



Hypoxia-responsive soft biohybrid bacteria for breast cancer therapy

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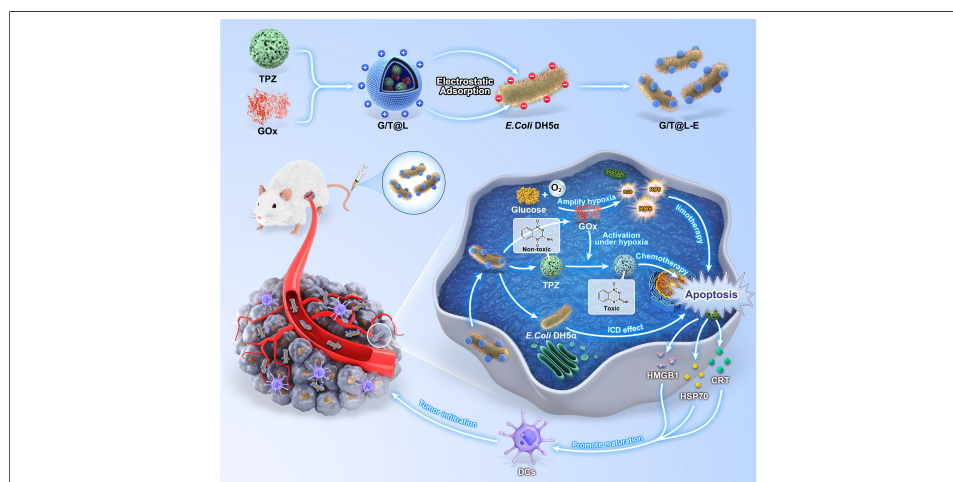
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Abstract

Because of their distinct physical and chemical characteristics, soft materials with low modulus, great deformability, and large compliance have progressively emerged as a significant area of study. However, the responsiveness of traditional soft materials is inherently limited by passive diffusion and equilibrium thermodynamics. Thus, by incorporating stimuli-responsive artificial materials into motile bacterial species with anticancer properties, a soft biohybrid bacterial system has been created, converting soft materials from static scaffolding into adaptable, living systems. Specifically, the surface of *Escherichia coli* DH5α is modified by liposomes co-loaded with glucose oxidase and Tirapazamine (TPZ). The soft biohybrid bacterial system uses anaerobic targeting to

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deliver anticancer drugs and catalyzes glucose in the tumor microenvironment, consuming local oxygen in the process. The enhanced hypoxic microenvironment fully activates TPZ, significantly boosting its cytotoxic effect on tumor cells. Moreover, the soft biohybrid bacterial system further induces strong immunogenic cell death, which enhances the therapeutic effect while minimizing side effects on normal tissues. This innovative strategy offers a promising solution to overcome the therapeutic challenges associated with traditional soft materials.

INTRODUCTION

Due to their significance in areas including biotechnology, energy, and electronics, soft materials have been the focus of much current study^[1]. Soft materials, such as hydrogels^[2], liquid crystals^[3], and polymer networks^[4], derive their functionality from reversible supramolecular interactions and mesoscale architectures. When subjected to external forces, soft materials with low stiffness or elasticity are easily deformed, which allows them to conform to complex topographies and distribute stress evenly^[5].

However, their responsiveness is inherently limited by passive diffusion and equilibrium thermodynamics^[6], lacking the ability to harvest energy actively, sense gradients, or undergo programmed morphological adaptation. For example, single-phase hydrophilic polymer networks are typically the foundation of current hydrogels with great mechanical performance, but their lack of adaptive characteristics still limits their use in complicated situations^[7]. Additionally, these robust hydrogel networks lack the requisite control to change shape in complex environments, unlike some biological tissues like muscles and cartilages^[8].

