loading efficiency of ALA@lipo in the scaffold was determined to be $49.6 \pm 6.9\%$. The profile of ALA release from the ALA@lipo/Au/Gel/PGA composite scaffold was investigated by incubating it in 37 °C PBS without or with periodic NIR laser irradiation. Periodic NIR laser irradiation was conducted using an 805 nm NIR laser at a density of 1.5 W/cm^2 for 10 min every 24 h. In the absence of laser irradiation, ALA exhibited slow release, with only $11.6 \pm 0.1\%$ of the ALA released over 192 h. Notably, laser irradiation significantly accelerated the release rate (**Figure 4D**). After the first 10-min laser irradiation cycle, $33.4 \pm 5.3\%$ of the ALA was released. In the subsequent laser irradiation on/off cycles, the ALA release rates were notably higher during the laser irradiation on periods and slower during the off periods. After seven irradiation cycles, the cumulative release reached $88.4 \pm 3.3\%$. These results indicated that the local temperature increase induced by NIR laser irradiation effectively accelerated ALA release from the ALA@lipo/Au/Gel/PGA composite scaffold. The increased release was attributed primarily to the photothermal effect of the AuNRs, which increased the local temperature during NIR laser irradiation. ALA release from the ALA@lipo/Au/Gel/PGA composite scaffolds could be accelerated or slowed by switching on or off the NIR laser irradiation.

***In vitro* anticancer effects of the composite scaffolds**

The anticancer effect of the composite scaffolds was first investigated by *in vitro* cell culture. The 635 nm laser was used as the excitation light source for PDT due to its good tissue penetration depth and effective activation capability. Transwell plates were used to simulate the cancer cells in the scaffolds and the cancer cells far from the scaffolds (**Figure 5A**). MDA-MB-231-Luc cancer cells were seeded in the composite scaffolds and cultured in the inserts of transwell plates, which simulated cells in the composite scaffolds. The cells were also seeded in the bottom wells of the transwell plates to simulate cells far from the composite scaffolds. First, the cell/scaffold constructs were removed from the inserts and subjected to 10 min of 805 nm NIR laser irradiation at an intensity of 1.5 W/cm². After irradiation, the cell/scaffold constructs were returned to the transwell inserts and incubated at 37 °C for 4 h. Subsequently, both the scaffold/cell constructs and the bottom wells of the transwell system were irradiated with a 635 nm laser at an intensity of 0.05 W/cm² for 10 min. This is one combination irradiation cycle (805 nm laser irradiation followed by 635 nm laser irradiation).

After the first cycle of combination irradiation, the intracellular ROS level of cells cultured in the composite scaffolds was evaluated using a ROS probe. As shown in **Figure S4A**, the cells cultured in the Gel/PGA scaffold did not produce any green fluorescence from ROS regardless of NIR laser irradiation at either 805 nm or 635 nm. Importantly, under the combination irradiation, the cells cultured in the ALA@lipo/Au/Gel/PGA composite scaffold exhibited strong green fluorescence. The fluorescence intensity of cells cultured in the ALA@lipo/Au/Gel/PGA composite scaffold was significantly higher than that of other conditions (**Figure S4B**). The results suggested that the photothermally released ALA could be internalized by cells and activated under 635 nm laser irradiation to generate ROS.

The combined irradiation procedure was repeated every 24 h for a total of two cycles. Finally, the viability of cells in the composite scaffolds was assessed *via* Calcein-AM/PI staining, where live cells emitted green fluorescence and dead cells emitted red fluorescence (**Figure 5B**). In the groups without any laser treatment, the cells in all the

scaffolds presented intense green fluorescence, and no red fluorescence was observed, indicating the high viability of the cells. These results suggested that all the scaffolds were biocompatible and did not inherently induce cell death. After the first 805 nm NIR laser irradiation, the cells in the Gel/PGA and ALA@lipo/Gel/PGA scaffolds maintained high green fluorescence, indicating that the absence of AuNRs had no photothermal effect. In contrast, red fluorescence was prominently observed in the Au/Gel/PGA and ALA@lipo/Au/Gel/PGA scaffolds, indicating significant cell death due to effective photothermal ablation induced by the AuNRs following 805 nm NIR laser irradiation. After the first combination irradiation cycle (805 nm laser irradiation followed by 635 nm laser irradiation), more dead cells were observed in the ALA@lipo/Gel/PGA composite scaffold. After the second combination irradiation cycle, more dead cells were observed, which was due to the synergistic effects of the AuNR-based PTT and ALA-based PDT.

