

Enhancing the therapeutic efficacy of photodynamic therapy in colon cancer based on an ECN-mediated targeted drug delivery strategy

Kai Wang^{1,2,§}, Huihui Bian^{2,§}, Tao Ye^{1,§}, Fusheng Shang², Chenxi Zhang², Dagui Chen², Li Su², Heng Yin³✉, and Liang Zhao¹✉

¹Shanghai Baoshan Luodian Hospital, School of Medicine, Shanghai University, Shanghai 201908, China

²Institute of Translational Medicine, Shanghai University, Shanghai 200444, China

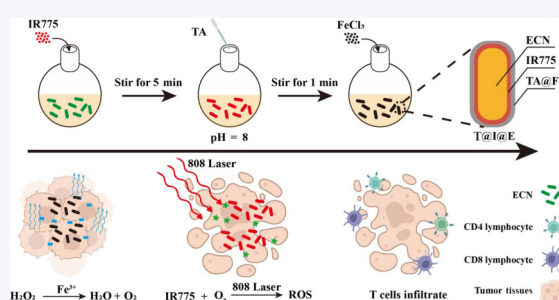
³Wuxi Affiliated Hospital of Nanjing University of Chinese Medicine, Wuxi 214000, China

§ Kai Wang, Huihui Bian, and Tao Ye contributed equally to this work.

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ABSTRACT: Photodynamic therapy (PDT) has attracted considerable interest in tumor treatment due to its precise temporal and spatial control. However, the off-target effects of photosensitizers and the hypoxic tumor microenvironment limit the production of reactive oxygen species (ROS), reducing PDT effectiveness. To address this challenge, we utilized *Escherichia coli* Nissle 1917 (ECN) as host cells and employed tannate (TA) and FeCl₃ in a bio-warp method to encapsulate IR775 on the surface of ECN forming TA@Fe@IR775@ECN (notes as T@I@E). At the tumor site, Fe³⁺ decomposes H₂O₂ to release O₂ within the tumor microenvironment, which interacts with IR775 to increase ROS production locally. Compared to non-living drug delivery methods, T@I@E actively targets tumor cells, achieving approximately 6-fold higher accumulation of IR775 at the tumor site and alleviating tumor hypoxia to enhance PDT efficacy. The favorable biocompatibility of T@I@E further supports its potential for clinical application, establishing T@I@E as a promising candidate for tumor therapy as a living material.

KEYWORDS: nanocarriers, photodynamic therapy, *Escherichia coli* Nissle 1917, targeted drug delivery, cancer therapy



1 Introduction

In recent years, the concept of precision therapy has been applied in tumor treatment [1]. Photodynamic therapy (PDT), as an emerging strategy, offers high temporal and spatial control, positioning it as a potential precision approach for tumors [2]. Traditional PDT primarily involves administering photosensitizers to accumulate at the tumor site, followed by irradiation with specific laser wavelengths, triggering reactions with oxygen to produce cytotoxic reactive oxygen species (ROS), which induce oxidative damage and apoptosis in tumor cells [3]. However, several limitations hinder PDT in oncology, including insufficient targeting specificity and rapid metabolism of photosensitizers by the liver, leading to low accumulation at the tumor site [4]. Additionally, the limited

penetration of short-wavelength lasers further restricts PDT use [5]. Tumor hypoxia, resulting from the uncontrolled metabolism of tumor cells and poorly developed vascular structures, presents another major challenge, as the oxygen concentration within tumors is significantly reduced, reaching only 3% of that in normal tissues [6]. This reduction in oxygen compromises PDT effectiveness.

To overcome these challenges, various nanocarriers, such as liposomes [7], silica nanoparticles [8], albumin-based manganese dioxide [9], and micelles, have been employed for photosensitizer delivery. Due to their small size (10–200 nm) and the leaky vasculature of tumor sites, these nanocarriers utilize the enhanced permeability and retention (EPR) effect to penetrate and accumulate within tumors, thereby improving PDT outcomes [10]. Additionally, oxygen delivery strategies, such as fluorinated carbon and hemoglobin-based oxygen carriers, and small molecule mitochondrial inhibitors, have been proposed to address tumor hypoxia [7, 11, 12]. Although these methods have moderately enhanced PDT, the reticuloendothelial system in the liver often captures smaller nanoparticles (under 100 nm), followed by renal

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✉ Address correspondence to Heng Yin, wxzy011@njucm.edu.cn; Liang Zhao, zhaoliangphar@shu.edu.cn

clearance, resulting in limited drug utilization efficiency [13]. Thus, developing efficient drug delivery carriers remains an urgent focus in this field.

Escherichia coli Nissle 1917 (ECN) has emerged as a living drug delivery system [14]. Compared to non-living nanocarriers, the living delivery strategy based on ECN can actively target the tumor site through chemotaxis factors [15], enhancing the tumor targeting ability [16–18]. Meanwhile, ECN can penetrate the hypoxic core of tumors tissues and colonize the tumor site, overcoming the tolerance due to dense extracellular matrix of tumor tissues and improving drug penetration [15]. Recently, various delivery systems have been designed by surface chemical modification of ECN to deliver drugs such as chemicals drug [19], proteins [20], and antibodies [21], marking a promising step in oncology.

In this study, we used ECN as host cells to deliver the near-infrared photosensitizer IR775. Initially, IR775 was successfully adsorbed onto the surface of ECN, forming IR775@ECN. To further enhance the stability of IR775@ECN and prevent premature release of IR775, we coated the ECN surface with TA@Fe³⁺, an organo-metallic structure similar to a metal-organic framework (MOF) resulting in the stable composite T@I@E (TA@IR775@ECN). Upon reaching the tumor site, the surface TA@Fe³⁺ reacted with overexpressed H₂O₂ in the tumor microenvironment, generating oxygen to reverse tumor hypoxia. Concurrently, IR775 was released into the tumor tissue, and upon irradiation with an 808 nm laser, ROS was generated, leading to tumor cell death. *In vivo* experiments demonstrated that T@I@E effectively inhibited CT26 tumor cell growth. Importantly, treatment with T@I@E did not cause significant damage to major organs in the treated mice, indicating good biocompatibility. Therefore, T@I@E not only improves tumor oxygenation but also enhances IR775 accumulation at the tumor site, thereby improving PDT effectiveness.

2 Experimental

2.1 Synthesis and characterization of T@I@E

T@I@E was synthesized in a two-step reaction. Briefly, 1×10^8 colony forming units (CFU) of ECN was resuspended in H₂O by vortexing. Then, 1 mg of IR775 was added and stirred at room temperature for 5 min. Afterward, TA was added to this buffer and vortexed for an additional 30 s, followed by the addition of 10 mM FeCl₃ solution with stirring for 10 s. The resulting mixture was washed three times with PBS and stored at 4 °C. The research approach and experimental procedure of the article are illustrated in Scheme 1.

T@I (TA@IR775) was synthesized by similar reaction. Briefly, 1 mg of IR775 was added and stirred at room temperature for 5 min. Afterward, TA was added to this buffer and vortexed for an additional 30 s, followed by the addition of 10 mM FeCl₃ solution with stirring for 10 s. The resulting mixture was washed three times with PBS and stored at 4 °C.

The morphology of ECN and T@I@E was observed by transmission electron microscope (TEM) (Thermo Fisher Scientific, USA). Changes in zeta potential and particle size of ECN and T@I@E were analyzed using a Zetasizer Nano ZS ZEN 3600 (Malvern, UK). Colocalization of IR775 with ECN was detected by ultraviolet-visible (UV-VIS) or confocal laser scanning microscope (CLSM).