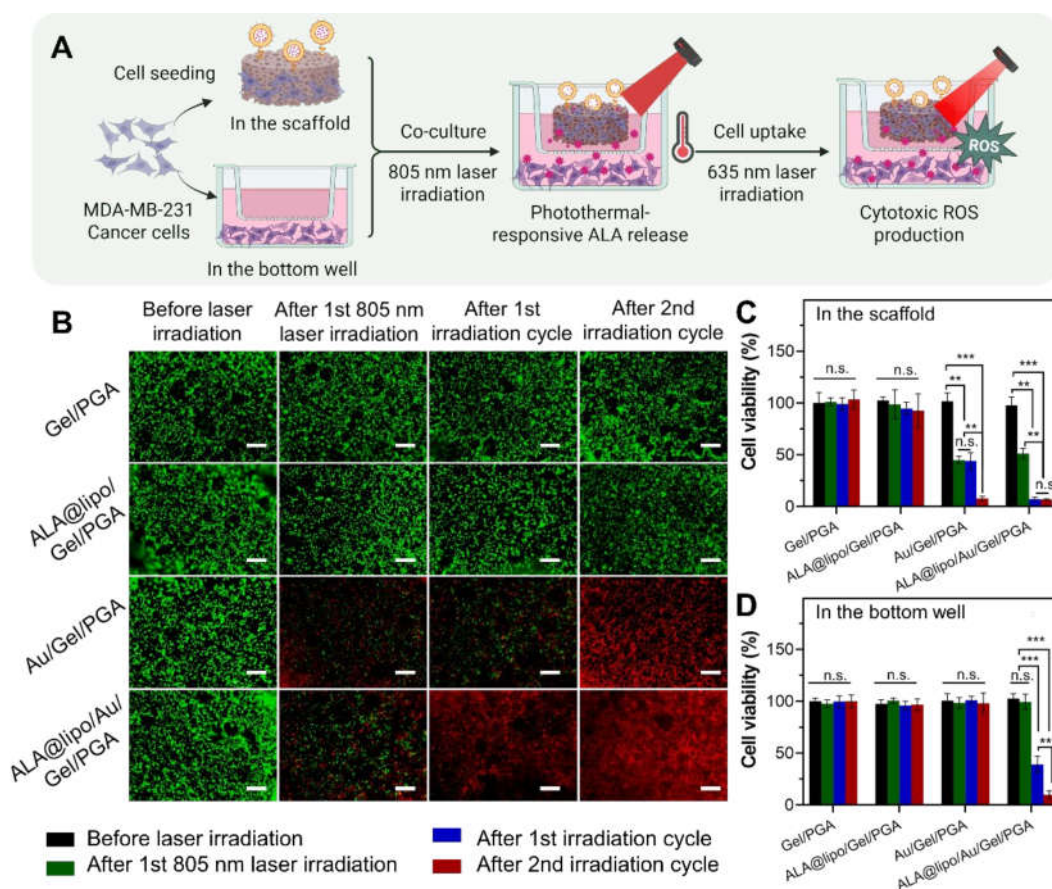


Figure 5. *In vitro* anticancer effects of the composite scaffolds. (A) Schematic

illustration of the *in vitro* cell culture experimental procedure. (B) Fluorescence images of Calcein-AM/PI-stained MDA-MB-231 cancer cells in the Gel/PGA, ALA@lipo/Gel/PGA, Au/Gel/PGA and ALA@lipo/Au/Gel/PGA composite scaffolds before laser irradiation and after the first 805 nm NIR laser irradiation, the first cycle of combination laser irradiation and the second cycle of combination laser irradiation. Green fluorescence indicates living cells, whereas red fluorescence indicates dead cells. Scale bar: 200 μm . The viability of MDA-MB-231 cancer cells cultured (C) in the composite scaffolds or (D) in the bottom wells of transwell plates before or after laser irradiation. The data are presented as the means $\pm$ S.D.s ($n = 3$). Significant differences: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; and N.S. = no significant difference

Cell viability was further quantified by a WST-1 assay (**Figure 5C and 5D**). Laser irradiation had no effect on the viability of the cells cultured in either the Gel/PGA composite scaffold or the corresponding bottom wells, which was likely due to the absence of either the AuNRs or ALA. The cells cultured in the ALA@lipo/Gel/PGA composite scaffold and its corresponding bottom wells were not affected by the laser irradiation either. This result is likely because there was no AuNRs in the ALA@lipo/Gel/PGA composite scaffold, and the amount of ALA released was insufficient without photothermal stimulation. Notably, after the 805 nm laser irradiation, significant cytotoxicity was observed in the Au/Gel/PGA composite scaffold. The viability of the cells on the Au/Gel/PGA composite scaffold decreased significantly due to the anticancer effect of the AuNR-based PTT. However, the cells in the corresponding bottom wells were not affected by the 805 nm laser irradiation because the cells in the bottom wells were far from the composite scaffold, and the AuNR-based PTT could not reach the distant cells in the bottom wells.

Interestingly, the viability of cells cultured in both the ALA@lipo/Au/Gel/PGA composite scaffold and the corresponding bottom wells decreased significantly after combination laser irradiation. After two cycles of combination laser irradiation, the viability of the cells in the ALA@lipo/Au/Gel/PGA composite scaffold decreased to a minimal level, which was due to the synergistic effects of the AuNR-based PTT and

ALA-based PDT. The viability of cells in the corresponding bottom wells decreased to a very low level, which was due to the PDT effect of the ALA released during the 805 nm NIR laser irradiation. The ALA@lipo/Au/Gel/PGA composite scaffold accelerated ALA release upon 805 nm NIR laser irradiation (**Figure 4D**). The released ALA could diffuse to the bottom wells to kill the cells following 635 nm laser irradiation.

***In vivo* anticancer effects of the composite scaffolds**

The *in vivo* anticancer effects of the composite scaffolds were evaluated using a whole-body bioluminescence *in vivo* imaging system (IVIS). The combination cycle of 805 nm NIR laser irradiation and 635 nm laser irradiation (4 h after 805 nm NIR laser irradiation) was repeated three times (**Figure 6A**). The temperatures of the Gel/PGA and ALA@lipo/Gel/PGA groups were slightly increased. In contrast, the Au/Gel/PGA and ALA@lipo/Au/Gel/PGA groups displayed rapid temperature increases due to the photothermal effects of the AuNRs. After 10 min 805 nm NIR laser irradiation, the temperature increased by 14.2 °C and 15.6 °C, respectively. The results confirmed that the Au/Gel/PGA and ALA@lipo/Au/Gel/PGA composite scaffolds maintained their high photothermal conversion efficiency after subcutaneous implantation in mice.

Bioluminescence imaging was performed before laser irradiation and after each laser irradiation cycle (**Figure 6D**). Before laser irradiation, strong bioluminescence signals were detected at the scaffold implantation sites in all the mice, confirming the viability of the MDA-MB-231-Luc cancer cells post-implantation. In the Gel/PGA scaffold group, bioluminescence remained high across all three laser irradiation cycles, indicating that the Gel/PGA scaffold lacked antitumour efficacy. The ALA@lipo/Gel/PGA scaffold group did not show a significant reduction in bioluminescence intensity. Although the ALA@lipo/Gel/PGA composite scaffolds slowly released ALA, the amount of ALA released was too low to achieve an anticancer effect during irradiation with a 635 nm laser. In mice implanted with the Au/Gel/PGA scaffold, bioluminescence signals in the central scaffold disc were gradually eliminated during the three laser irradiation cycles, indicating the effective photothermal ablation of cancer cells within the central region. However, strong bioluminescence signals

persisted in the surrounding gelatin ring, suggesting that the thermal effect generated by the central scaffold disc could not kill distant cancer cells in the outer ring. In contrast, mice implanted with the ALA@lipo/Au/Gel/PGA composite scaffold exhibited superior therapeutic outcomes. After the first laser irradiation cycle, the bioluminescence signals in the central scaffold disc diminished. After the second laser irradiation cycle, the bioluminescence signals in the central scaffold disc disappeared. Moreover, the bioluminescence intensity in the surrounding scaffold ring gradually decreased and was completely extinguished after three laser irradiation cycles. This result should be due to the synergistic effects of AuNR-based PTT and ALA-based PDT because ALA release can be accelerated during 805 nm NIR laser irradiation. The bioluminescence signal of mice was also quantitatively analysed. As shown in **Figure S5**, the mice implanted the Gel/PGA and ALA@lipo/Gel/PGA scaffolds showed a constant intensity of bioluminescent signal during the animal experiment period. The mice implanted with the Au/Gel/PGA composite scaffolds exhibited a decrease of bioluminescent signal after 3 cycles of combination laser irradiation, which was still strong. In contrast, the bioluminescent signal in the mice implanted with the Au/ALA@lipo/Gel/PGA composite scaffold decreased with time, and eventually disappeared after 3 cycles of combination laser irradiation. These results indicated that the ALA@lipo/Au/Gel/PGA scaffolds exerted excellent anticancer effects in both the central and peripheral regions under sequential 805 nm and 635 nm laser irradiation. NIR laser irradiation at a wavelength of 805 nm increased the local temperature and accelerated release amount of ALA that diffused to the surrounding scaffold ring. The diffused ALA in the surrounding scaffold ring killed the cancer cells upon 635 nm laser irradiation.

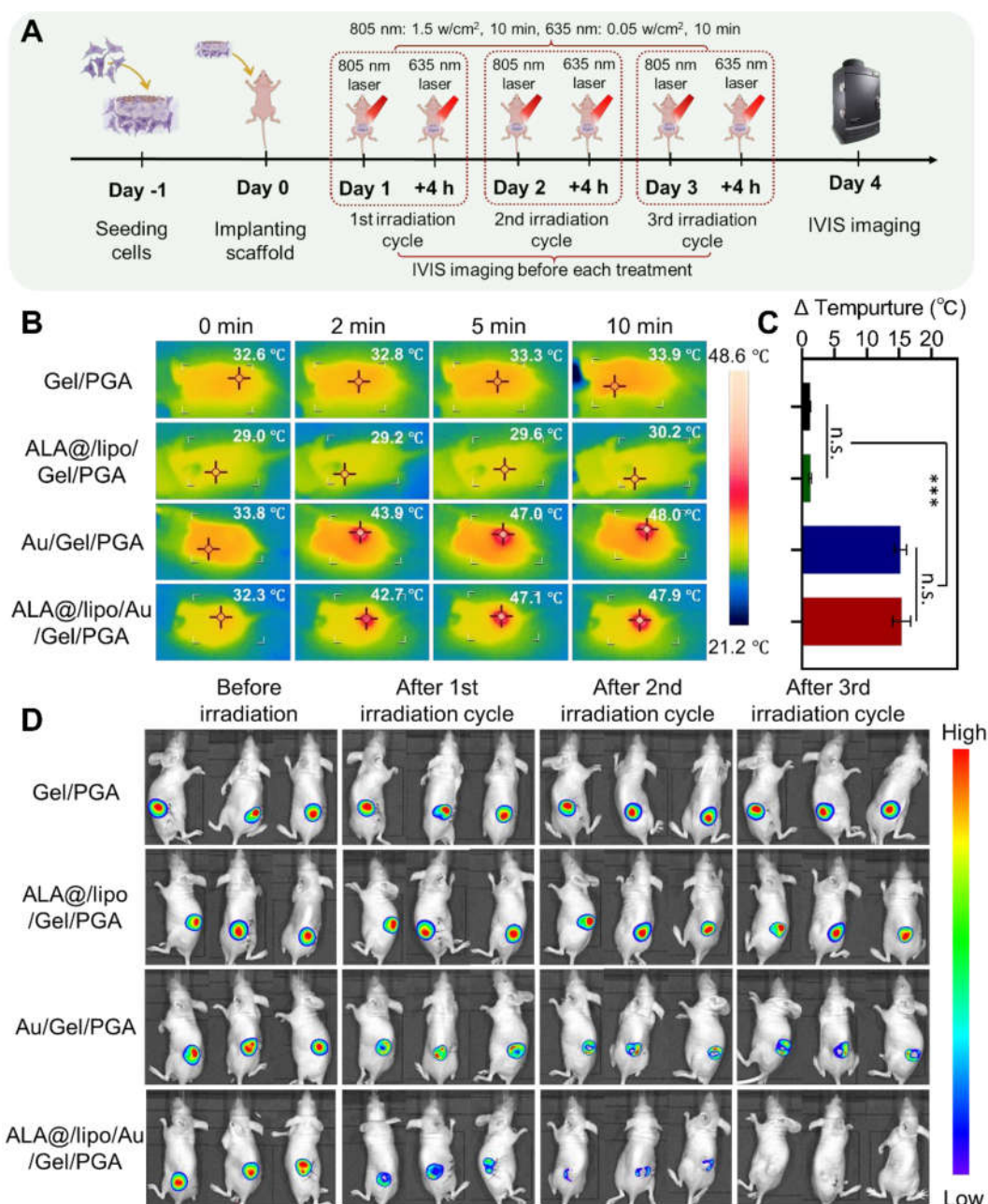


Figure 6 *In vivo* anticancer effects of the composite scaffolds. (A) Schematic illustration of the *in vivo* animal experimental procedure. (B) Thermographic images of the mice and (C) temperature increase at the irradiated sites of the mice. (D) Whole-body bioluminescence images of the mice before and after laser irradiation. The mice were subcutaneously implanted with the MDA-MB-231 cancer cell/scaffold constructs of the Gel/PGA, ALA@lipo/Gel/PGA, Au/Gel/PGA and ALA@lipo/Au/Gel/PGA composite scaffolds. The data are presented as the means $\pm$ S.D.s ($n = 3$).