2.2 Drug release behavior of T@I@E in different buffers

The drug release behavior of T@I@E was tested by adding 2 mL of T@I@E to a dialysis tube (molecular weight cutoff: 3.5 kDa) and stirring in 50 mL of PBS (pH 7.4 or pH 6) at 300 rpm at room temperature. At various time points, 1 mL of PBS was removed for UV-VIS analysis to determine IR775 concentration, and 1 mL of fresh PBS was added to maintain a constant volume of 50 mL. The cumulative release rate of IR775 was calculated according to the standard curve.

2.3 Growth curve of ECN and T@I@E

1×10^4 ECN or T@I@E were added into 4 mL Luria-Bertani (LB) media incubation at shake cultivation, respectively. At different time points, 0.5 mL LB was taken out for UV-VIS at 600 nm. The curve of ECN or T@I@E was drawn by GraphPad 8.0.1.

2.4 CRT expression after treatment with T@I@E

CT26 tumor cells (density: 3×10^6 cells per well) were seeded onto a 20 mm CLSM plate. After overnight incubation, cells were treated with medium containing 10 ng of IR775 or T@I@E for 4 h. An 808 nm laser was applied to the plate for 30 s (laser density: 0.5 mW/cm²), followed by an additional 6-h incubation. Cells were then collected, stained with 4',6-diamidino-2-phenylindole (DAPI) or phycoerythrin (PE)-anti calreticulin (CRT) antibody for 30 min, washed three times with PBS, and imaged by CLSM. In a parallel experiment, CT26 cells were seeded in a 6-well plate, treated with IR775 or T@I@E under 808 nm laser exposure, and stained with PE-anti CRT antibody. CRT expression was detected using a flow cytometer (Thermo Fisher, Attune™ CytPix™).

2.5 Enhanced ROS production by T@I@E *in vitro*

CT26 tumor cells (density: 3×10^6 cells per well) were seeded in a 6-well plate and treated with IR775 or T@I@E for 2 h. Cells were then exposed to an 808 nm laser for 30 s. 2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA) was added to the plate, and cells were incubated for an additional 10 min. Cells were collected, stained with DAPI for 5 min, washed three times with PBS, and imaged by CLSM.

2.6 Cell cytotoxicity assay

Cell cytotoxicity was assessed using the cell counting kit-8 (CCK-8) assay. CT26 tumor cells (density: 1×10^5 cells per well) were seeded in 96-well plates. Various concentrations of T@I (TA@IR775), T@E (TA@Fe³⁺@ECN), and T@I@E were added to the medium and incubated for 6 h. Afterward, cells were either exposed or not exposed to an 808 nm laser for 30 s and then incubated for an additional 24 h. The medium was removed, cells were washed three times with PBS, and fresh medium containing 10% CCK-8 was added to each well. Following a 3-h incubation, cell viability was measured using UV-VIS at 488 nm.

2.7 Biodistribution of T@I@E

CT26 tumor cells (1×10^7 cells per mouse) were subcutaneously injected into the forelimb of mice. When tumor volume reached approximately 100 mm³, mice were intravenously injected with T@I or T@I@E (IR775 concentration: 5 mg/kg). Biodistribution of T@I and T@I@E were monitored at designated time points using a multi-mode live imaging system (IVIS Lumina XRMS Series III, PerkinElmer, USA). Additionally, the distribution of IR775 and

ECN in major organs and tumor tissues was analyzed by UV–VIS and spread plate methods, respectively.

The heart, liver, spleen, lungs, kidneys, and tumor tissues were collected and ground, and PBS was added to achieve a concentration of 1 mg/mL (tumor powder mass/PBS). A 0.1 mL aliquot of the solution was plated, and after 24 h of incubation, the number of ECN colonies was observed.

To evaluate the reversal of tumor hypoxia *in vivo*, CT26 mouse models were injected with either T@I or T@I@E. After 24 h, the mice were sacrificed, and tumor tissues were collected. The tumor tissues were embedded in optimal cutting temperature (OCT) gel, stored at -20°C , and sectioned into 10 μm slices. These sections were stained with fluorescein isothiocyanate (FITC)-anti hypoxia inducible factor-1 α (HIF-1 α) antibody for 2 h, followed by DAPI staining for 5 min. Fluorescence images of HIF- α were obtained by CLSM.

2.8 *In vivo* antitumor efficacy of T@I@E in CT26 tumors

CT26 bilateral tumor models were used to assess antitumor effectiveness. When the primary tumor volume reached approximately 100 mm³, mice were injected via the tail vein with PBS, PBS (+laser), T@I, T@I (+laser), T@E, or T@E (+laser), T@I@E, or T@I@E (+laser) (IR775 concentration: 5 mg/kg; ECN: 1×10^7). Tumor volume and body weight were recorded every two days for 14 days. After 14 days, a subset of mice was sacrificed to collect tumor tissues, blood, and major organs (heart, liver, spleen, lungs, kidneys). The remaining mice were monitored to assess overall survival.

2.9 Evaluation of tumor apoptosis and proliferation *in vivo*

After 14 days of treatment, tumor tissues were collected and embedded in OCT gel. Hematoxylin and eosin (H&E) staining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays were performed to assess tumor morphology and apoptosis. Tumor cell proliferation was evaluated by staining slices with Ki67 antibody for 1 h, followed by observation of fluorescence images by CLSM.

Similarly, CD3⁺, CD4⁺, and CD8⁺ T lymphocytes were stained with FITC-anti CD3⁺, CD4⁺, and CD8⁺ antibodies for 2 h, followed by DAPI staining for 5 min. Fluorescence images were obtained by CLSM.

2.10 Biosafety evaluation of T@I@E

The biosafety of T@I@E was evaluated in mouse plasma after 14 days of treatment. Hepatorenal toxicity was assessed by measuring aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum creatinine (CRE), and urea nitrogen (BUN) using assay kits from Jiancheng Bioengineering Institute (Nanjing, China). Major organs were collected, fixed in 4% neutral formalin, embedded in paraffin, and stained with H&E for histological analysis.

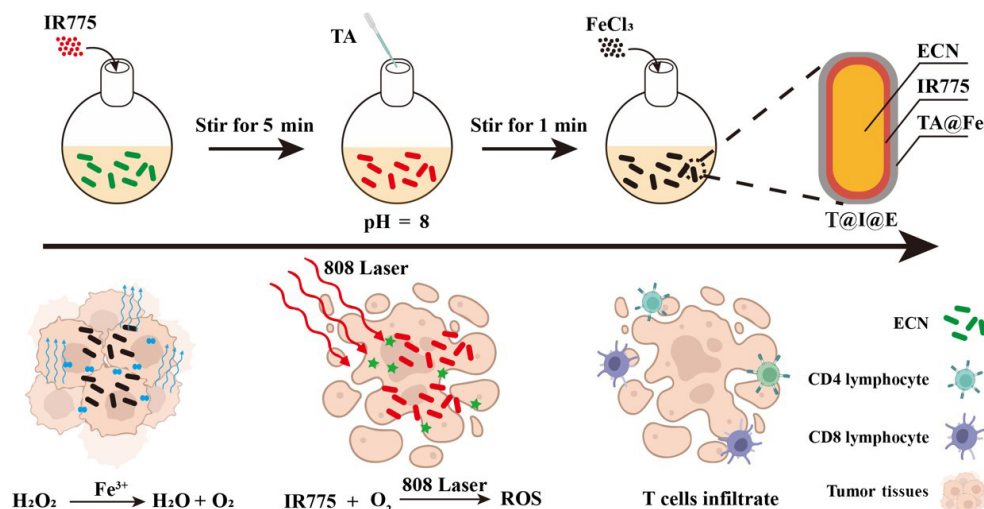
3 Results and discussion

3.1 Synthesis and characterization of T@I@E

We found that the hydrophobic IR775 could be effectively captured and adsorbed onto the surface of ECN. To enhance the stability of the complex, we applied a gentle biomineralization method to encapsulate the ECN surface in a protective layer. As shown in Figs. 1(a) and 1(b), compared to wild type ECN, the surface of T@I@E (TA@IR775@ECN) appeared rougher, likely due to the mineralization of TA@Fe on the ECN surface. In Fig. 1(c), elemental mapping of T@I@E revealed clear distributions of C, N, O and Fe, with Fe primarily surrounding the ECN. In contrast, Fig. S1 in the Electronic Supplementary Material (ESM) shows that in the elemental mapping image of ECN, considerable abundance of C, N, O elements could be detected, while Fe element was almost absent. These findings confirm that TA-Fe was successfully grafted onto the ECN surface in T@I@E, demonstrating effective modification.