The mice implanted with the ALA@lipo/Au/Gel/PGA composite scaffold were irradiated with 805 nm or 635 nm lasers individually to further evaluate the effect of the single treatment modalities. When the mice were subjected to 805 nm NIR laser irradiation alone, the bioluminescence signals in the central scaffold disc rapidly disappeared (**Figure S6A**). However, the bioluminescence signals persisted in the peripheral ring even after seven times of 805 nm laser irradiation. NIR laser irradiation (805 nm) could generate heat to increase the local temperature in the central scaffold disc to ablate the cancer cells in the central scaffold disc. However, the thermal energy dissipated too quickly to reach and ablate the distant cells in the surrounding scaffold ring. Conversely, when the mice were subjected to 635 nm irradiation alone, the bioluminescence signals remained even after seven times of irradiation (**Figure S6B**), highlighting that the slow ALA release from the ALA@lipo/Au/Gel/PGA composite scaffold without high-temperature stimulation was insufficient to achieve photodynamic therapy. The quantitative analysis of bioluminescent signals in different groups before and after laser irradiation was also performed, revealing trends consistent with those observed in the bioluminescence images (**Figure S6**).

Fifteen days after implantation, all the mice were euthanized to harvest the implanted scaffolds (**Figure S7**). The scaffolds maintained their original size and shape, the presence of surrounding connective tissue and neovascularization was observed, indicating good biocompatibility after implantation. These results conclusively showed that the ALA@lipo/Au/Gel/PGA composite scaffold, when subjected to stepwise 805 nm and 635 nm laser irradiation, exhibited superior synergistic photothermal–photodynamic antitumour effects. The extent of breast cancer cell ablation achieved with the combined laser irradiation was significantly greater than that achieved with either PTT or PDT alone, emphasizing its potential for comprehensive and effective cancer therapy.

Discussion

External radiation-driven phototherapy has been regarded as a spatiotemporally controllable treatment strategy for cancer, offering minimal invasiveness and high

selectivity³⁴. In this study, we developed a multifunctional platform of a photothermal composite scaffold integrated with photosensitizer-encapsulated thermosensitive liposomes (ALA@lipo/Au/Gel/PGA composite scaffold). The photothermal agent (AuNRs) was directly embedded into the scaffold, while the photosensitizer prodrug-encapsulated liposomes were anchored to the scaffold via amide bonds, enabling synergistic photothermal and photodynamic functions.

AuNRs, known for their excellent photothermal conversion capabilities, endowed the ALA@lipo/Au/Gel/PGA composite scaffold with superior photothermal properties. The temperature of the ALA@lipo/Au/Gel/PGA scaffold rapidly increased under 805 nm NIR irradiation, reaching 57.6 °C and 62.0 °C after 10 min at intensities of 1.5 and 2.0 W/cm², respectively. ALA, a precursor of the photosensitizer PpIX for PDT, provided the composite scaffold with photodynamic property. The ALA was encapsulated in thermosensitive liposomes for controlled release by 805 nm NIR irradiation. DPPC, with a phase transition temperature of ~42 °C, was used as the main lipid component of the thermosensitive liposomes. At normal body temperature (37 °C), the amount of ALA released was minimal, with only 11.6 ± 0.1% released after 8 days in PBS. However, under 805 nm NIR laser irradiation, the ALA release rate was accelerated significantly, with 88.4 ± 3.3% released after seven times of 805 nm NIR laser irradiation. The ALA@lipo/Au/Gel/PGA scaffold served as an efficient laser-triggered drug delivery system upon 805 nm NIR laser irradiation.

This multifunctional platform offered three key therapeutic advantages. First, the scaffold can be implanted post-surgery, ensuring the localized retention of nanoparticles and drugs at the tumour site. Second, upon 805 nm NIR laser irradiation, the scaffold induced effective photothermal ablation of residual cancer cells. Finally, the high-temperature environment generated by photothermal heating triggered the quick release of ALA from the thermosensitive liposomes, enabling PDT upon subsequent 635 nm laser activation, leading to efficient cancer cell eradication.

The *in vitro* and *in vivo* anticancer experiments showed that the ALA@lipo/Au/Gel/PGA composite scaffold produced distinct therapeutic results compared with those of the controls (Gel/PGA scaffold, Au/Gel/PGA scaffold and

ALA@lipo/Gel/PGA scaffolds). The Gel/PGA scaffold had no therapeutic effect because of the lack of both AuNRs and ALA. The Au/Gel/PGA scaffold could generate a high-temperature environment upon 805 nm NIR laser irradiation and effectively ablated the cancer cells in the scaffold. However, the limited thermal diffusion had less impact on distant cancer cells (e.g., the cells in the transwell bottom wells or scaffold rings). The ALA@lipo/Gel/PGA scaffold did not show evident photodynamic efficacy because ALA release without thermal stimulation was too slow to accumulate sufficient PpIX for ROS generation. In contrast, the ALA@lipo/Au/Gel/PGA composite scaffold, under stepwise 805 nm and 635 nm laser irradiation, exerted significant anticancer effects. NIR laser irradiation at 805 nm increased the local temperature and accelerated ALA release. Subsequent 635 nm laser irradiation activated PpIX, producing cytotoxic ROS to kill cancer cells. Multiple laser irradiation cycles resulted in the effective eradication of cells both in the scaffold and far from the scaffold, highlighting the synergistic effects of PTT and PDT. Therefore, the ALA@lipo/Au/Gel/PGA composite scaffold holds great potential as an advanced therapeutic platform for postsurgical cancer treatment, providing controlled drug delivery and synergistic therapeutic effects. In this study, the AuNRs were used as a photothermal conversion agent to achieve PTT under NIR laser irradiation. The composite scaffold can be used for treatment of shallow tumours. Limitation of tissue penetration depth of laser irradiation remains an inevitable challenge for application of the composite scaffold to treat deep-sited tumours. For the treatment of deep-sited tumours, other functional nanoparticles such as ultrasound-responsive nanoparticles or magnetic nanoparticles can be used to replace the AuNRs for preparation of the same multifunctional platforms.

Conclusions

In this study, an ALA@lipo/Au/Gel/PGA composite scaffold was developed for the synergistic PTT and PDT of cancers. The composite scaffold integrated thermosensitive liposomes carrying a photosensitizer precursor with AuNRs, gelatin and PGA and used ice particulates as porogen templates to achieve controllable pore size and good interconnectivity. Upon 805 nm NIR laser irradiation, the composite

scaffold exhibited excellent photothermal performance and increased ALA release. Following irradiation with a 635 nm NIR laser, the composite scaffold showed excellent photodynamic effects. In vitro and in vivo experiments confirmed that the composite scaffold enabled stepwise photothermal and photodynamic therapy under the combined 805 nm and 635 nm NIR laser irradiation, effectively eliminating both the cancer cells in the scaffold and the cancer cells far from the scaffold, resulting in superior therapeutic outcomes compared with single 805 nm or 635 nm NIR laser irradiation. The ALA@lipo/Au/Gel/PGA composite scaffold represents a promising approach for postsurgical cancer treatment.

Materials and methods

Reagents and chemicals

Sodium borohydride (NaBH_4), cetyltrimethylammonium bromide (CTAB), L-ascorbic acid, chloroauric acid (HAuCl_4), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), polyglutamic acid (PGA), and L-glycine were purchased from Sigma-Aldrich. Hydrochloric acid (HCl), silver nitrate (AgNO_3), 5-aminolevulinic acid hydrochloride (ALA), 2-(N-morpholino)ethanesulfonic acid (MES), acetic acid (HAc), 4-dimethylaminobenzaldehyde, D-luciferin and isoflurane solutions were purchased from Wako Pure Industries. Gelatin was purchased from Nitta Gelatin, Inc. 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), cholesterol (Chol), and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG-NH₂) were purchased from Avanti Polar Lipids, Inc. Phosphate-buffered saline (PBS, 10x, pH 7.4) was obtained from Nacalai Tesque, Inc. Foetal bovine serum (FBS), high-glucose Dulbecco's modified Eagle's medium (DMEM) and penicillin-streptomycin were acquired from Thermo Fisher Scientific, Inc. The Calcein-acetoxymethyl ester/propidium iodide (calcein-AM/PI) double-staining kit, water-soluble tetrazolium-1 (WST-1 reagent) and ROS assay kit were purchased from Dojindo, Ltd. All chemicals were used without further purification.

Synthesis and characterization of the Au nanorods

AuNRs were synthesized using the seed-growth method as previously described³⁵. First, 0.6 mL of a freshly prepared NaBH₄ (0.01 M) solution was rapidly added to a stirred mixture of HAuCl₄ (0.25 mL, 0.01 M) and CTAB (7.5 mL, 0.1 M) to obtain the gold seed solution. Then, 50 mL of a CTAB (0.1 M) solution was prepared and stirred continuously while HAuCl₄ (2.5 mL, 0.01 M), HCl (1 mL, 1 M), AgNO₃ (0.55 mL, 0.01 M), and L-ascorbic acid (0.4 mL, 0.1 M) were sequentially added. Finally, 0.12 mL of the prepared gold seed solution was introduced into the mixture and then left undisturbed at room temperature overnight to allow for the growth of the AuNRs. The resulting AuNRs were collected by centrifugation (13,000 rpm, 15 min), washed with Milli-Q water, and redispersed in Milli-Q water for subsequent experiments. The Au nanorods were characterized using transmission electron microscopy (TEM, JEOL 2011F, Japan) and an ultraviolet-visible-near infrared (UV-Vis-NIR) spectrophotometer (Shimadzu UV-2600, Japan). The longitudinal length and transverse length of the Au nanorods were measured from the TEM images using an ImageJ software. 100 nanoparticles were used for the measurement to show their size distribution and to calculate the means and standard deviations.

Synthesis of ALA-encapsulated liposomes

ALA-encapsulated liposomes were prepared using a thin-film hydration method with DPPC, Chol, and DSPE-PEG-NH₂ as the raw materials. First, DPPC (1.2 mL, 5 mg/mL), Chol (0.16 mL, 5 mg/mL) and DSPE-PEG-NH₂ (0.64 mL, 5 mg/mL) were dissolved in a chloroform/methanol mixture (v/v=9:1) and thoroughly mixed in a clean round-bottom flask. The molar ratio of DPPC/Chol/DSPE-PEG-NH₂ was maintained at 7:2:1. The flask was then connected to a rotary evaporator and incubated under reduced pressure for 30 min in a 45 °C water bath until a thin lipid film formed. The resulting lipid film was further dried overnight in a vacuum desiccator to ensure complete solvent removal. 1 mL of the ALA solution in PBS (10 mg/mL) was added to hydrate the lipid film and incubated for 30 min, then subjected to ultrasonication at 60 °C for 30 min to facilitate encapsulation. The resulting dispersion was extruded through a 200 nm polycarbonate membrane at 60 °C to obtain uniform liposomes. The extruded liposome suspension was incubated at 4 °C overnight, then centrifuged (20,000 rpm, 15

min) and washed with PBS. The final ALA-encapsulated liposomes were resuspended in PBS and stored at 4 °C for further use. As a control, DPPC/Chol/DSPE-PEG-NH₂ liposomes were prepared using the same method but without the addition of ALA during the hydration process. The size distributions of the ALA-encapsulated liposomes and the control liposomes were measured by dynamic light scattering (DLS; Otsuka Electronics Co., Ltd., Japan).