Further analysis with a Malvern particle size analyzer (Fig. 1(d)) showed that the particle size of wild-type ECN was 0.9 μm , which increased to 1.1 μm after TA@Fe coating, consistent with TEM results and indicating the successful synthesis of T@I@E. Changes in zeta potential before and after biomineralization are presented in Fig. 1(e). The zeta potential of ECN shifted from -17.26 ± 0.62 to -24.40 ± 1.49 mV following biomineralization, indicating successful mineralization of TA@Fe on the ECN surface to obtained T@I@E.

To verify IR775 loading onto the ECN surface, we conducted UV–VIS analysis, detecting the characteristic absorption peak of IR775. As shown in Fig. S2 in the ESM, T@I@E exhibited a distinct IR775 absorption peak at 750 nm. Co-localization of IR775 and



Scheme 1 Schematic illustration of the synthesis route of T@I@E, and the mechanism of reversal of tumor hypoxia and enhanced ROS production.

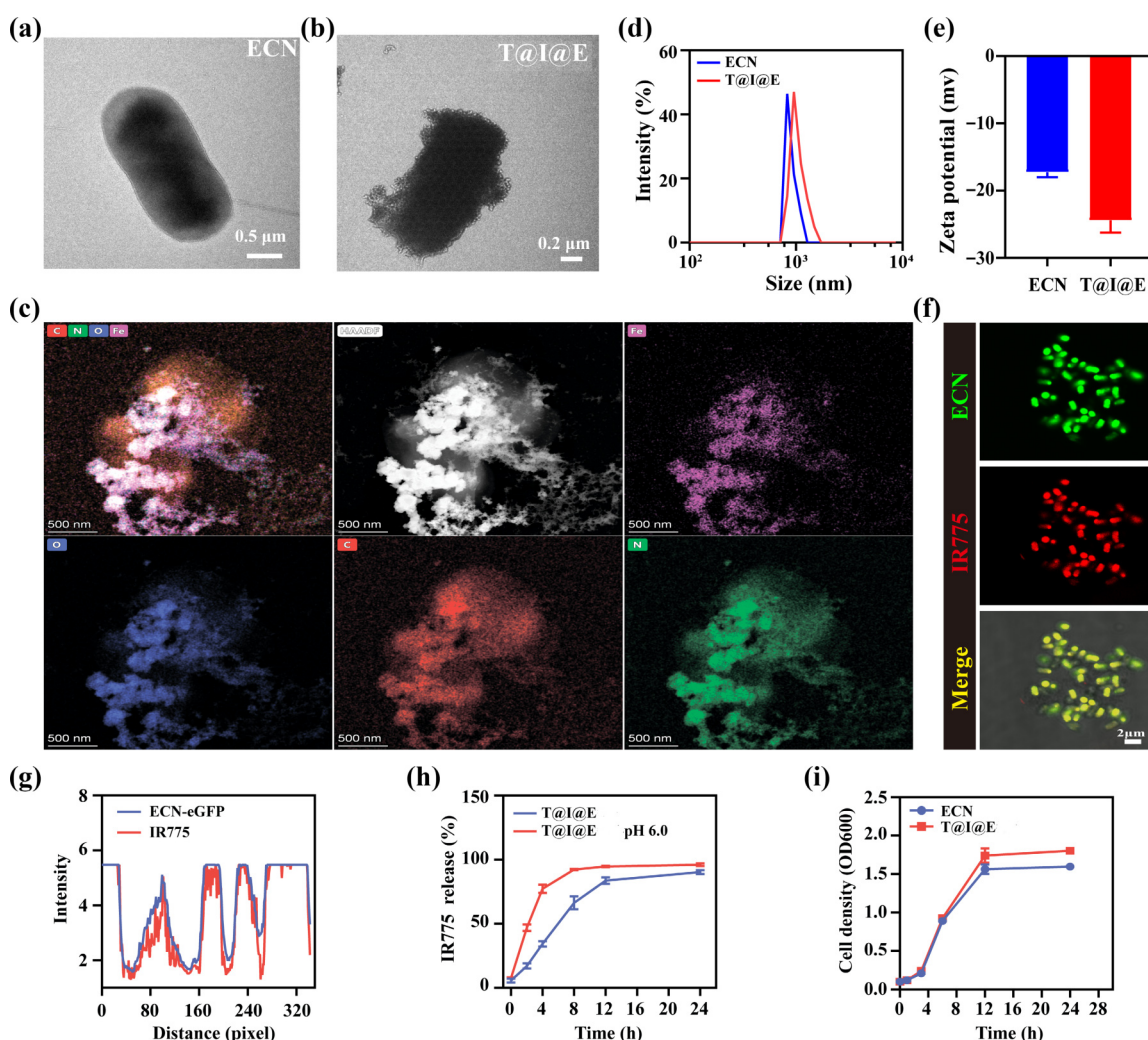


Figure 1 Characterization of T@I@E. Representative TEM image of (a) ECN or (b) T@I@E, scale bar: 0.5 μm in (a), 0.2 μm in (b). (c) Elemental mapping of T@I@E. C, N, O and Fe elements could be detected with considerable abundance. (d) The change of size of ECN and T@I@E. (e) The change of zeta potential of ECN, T@E (TA@Fe@ECN), and T@I@E. (f) Co-localization and (g) quantity analysis of IR775 and ECN, scale bar: 2 μm . (h) IR775 release from T@I@E in PBS buffer (pH = 6.0). (i) The growth behavior of ECN, and T@I@E in LB medium.

ECN was demonstrated in Figs. 1(f) and 1(g), where the red fluorescence of IR775 overlapped with the green fluorescence of ECN, confirming successful IR775 encapsulation on the surface of ECN. We further examined the drug release behavior of T@I@E, as shown in Fig. 1(h). In PBS solution, approximately 80% of IR775 was released after 12 h, while in PBS (pH 6), nearly 80% was released within 4 h, indicating the acidic tumor microenvironment-responsive drug release characteristic of T@I@E.

The stability of T@I@E was also tested (Fig. S3 in the ESM). In H_2O solution, the particle size of T@I@E remained stable over 50 min, while in PBS and 5% FBS, the particle size gradually decreased over time. This reduction may be due to ECN growth in solution, leading to outer membrane rupture and size reduction. To assess ECN activity within T@I@E, a growth curve was shown in Fig. 1(i), indicating that T@I@E exhibited a growth profile similar to wild-type ECN, suggesting that encapsulation did not impair ECN activity and supports its potential for active targeting.

3.2 T@I@E enhances ROS production and induces immunogenic cell death in CT26 cells

ROS can cause oxidative damage to cells, leading to immunogenic

cell death in tumor cells and the release of related antigens such as CRT, ATP, and high mobility group protein B1 (HMGB1). CRT, a commonly studied antigen, displays “eat me” signals that immune cells recognize after tumor cell damage.

To determine if T@I@E induces CRT expression, we first assessed CRT levels in CT26 cells using immunofluorescence. As shown in Fig. 2(a), T@I@E treatment resulted in increased red fluorescence. Among treatment groups, T@I@E + Laser showed the highest red fluorescence intensity, attributed to ROS production triggered by irradiation, which ultimately led to immunogenic cell death. T@E (TA@Fe@ECN) also induced CRT expression, possibly due to the immunogenic cell death induced by toxic factors released from ECN.