Preparation of the Au/Gel/PGA Composite Scaffold

The Au/Gel/PGA composite scaffold was fabricated using ice particulates as a porogen. To obtain uniformly sized ice particulates, ultrapure water was sprayed into liquid nitrogen and sieved at -4 °C to isolate ice particulates with diameters ranging from 250-355 µm. Gelatin was dissolved in a 35% HAc (w/v) solution, followed by the addition of PGA and the AuNRs. The final concentrations of gelatin, AuNRs and PGA were 4% (w/v), 2 mM, and 0.4% (w/v), respectively. The ice particulates and the prepared Au/Gel/PGA mixture were precooled at -5 °C and then mixed at a ratio of 7:3 (w/v) in a mould. The mixture was sequentially frozen at -20 °C for 12 h and then at -80 °C for 6 h. The frozen samples were lyophilized using a freeze-dryer (FDU-2200, Tokyo, Japan) to obtain a porous scaffold. The obtained scaffold was washed with ethanol and then sequentially immersed in 95%, 90%, and 85% ethanol solutions containing EDC (50 mM), NHS (20 mM), and MES (0.1% w/v) for 8 h each to induce cross-linking. After cross-linking, the Au/Gel/PGA composite scaffold was thoroughly washed with water and lyophilized again. As a control, a Gel/PGA composite scaffold without AuNRs adding was prepared using the same method. The pore structure of the composite scaffolds was analysed by scanning electron microscopy (SEM; Hitachi S-4800, Japan). The composite scaffolds were cut into discs with a diameter of 8 mm and a thickness of 2 mm for further use.

Preparation of ALA-functionalized composite scaffolds

The Gel/PGA and Au/Gel/PGA scaffold discs were immersed in an EDC/NHS (50 mM/20 mM) solution at room temperature for 6 h to activate the carboxyl groups. After activation, the scaffolds were washed with PBS 3 times to remove the residual reagents. Then, 100 µL of the ALA-encapsulated liposome solution (10 mg/mL counted by ALA)

was added to each scaffold disc, followed by an incubation with gentle shaking at room temperature for 12 h to facilitate binding. After the reaction, the scaffold discs were washed with PBS to remove unbound liposomes, and the obtained scaffolds were immersed in a 0.1 M glycine solution overnight to terminate the activation of the carboxyl groups.

Photothermal Conversion Performance of the Composite Scaffolds

The photothermal conversion properties of the Gel/PGA, ALA@lipo/Gel/PGA, Au/Gel/PGA and ALA@lipo/Au/Gel/PGA scaffolds were evaluated. After adding 100 μ L of Milli-Q water to each scaffold disc, the hydrated samples were irradiated with an 805 nm NIR laser at different power intensities (1.5 and 2 W/cm²) for 10 min. During irradiation, the temperature of each scaffold disc was recorded every 30 s using an infrared thermometer (Asone Corp., Osaka, Japan). Three samples from each group were used for the measurement.

Characterization and drug loading efficiency of liposomes

The quantification of ALA was performed using a previously reported colorimetric method ³⁶. Briefly, a series of ALA standard solutions were mixed with ethyl acetoacetate (400 μ L) and HAc-sodium acetate buffer (2 mL, pH=4.6). The mixture was incubated at 100 °C for 12 min to induce condensation. After cooling to room temperature, the Ehrlich reagent, which was freshly prepared by dissolving 1 g of 4-dimethylaminobenzaldehyde in a mixture containing 25 mL of 95% ethanol and 25 mL of concentrated hydrochloric acid, was added for chromogenic reactions. The absorbance was detected at 554 nm by using the UV-Vis-NIR spectrophotometer. The liposome membranes were completely disrupted using an ultrasonic cell dismembrator, thereby ensuring complete ALA release for the quantification of the encapsulation efficiency. Three samples from each group were used for the measurement to calculate the means and standard deviations.

ALA release from liposomes and ALA@liposome-loaded composite scaffolds

The ALA-encapsulated liposomes were suspended in 1 mL of PBS within a dialysis bag (3.5 kDa MWCO) and incubated at a 37 °C constant temperature, or a periodic temperature variation between 37 °C and 42 °C to investigate the effect of temperature

on ALA release from the liposomes. Dialysis was performed under constant stirring in 4 mL of PBS. At the designated time points, 1 mL of PBS was collected from the dialysis buffer solution, and 1 mL of fresh PBS was added. The amount of released ALA in the collected PBS samples was quantified, and the cumulative release profile was calculated. ALA release from the composite scaffolds under periodic laser irradiation was analysed by immersing each ALA@lipo/Au/Gel/PGA scaffold disc in 400 μ L of PBS and incubating them at 37 °C with constant shaking. After an initial 24-h incubation, the scaffold discs were irradiated with an 805 nm NIR laser at 1.5 W/cm² for 10 min. Laser irradiation was performed repeatedly every 24 h. At each designated time point before and after irradiation, 200 μ L of PBS was collected and replaced with 200 μ L of fresh PBS. The amount of released ALA was quantified, and the cumulative release profile was determined. ALA release from nonirradiated ALA@lipo/Au/Gel/PGA composite scaffolds incubated at 37 °C was also measured. Three samples from each group were used for the measurement to calculate the means and standard deviations.

***In vitro* cellular experiments using the composite scaffolds**

MDA-MB-231-Luc breast cancer cells (JCRB, Osaka, Japan) were used for the *in vitro* cell test. The cells were harvested by trypsinization and resuspended in DMEM at a concentration of 3×10^6 cells/mL. 100 μ L of the cell suspension was seeded onto one side of each sterilized scaffold disc and incubated in a humidified incubator (5% CO₂, 37 °C) for 6 h. After the incubation, another 100 μ L of the cell suspension was seeded onto the opposite side, followed by an additional 6-h incubation. The cells were also seeded in the bottom wells of a 24-well plate and incubated for 6 h. The cell-seeded scaffold discs were then transferred into transwell inserts and cocultured with the cells in the bottom wells. The transwell insert contained an 8 μ m microporous polyester membrane, ensuring the diffusion of the released ALA.

After 12 h of incubation, the cell/scaffold constructs were removed from the inserts and irradiated with an 805 nm NIR laser (1.5 W/cm²) for 10 minutes. After irradiation, the scaffold discs were immediately placed back into the inserts and cultured in serum-free DMEM for an additional 4 h. Subsequently, the cell/scaffold constructs were treated

with a ROS assay kit for 20 min, followed by exposure to a 635 nm NIR laser (0.05 W/cm², 10 min) to observe the fluorescence intensity of ROS under a fluorescence microscope (Olympus, Japan). The mean fluorescence intensity was measured by using an ImageJ software. Three samples from each group were used for the measurement to calculate the means and standard deviations.