Further verification of CRT expression was conducted using flow cytometry. As shown in Fig. 2(b), CRT-positive CT26 cells increased dose-dependently with T@I@E concentration, rising from 1.6% to 58.4%. This increase is primarily due to IR775 on T@I@E reacting with oxygen to generate various ROS concentrations, inducing immunogenic cell death in CT26 cells. ATP and HMGB1 release were also tested, compared to CRT, the levels of HMGB and ATP in the cells decreased after treatment (Fig.

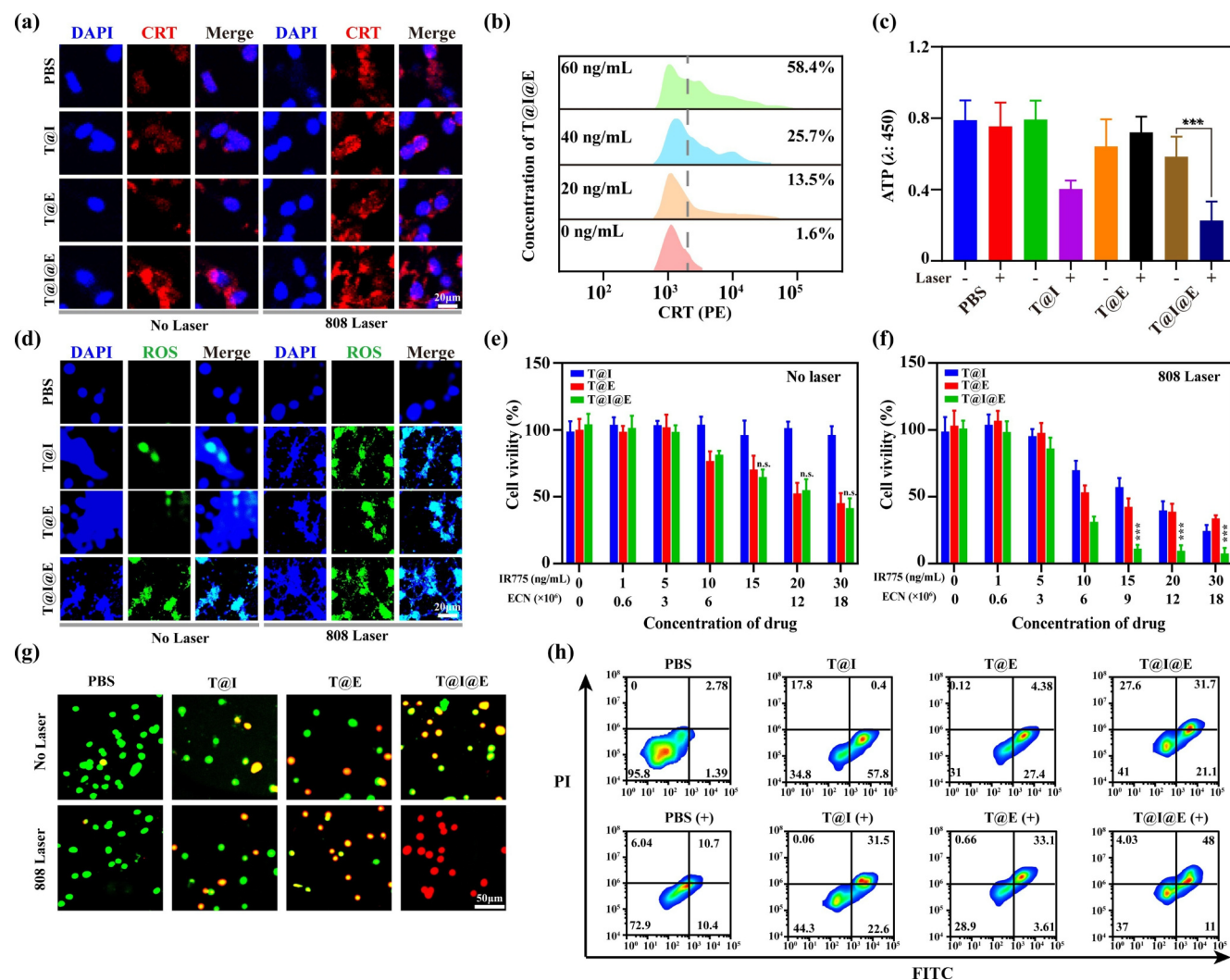


Figure 2 T@I@E enhances the production of ROS and induces cell toxicity *in vitro*. (a) Representative image of CRT expression after various treatment. scale bar: 50 μ m. (b) Flow cytometry quantified analysis expression level of CRT after various concentrations of T@I@E treated. (c) ATP release after various treatments. (d) Representative images of ROS level after various treatments. Cell viability of CT26 cells as detected by CCK-8 following treatment (e) without laser and (f) with laser ($n = 3$). (g) Cell apoptosis of CT26 cells co-incubated with various drugs for 24 h. (h) Flow cytometry of CT26 cells co-incubated with different drugs for 24 h. Statistical analysis was performed using two-tailed Student's *t*-tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

2(c) and Fig. S4 in the ESM). ROS production was measured using the DCFH-DA probe, as shown in Fig. 2(d). After T@I@E treatment, an increase in green fluorescence indicated enhanced ROS production, attributable to T@I@E's capacity to decompose H_2O_2 and boost ROS levels. These findings confirm that T@I@E promotes CRT expression through increased ROS production.

Cytotoxicity of T@I@E was evaluated in Figs. 2(e) and 2(f), without laser irradiation, T@I@E at concentrations below 10 ng/mL showed no significant cytotoxicity. However, at concentrations above 10 ng/mL, T@I@E inhibited CT26 cell growth, likely due to ECN's effect on cell growth. Under laser irradiation, even at 10 ng/mL, T@I@E significantly reduced cell viability, indicating strong cytotoxic effects under laser activation. CLSM imaging of cell apoptosis under various treatments is shown in Fig. 2(g). Following laser exposure, T@I@E caused significant cell death (indicated by red fluorescence). These results align with Fig. 2(h), demonstrating that T@I@E combined with laser exposure induced the highest tumor cell apoptosis compared to PBS or other treatments, effectively inhibiting tumor cell growth.

3.3 Pharmacokinetics and *in vivo* drug distribution of T@I@E

ECN, a facultative anaerobic probiotic, can survive in hypoxic environments. To assess its specific colonization at the tumor site *in vivo*, we employed an animal imaging system to monitor T@I@E biodistribution in mice, as shown in Fig. 3(a). After treatment, T@I@E demonstrated targeted colonization at the tumor site. Compared to T@I, T@I@E exhibited higher tumor accumulation 24 h post-treatment (Figs. 3(b) and 3(c)), which may be attributed to its enhanced *in vivo* stability. Additionally, T@I@E prolonged blood circulation time, further increasing drug accumulation at the tumor site (Fig. S5 in the ESM). Mice were then euthanized, and major organs were collected to measure IR775 concentrations. As shown in Figs. 3(d) and 3(e), the concentration of IR775 at the tumor site in the T@I@E-treated group was approximately three times higher than that in the T@I-treated group, indicating T@I@E's active tumor-targeting ability. Further confirmation of ECN biodistribution demonstrated significantly higher ECN levels at the tumor site than in other tissues (Figs. 3(f) and 3(g)), reinforcing the

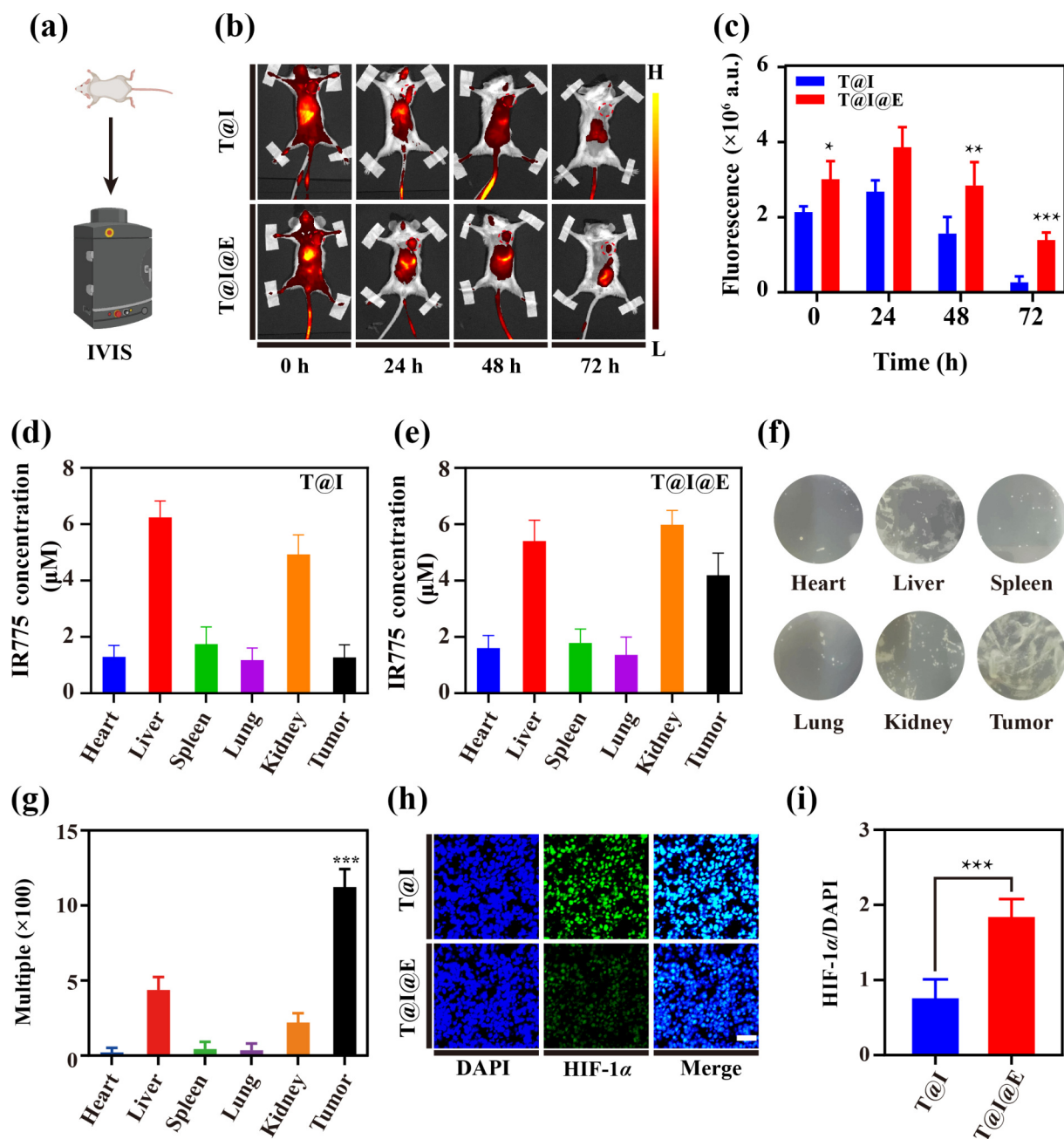


Figure 3 Pharmacokinetics and bio-distribution of T@I@E *in vivo*. (a) Schematic diagram of the pharmacokinetics experimental design. (b) Real-time fluorescence images and (c) quantity analysis of CT26 tumor-bearing mice following an intravenous injection with T@I or T@I@E at different time points, the quantitative sites were marked in red. The intensity of IR775 after treated with (d) T@I or (e) T@I@E in major organs *ex vivo*. (f) Bio-distribution and (g) quantity analysis of ECN in heart, liver, spleen, lung, kidney, and tumor. (h) The expression and (i) quantity analysis of HIF-1 α in tumor site after various treatments, scale bar: 50 μ m. Statistical analysis was performed using two-tailed Student's t-tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

tumor-targeting capability of T@I@E and supporting increased drug accumulation. Finally, T@I@E treatment significantly improved tumor hypoxia (Figs. 3(h) and 3(i)) and the level of ROS (Figs. S6 and S7 in the ESM) due to its catalytic decomposition of H_2O_2 , generating O_2 *in vivo*.

3.4 *In vivo* anti-tumor effect of T@I@E

The CT26 mouse tumor model was established by subcutaneous xenograft. When the tumor volume reached 100 mm³, mice were randomly divided into eight groups. The treatment regimen is illustrated in Fig. 4(a), and the changes in tumor volume

throughout the treatment are depicted in Fig. 4(b). The PBS, PBS + Laser, and T@I groups exhibited rapid tumor growth after day 5. In contrast, the T@I + Laser and T@I@E + Laser groups showed tumor suppression effects, with T@I@E + Laser demonstrating the strongest tumor inhibitory effect, while T@E + Laser and T@I@E achieved only 33% inhibition.

Tumor tissue mass from the *ex vivo* analysis is shown in Fig. 4(c), where mice treated with T@I@E + Laser had lower tumor tissue weights compared to other groups. After 14 days of treatment, mice body weights remained stable (Fig. 4(d)), indicating good biocompatibility of T@I@E. Finally, T@I@E treatment