To evaluate the *in vitro* therapeutic efficacy of the composite scaffolds, the cell/scaffold constructs were irradiated with an 805 nm NIR laser (1.5 W/cm²) for 10 min. After irradiation, the cell/scaffold discs were immediately returned to the culture inserts. After 4 h of incubation, the cell/scaffold constructs and the cells in the bottom wells were irradiated with a 635 nm laser (0.05 W/cm²) for 10 min. This is one cycle of combined irradiation procedure. The combination NIR laser irradiation was repeated every 24 h for a total of two cycles. After the 2nd cycle of combination laser irradiation, the cells were cultured for another 24 h and then used for live/dead staining and the WST-1 assay. A Calcein-AM/PI staining kit was used to visualize the live and dead cells in the scaffold followed by the manufacturer's instructions. The stained cells were observed with a fluorescence microscope (Olympus, Japan). The WST-1 assay was performed to quantify the viability of cancer cells in the scaffolds and in the bottom wells of the transwell plate. The cell/scaffold constructs were transferred into new 24-well plates containing 1 mL of WST-1 reagent (diluted 1:10 in medium) per well. The old culture medium in the bottom wells of the transwell plate was replaced with 1 mL of WST-1 reagent (diluted 1:10 in medium). After 3 h of incubation, the absorbance of the WST-1 solution at 440 nm was measured using a microplate reader (Benchmark Plus, Bio-Rad, Hercules, CA, USA). Three samples from each group were used for the measurement to calculate the means and standard deviations.

In vivo anticancer effects of the composite scaffolds

The animal experimental protocols were approved by the Animal Experiments Committee of the National Institute for Materials Science (accreditation No. 82-2025-3) and were performed in accordance with the committee guidelines. Six-week-old female BALB/c nude mice were purchased from Charles River Laboratories (Yokohama, Japan) and used for the animal experiments. Round discs (diameter: 8 mm,

thickness: 2 mm) of the Gel/PGA, ALA@lipo/Gel/PGA, Au/Gel/PGA and ALA@lipo/Au/Gel/PGA composite scaffolds were used for the animal experiments. Additionally, the Gel/PGA composite scaffold was cut into ring-shaped structures with an inner diameter of 8 mm, an outer diameter of 10 mm and a thickness of 2 mm.

The MDA-MB-231-Luc cancer cells were seeded in the scaffold discs, as described above (6×10^5 cells/scaffold). The cells were also seeded in the Gel/PGA scaffold rings (5×10^6 cells/scaffold). After 12 h of incubation, the cell-seeded scaffold discs were placed into the inner cavity of the scaffold rings and subcutaneously implanted into the mice. At one day post-implantation, the implantation site was irradiated with an 805 nm NIR laser (1.5 W/cm^2) for 10 min. 4 h later, the site was further irradiated with a 635 nm laser (0.05 W/cm^2) for 10 min. The implantation site was irradiated every day under the same irradiation conditions as those used for the first irradiation, and a total of three treatment cycles were conducted.

During the 805 nm NIR laser irradiation on the first day, infrared thermal images and temperature changes were recorded using an infrared camera. Cell viability was assessed *in vivo* by administering the mice a D-luciferin solution *via* intraperitoneal injection at the designated time points before and after each laser irradiation cycle. At 15 min postinjection, the mice were anaesthetized with 2% isoflurane, and bioluminescence imaging was performed using the *in vivo* imaging system (IVIS, Lumina III, PerkinElmer, MA, Japan). Each experimental group consisted of three mice.

Statistical analysis

All the quantitative experiments were performed in triplicate ($n = 3$). All the results are presented as the means $\pm$ standard deviations (S.D.s). Statistical analyses were conducted using one-way analysis of variance (ANOVA) with GraphPad Prism 9 software. Significance levels were determined based on the P values: * $P < 0.05$ (significant), ** $P < 0.01$ (moderately significant), and *** $P < 0.001$ (highly significant).

Author contributions

Conceptualization: G.C., X.L., Y.Y. Funding acquisition: G.C., N.K. Project administration: G.C. Resources: G.C. Supervision: G.C. Data curation: G.C., X.L., N.K.

Formal analysis: G.C., X.H., H.C., M.W., T. Z., T.Y., Investigation: G.C., X.H., H.C., M.W., T. Z., T.Y., M.H., M.T. Methodology: G.C., X.H., H.C., M.W., T.Y., N.K., Y.Y. Validation: G.C., X.L., N.K. Software: X.L., T.Y., Visualization: X.L. Writing-original draft: All coauthors contributed to original draft. Writing-review and editing: All coauthors contributed to reviewing and editing.

Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online.

Reference:

- 1 R. L. Siegel, T. B. Kratzer, A. N. Giaquinto, H. Sung and A. Jemal, *CA-Cancer J. Clin.*, 2025, **75**, 10–45.
- 2 A. N. Giaquinto, H. Sung, L. A. Newman, R. A. Freedman, R. A. Smith, J. Star, A. Jemal and R. L. Siegel, *CA-Cancer J. Clin.*, 2024, **74**, 477–495.
- 3 W. Budach, C. N. Kraemling, S. Wollandt, D. Jazmati, B. Tamaskovics, S. Corradini, E. Boelke, A. Haussmann, W. Audretsch and C. Matuschek, *J. Clin. Oncol.*, 2024, e12652.
- 4 T. F. Stoop, R. T. Theijse, L. W. F. Seelen, B. Groot Koerkamp, C. H. J. van Eijck, C. L. Wolfgang, G. van Tienhoven, H. C. van Santvoort, I. Q. Molenaar, J. W. Wilmink, M. Del Chiaro, M. H. G. Katz, T. Hackert and M. G. Besselink, *Nat. Rev. Gastroenterol. Hepatol.*, 2024, **21**, 101–124.
- 5 D. De Ruyscher, G. Niedermann, N. G. Burnet, S. Siva, A. W. M. Lee and F. Hegi-Johnson, *Nat. Rev. Dis. Primers*, 2019, **5**, 1–20.

- 6 N. Vasan, J. Baselga and D. M. Hyman, *Nature*, 2019, **575**, 299–309.
- 7 S. Tohme, R. L. Simmons and A. Tsung, *Cancer Res.*, 2017, **77**, 1548–1552.
- 8 N. B. Kouzminova, A. Aggarwal, S. Aggarwal and A. Y. Lin, *J. Clin. Oncol.*, 2009, **27**, e11533–e11533.
- 9 M. Z. Quazi, J. Park and N. Park, *Technol. Cancer Res. Treat.*, 2023, **22**, 1–6.
- 10 H. Shi and P. J. Sadler, *Br. J. Cancer*, 2020, **123**, 871–873.
- 11 M. Overchuk, R. A. Weersink, B. C. Wilson and G. Zheng, *ACS Nano*, 2023, **17**, 7979–8003.
- 12 Y. Xiong, Y. Rao, J. Hu, Z. Luo and C. Chen, *Adv. Mater.*, 2023, 2305140.
- 13 Y. Ren, Y. Yan and H. Qi, *Adv. Colloid Interface Sci.*, 2022, **308**, 102753.
- 14 J. Xie, Y. Wang, W. Choi, P. Jangili, Y. Ge, Y. Xu, J. Kang, L. Liu, B. Zhang, Z. Xie, J. He, N. Xie, G. Nie, H. Zhang and J. S. Kim, *Chem. Soc. Rev.*, 2021, **50**, 9152–9201.
- 15 M. Dirak, C. M. Yenici and S. Kolemen, *Coordin. Chem. Rev.*, 2024, **506**, 215710.
- 16 M. Zhu, P. Wang, B. Chen, L. Shi, R. Long, S. Wang and Y. Liu, *Mater. Des.*, 2024, **237**, 112618.
- 17 D. Thankachan, R. Anbazhagan, H. C. Tsai, V. T. T. Dinh, H. T. Gebrie, S. L. Kitaw, Y. W. Ahmed, B. E. Anley, Y.-S. Liao, W.-L. Chen and J.-K. Chen, *Dyes Pigments*, 2024, **221**, 111812.
- 18 F. Danhier, *J. Control. Rel.*, 2016, **244**, 108–121.
- 19 L. Wang, S. Quine, A. N. Frickenstein, M. Lee, W. Yang, V. M. Sheth, M. D. Bourlon, Y. He, S. Lyu, L. Garcia-Contreras, Y. D. Zhao and S. Wilhelm, *Adv. Funct. Mater.*, 2024, **34**, 2308446.
- 20 M. A. Beach, U. Nayanathara, Y. Gao, C. Zhang, Y. Xiong, Y. Wang and G. K. Such, *Chem. Rev.*, 2024, **124**, 5505–5616.
- 21 R. Sun, M. Wang, T. Zeng, H. Chen, T. Yoshitomi, M. Takeguchi, N. Kawazoe, Y. Yang and G. Chen, *Bioact. Mater.*, 2025, **44**, 205–219.
- 22 L. Sutrisno, H. Chen, Y. Chen, T. Yoshitomi, N. Kawazoe, Y. Yang and G. Chen, *Biomater.*, 2021, **275**, 120923.
- 23 L. Guo, Q. Zhao, L. Zheng and M. Wang, *Adv. Healthc. Mater.*, 2023, **12**, 2302484.
- 24 X. Chen, B. B. Mendes, Y. Zhuang, J. Conniot, S. Mercado Argandona, F. Melle, D. P. Sousa, D. Perl, A. Chivu, H. K. Patra, W. Shepard, J. Conde and D. Fairen-Jimenez, *J. Am. Chem. Soc.*, 2024, **146**, 1644–1656.
- 25 X. Gao, B. R. Caruso and W. Li, *Gels*, 2024, **10**, 479.
- 26 D. I. Jeong, H. J. Kim, S. Y. Lee, S. Kim, J. W. Huh, J.-H. Ahn, M. Karmakar, H.-J. Kim, K. Lee, J. Lee, H.-J. Ko and H.-J. Cho, *J. Control. Rel.*, 2024, **366**, 142–159.
- 27 H. Chen, R. Sun, T. Zeng, J. Zheng, T. Yoshitomi, N. Kawazoe, Y. Yang and G. Chen, *Biomater. Sci.*, 2022, **10**, 7042–7054.
- 28 B.-Y. Li, T.-Y. Lin, Y.-J. Lai, T.-H. Chiu and Y.-C. Yeh, *Small*, 2024, 2407420.
- 29 G. Kuang, X. Lin, J. Li, W. Sun, Q. Zhang and Y. Zhao, *Chem. Eng. J.*, 2024, **489**, 151253.
- 30 R. Sun, H. Chen, L. Sutrisno, N. Kawazoe and G. Chen, *Sci. Technol. Adv. Mater.*, 2021, **22**, 404–428.
- 31 K. McNicholas, M. N. MacGregor and J. M. Gleadle, *Br. J. Cancer*, 2019, **121**, 631–

639.

- 32 V. Sharma, K. Park and M. Srinivasarao, *Mater. Sci. Eng R Rep.*, 2009, **65**, 1–38.
- 33 T. Ta and T. M. Porter, *J. Control. Rel.*, 2013, **169**, 112–125.
- 34 Y. Wang, K. Ma, M. Kang, D. Yan, N. Niu, S. Yan, P. Sun, L. Zhang, L. Sun, D. Wang, H. Tan and B. Z. Tang, *Chem. Soc. Rev.*, 2024, **53**, 12014–12042.
- 35 H. Chen, R. Sun, J. Zheng, N. Kawazoe, Y. Yang and G. Chen, *J. Mater. Chem. B*, 2022, **10**, 4771–4782.
- 36 K. Tomokuni and M. Ichiba, *Sangyo Igaku*, 1988, **30**, 52–53.