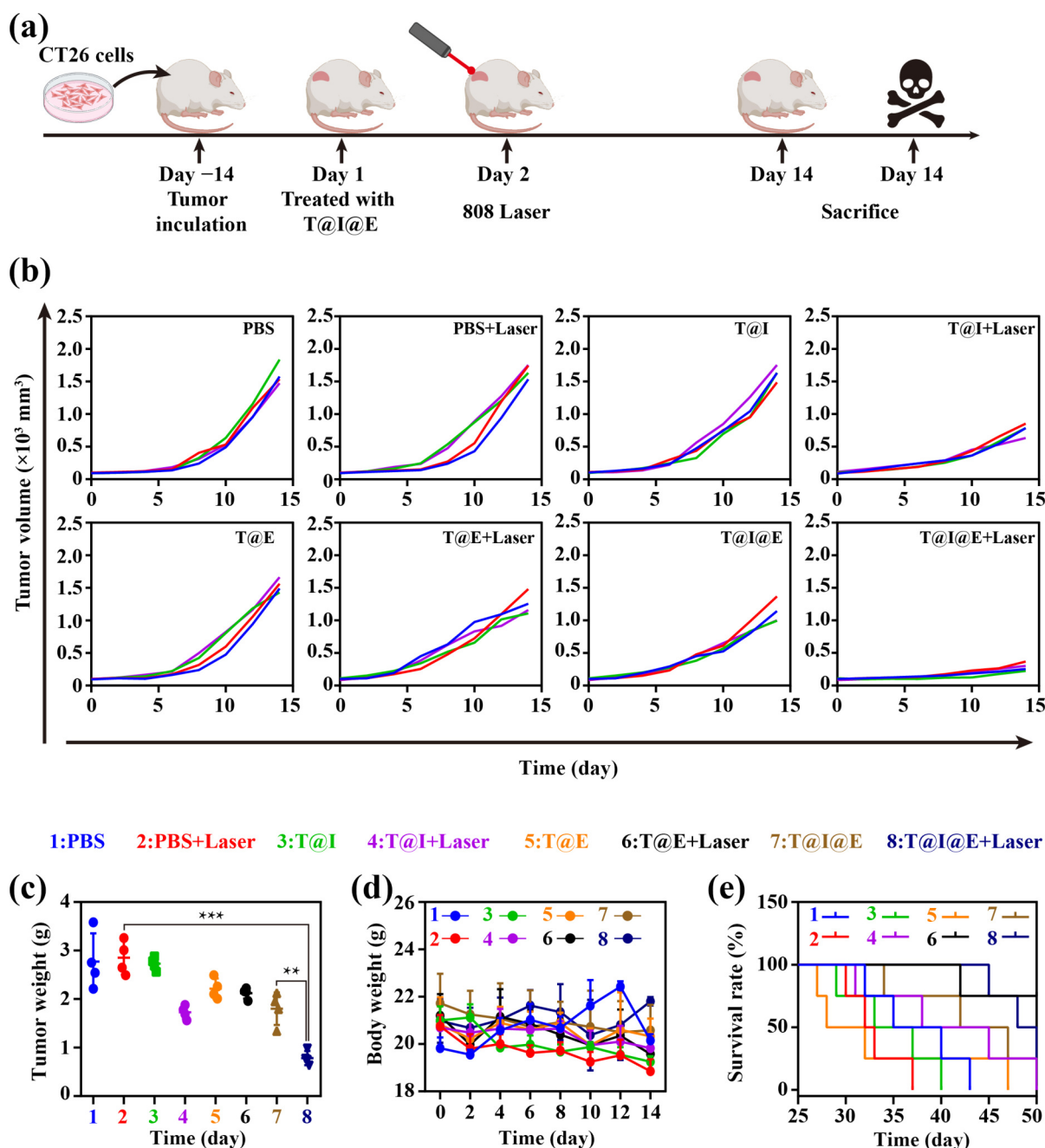


Figure 4 *In vivo* anti-tumor efficacy of T@I@E in CT26 tumor-bearing mice. (a) Schematic diagram of the experimental design. (b) Major tumor growth curves of CT26 tumor-bearing mice after being treated with different treatments ($n = 4$). (c) Excised CT26 tumors weight after treatment for 14 days. (d) Body weight curves of CT26 tumor-bearing mice after being treated with different treatments. (e) Survival rates of the mice after the different treatments. Data were represented as the mean $\pm$ SD. Statistical analysis was performed using two-tailed Student's *t*-tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

extended mouse survival (Fig. 4(e)), demonstrating its effective anti-tumor activity. These data collectively indicate that T@I@E effectively inhibits tumor growth and prolongs survival in mice.

3.5 *In vivo* anti-tumor mechanism of T@I@E

To investigate the anti-tumor mechanism of T@I@E, we analyzed tumor tissue sections from mice treated with various groups after 14 days (Fig. 5(a)). We assessed the cell proliferation marker Ki67 and the cell apoptosis marker TUNEL. As shown in Figs. 5(b)–5(e), without 808 nm laser irradiation, the T@I-treated group exhibited fluorescence intensities similar to the PBS group, indicating that

T@I did not significantly impact cell proliferation or apoptosis. Conversely, T@I@E demonstrated cytotoxicity by inhibiting tumor cell growth and promoting apoptosis, likely due to the inherent anti-tumor activity of ECN. Under 808 nm laser irradiation, both the T@I and T@I@E groups showed cytotoxic effects; however, T@I@E exhibited stronger green fluorescence (TUNEL) and reduced red fluorescence (Ki67), signifying a superior therapeutic effect compared to T@I. This improvement is attributed to the enhanced accumulation of T@I at the tumor site by T@I@E, as well as the TA-Fe complex reversing tumor hypoxia and enhancing PDT efficacy through the Fenton reaction.

PDT generates ROS at the tumor site, causing oxidative damage

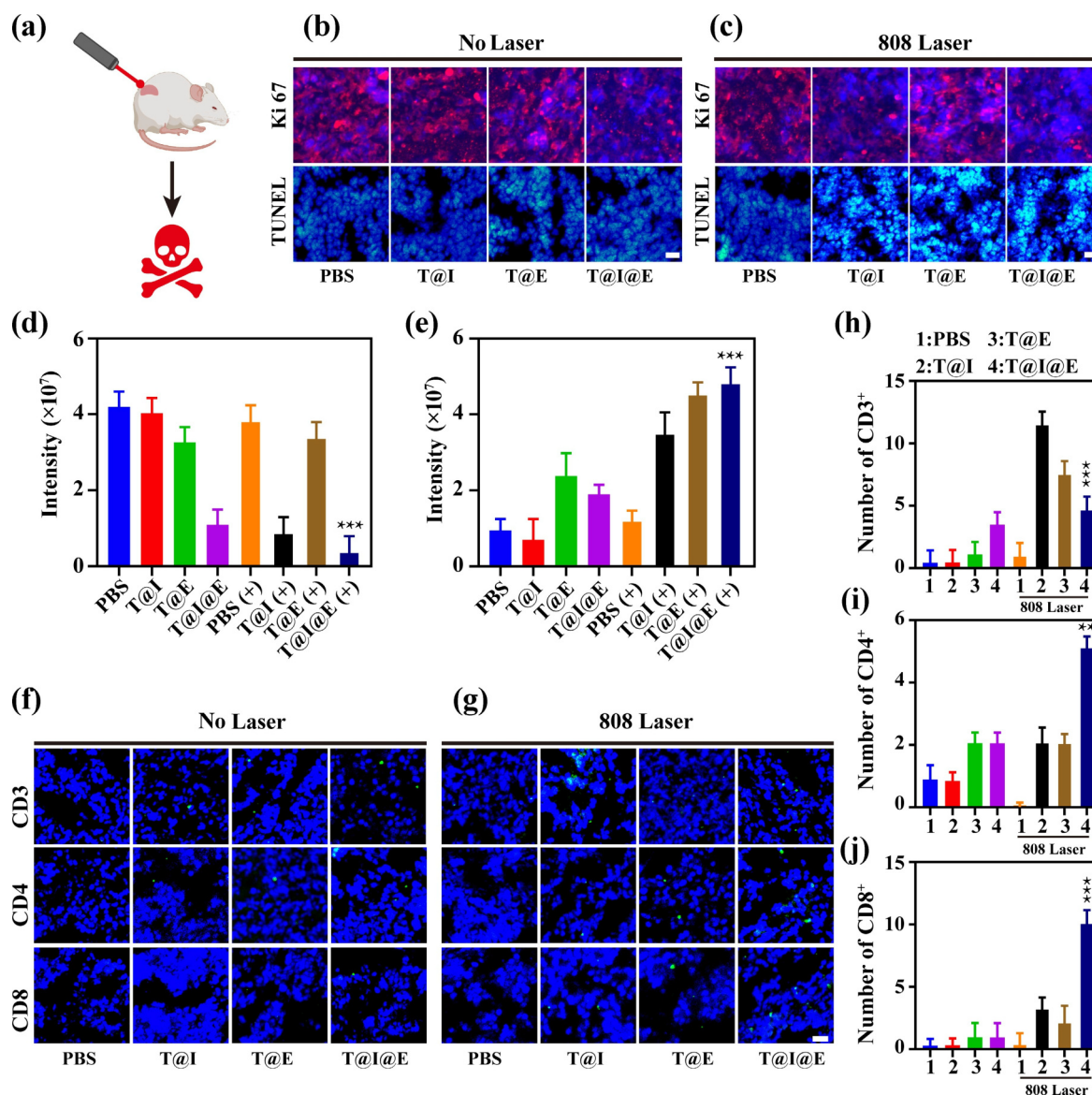


Figure 5 T@I@E recruit T cell infiltration and induce CT26 cells apoptosis *in vivo*. (a) Schematic diagram of the experimental design. Representative images of Ki67 staining, and TUNEL staining (b) with or (c) without laser treatment, scale bars: 50 μm . Quantification of Ki67 staining, and TUNEL staining (d) with or (e) without laser treatment. Representative fluorescence images of CD3⁺ T, CD4⁺ T, and CD8⁺ T cells in CT26 tumor slices (f) without or (g) with laser treatment, scale bar: 50 μm . Quantification of (h) CD3⁺ T, (i) CD4⁺ T, and (j) CD8⁺ T after various treatments. Data was presented as the mean $\pm$ SD. Statistical analysis was performed using two-tailed Student's t-tests. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

to tumor cells, inducing immunogenic cell death, and recruiting immune cells. Thus, we examined the presence of CD3⁺, CD4⁺, and CD8⁺ T lymphocytes in tumor tissues after treatment, as illustrated in Figs. 5(f) and 5(g). Compared to groups without laser irradiation, those with laser exposure displayed increased infiltration of CD3⁺, CD4⁺, and CD8⁺ positive T lymphocytes at the tumor site, quantitatively analyzed in Figs. 5(h)–5(j). These results indicate that T@I@E reverses the tumor's immune-suppressive microenvironment and enhances immune cell activity.

3.6 Immunohistochemical analysis

Under 808 nm laser irradiation, T@I@E produces ROS, inducing immunogenic cell death in tumor cells and promoting CRT expression, which recruits immune cells to infiltrate the tumor site. This establishes a combined photodynamic and immune therapy strategy. The ensuing immune response prompts immune cells to

secrete cytokines such as interleukin-6 (IL-6), IL-12, tumor necrosis factor- α (TNF- α), and interferon- γ (IFN- γ) at the tumor site. We measured these cytokines in mice during treatment.

The results, shown in Figs. 6(a)–6(h), indicate that even without laser irradiation, both T@E and T@I@E induced high expression of IL-6, IL-12, TNF- α , and IFN- γ due to ECN's role as an immune adjuvant, which stimulates the immune system via toll-like receptors. Under 808 nm laser irradiation, T@I showed strong cytokine induction. This results from ROS produced by PDT, which induces immunogenic cell death in CT26 cells, presenting "eat me" signals that recruit immune cells to the tumor site and thereby enhance cytokine expression. The combined stimulation from PDT and ECN led to the strongest cytokine expression, demonstrating that T@I@E under laser irradiation can effectively elicit an immune response.

Immunohistochemistry was then used to examine apoptosis

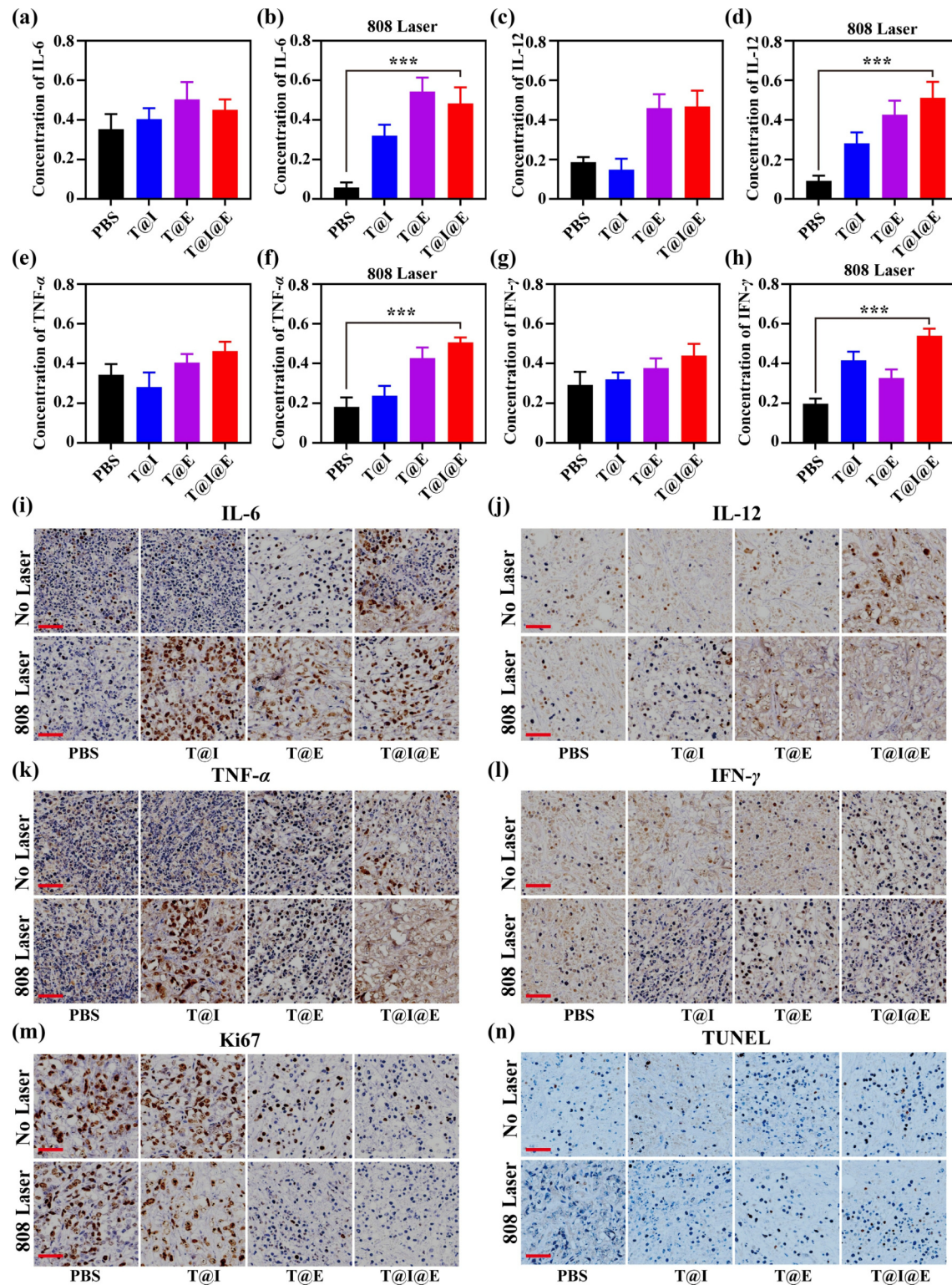


Figure 6 T@I@E induction of cytokine expression. The cytokines in the blood tested by ELISA after all various treatments: IL-6 (a) without and (b) with laser, IL-12 (c) without and (d) with laser, TNF-α (e) without and (f) with laser, IFN-γ (g) without and (h) with laser. Immunohistochemical analysis of (i) IL-6; (j) IL-12; (k) TNF-α and (l) IFN-γ at tumor site after various treatments. Immunohistochemical analysis of (m) Ki67 and (n) TUNEL after various treatments. Scale bars: 50 μm.

mechanisms at the tumor site. As shown in Figs. 6(i)–6(n), consistent with the enzyme linked immunosorbent assay (ELISA) results, T@I@E maximally recruited immune cells to infiltrate the tumor site. These effects are attributed both to the inflammatory response induced by ECN and to the immunogenic cell death

caused by PDT. Finally, tumor proliferation and apoptosis indicators were assessed. Compared to the PBS group, Ki67 expression was significantly decreased, while TUNEL expression was increased after T@I@E treatment. This indicates that laser-activated T@I@E can effectively inhibit tumor cell growth.

3.7 Biosafety of T@I@E

The biosafety of T@I@E was assessed by evaluating liver and kidney function and performing H&E staining on major organs before and after treatment. Results of liver and kidney function tests are shown in Figs. 7(a)–7(h). Compared to the PBS group, no significant changes were observed in liver function indicators (AST and ALT) following T@I@E treatment. Similarly, kidney function indicators (BUN and CRE) showed no significant alterations before and after treatment. Although mild toxicity was observed on the second day post-injection, all indicators returned to normal levels by the seventh day across all treatment groups. Additionally, no red blood cell hemolysis was observed at various T@I@E concentrations, as shown in Figs. S8 and S9 in the ESM.

To further confirm safety, H&E staining was conducted on

major organs. As illustrated in Fig. 7(i), no evidence of damage was observed in major organs, including the heart, liver, spleen, lungs, and kidneys, after 14 days of treatment. These results indicate that T@I@E exhibits good biosafety for tumor treatment.

3.8 Discussion

In our study, we successfully integrated IR775 into ECN, marking a significant advancement in the field. To ensure the resilience and longevity of this novel structure, we performed an additional encapsulation step using TA-Fe, a system similar to an MOF, to encapsulate ECN, resulting in the innovative composite T@I@E. This composite exhibited a strong responsiveness to the tumor microenvironment, facilitating precise and targeted drug release, as illustrated in Fig. 1. Notably, the encapsulation process preserved

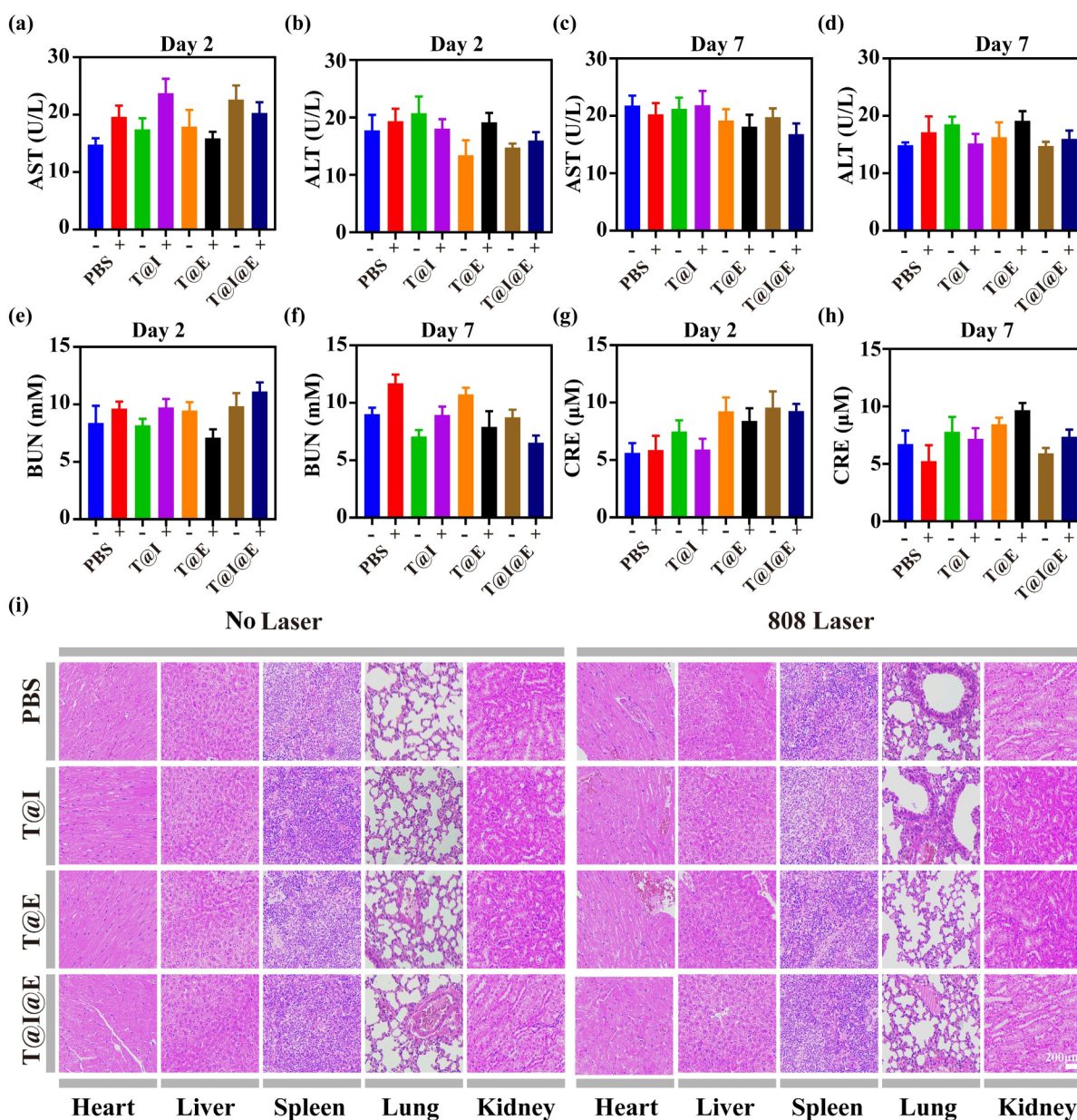


Figure 7 Biosafety tests of T@I@E on Balb/C mice. Serum biochemistry data of AST on (a) day 2 and (c) day 7, and ALT on (b) day 2 and (d) day 7 represent the liver function ($n = 3$). Serum biochemistry data of BUN on (e) day 2 and (f) day 7, and CRE on (g) day 2 and (h) day 7 represent the kidney function ($n = 3$). (i) H&E staining images of the major organs on day 14, scale bar: 200 μm . Data were presented as the mean $\pm$ SD.

ECN's functionality and enhanced its ability to actively target tumor tissues.

To evaluate T@I@E's antitumor potential, we conducted *in vitro* cytotoxicity assays (Fig. 2), which demonstrated its high efficacy in inducing apoptosis in CT26 tumor cells. Additionally, using advanced *in vivo* imaging technology, we investigated the pharmacokinetics of T@I@E, revealing its effectiveness in enhancing IR775 accumulation specifically at tumor sites after intravenous administration. This targeted delivery system underscores T@I@E's precision and efficacy in directing therapeutic agents to the tumor site.

Beyond its targeted delivery capabilities, T@I@E also modulated the tumor microenvironment. By catalyzing H_2O_2 decomposition through Fe^{3+} , it ameliorated tumor hypoxia, further inducing apoptosis in CT26 cells, as illustrated in Figs. 3 and 4. These combined mechanisms highlight the comprehensive antitumor properties of T@I@E.

Our *in vivo* experiments underscored T@I@E's potent antitumor efficacy without compromising the health of the mice, emphasizing its ability to suppress CT26 tumor growth while maintaining excellent biosafety, making it a promising candidate for clinical application. Collectively, these findings suggest the vast potential of T@I@E in cancer therapy, paving the way for more targeted and efficient antitumor strategies.

However, before clinical application can be achieved, some challenges remain. These include concerns over bacterial proliferation and the long-term stability of T@I@E post-encapsulation during storage. Continued refinements and optimizations are essential to ensure the safety, efficacy, and practicality of T@I@E for clinical use, setting the stage for its eventual clinical translation.

4 Conclusions

PDT and other precision therapeutic modalities have garnered significant attention in tumor management. However, challenges such as hypoxia and off-target effects continue to hinder the full potential of PDT. To address these obstacles, we utilized the tumor-targeting capabilities of ECN to develop a novel living delivery system, T@I@E, which effectively reverses tumor hypoxia and enhances drug accumulation at tumor sites. Rigorous experimentation has demonstrated the exceptional tumor-inhibitory efficacy of T@I@E, as shown both *in vitro* and *in vivo*.

Electronic Supplementary Material: Supplementary material (elemental mapping of ECN, the characteristic absorption peak and stability of T@I@E) is available in the online version of this article at <https://doi.org/10.26599/NR.2025.94907148>.

Data availability

Data is contained within the article and the Electronic Supplementary Material.

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Declaration of competing interest

The authors declare no conflict of interest.

Author contribution statement

L. S., L. Z., K. W., and H. H. B. conceived and designed the experiments; K.W., H. H. B., T. Y., F. S. S., C. X. Z., and D. G. C. participated in the experiments; K. W., H. H. B., and F. S. S. analyzed the data; K. W., H. H. B., T. Y., and D. G. C. wrote the manuscript; L. Z., L. S., and H. Y. supported this study. All the authors provided the final approval of the manuscript.

Informed consent

Not applicable.

Ethics statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and Approval by Ethics Committee of Shanghai University (approval No. 2024-016).

Use of AI statement

None.

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