The emerging paradigm of biohybrid soft materials^[9] addresses this gap by integrating living components (bacteria^[10], mammalian cells^[11], or organelles^[12]) with synthetic matrices because of the benefits of *in vivo* biodegradation, good water solubility, self-propelling capability, and designable targeting ability. The majority of motile microorganisms have built-in molecular motors that power filamentous systems, such as flagella^[13], for independent movement that increases the bacteria's ability to penetrate tumor tissue^[14]. Biohybrid devices have previously employed flagellated bacterial species such as *Escherichia coli* (*E. coli*)^[15], *Serratia marcescens*^[16], and magnetotactic bacteria such as *Magnetococcus marinus* (MC-1)^[17]. Microorganisms have several advantages, including their small size (usually between 1 and 3 μm), high-speed motility, and, most importantly, tumor-colonizing capabilities. Using magneto-aerotaxis to deliver anticancer medications, the biohybrid microbots' autonomous movement has been demonstrated. In a seminal study, Felfoul *et al.* demonstrated that drug-loaded nanoliposomes covalently bonded to the surface of MC-1 could exploit magneto-aerotaxis for targeted delivery into tumor hypoxic regions, achieving up to 55% cell penetration^[18]. Therefore, these bacteria can be considered ideal candidates to construct biohybrid soft drug delivery for antitumor drugs. Compared with the aforementioned bacteria, *E. coli* DH5 α , a low-pathogenic mutant strain of *E. coli* which is artificially selected and bred, is a relatively safe soft biovector to deliver drugs into cancer cells owing to its good biocompatibility and biodegradability^[19]. It is envisioned that remarkable tumor therapeutic outcomes would be achieved if *E. coli* DH5 α not only selectively colonizes tumor regions through its inherent tumor-targeting ability, but also delivers hypoxia-sensitive chemotherapeutic prodrugs to tumor hypoxic areas for full activation.

In this regard, we have developed a bacterium-based drug delivery soft biohybrid system using *E. coli* DH5 α as a vector to deliver hypoxia-activated prodrug Tirapazamine (TPZ) and glucose oxidase (GOx). The developed soft biohybrid system, called G/T@L-E, is fabricated by attaching liposomes co-loaded with GOx and TPZ to the surface of *E. coli* DH5 α . G/T@L-E imparts tumor-targeting capabilities to the liposomes, enabling them to selectively colonize tumor tissues and release their cargo of GOx and TPZ, thereby protecting normal tissues from the adverse effects. Once taken up by tumor cells, the released GOx catalyzes the oxidation of glucose to produce hydrogen peroxide (H_2O_2) and simultaneously consumes oxygen (O_2),

thereby increasing the reactive oxygen species (ROS) levels and further inducing intracellular hypoxia. Subsequently, due to the enhanced hypoxic tumor microenvironment, TPZ is activated and becomes cytotoxic to tumor cells, thus facilitating efficient chemotherapy. This marriage transforms soft materials from static scaffolds into adaptive, living systems.

EXPERIMENTAL

Preparation of G/T@L

Initially, 1 mg of GOx (Aladdin) and 1 mg of TPZ (Beyotime) were dissolved in 2 mL of phosphate-buffered saline (PBS) for subsequent use. Subsequently, DPPC (Ponsure Biotechnology), DSPE-mPEG2000 (Ponsure Biotechnology), cholesterol (Yuanye), and octadecylamine (Aladdin) were combined in a round-bottom flask at a fixed weight ratio of 6:2:1:1. The mixture was heated to 65 °C for 1 h under rotary evaporation to strip off the cholesterol, yielding a thin lipid film that was dried overnight in vacuo. Afterward, to hydrate and emulsify the film, 2 mL PBS containing 1 mg GOx and 1 mg TPZ was then added to the flask and spun for 1 h. The resulting dispersion was successively extruded through 1,000-, 400-, and 200-nm membranes, then dialyzed to afford G/T@L nanoparticles (~200 nm).

Construction of G/T@L-E

E. coli (Beyotime, China) was cultured in Luria-Bertani (LB) medium using a constant temperature shaker at 37 °C. Once the bacteria reached the logarithmic growth phase, they were harvested by centrifugation at 4 °C, 2,500 × g for 5 min. The bacterial pellet was then washed three times with PBS and resuspended to a concentration of 1×10^6 colony-forming units (CFU)/mL for subsequent experiments. Specifically, 1×10^6 CFU of *E. coli* was suspended in 1 mL of PBS, and G/T@L nanoparticles at various concentrations were added to the bacterial suspension. After gentle stirring for 1 h, the *E. coli* modified with G/T@L (denoted as G/T@L-E) was collected by centrifugation.

Characterization

The hydrodynamic diameter of G/T@L and the zeta potentials of G/T@L, *E. coli* DH5α, and G/T@L-E were measured in deionized water with a Malvern Zetasizer Nano ZSE (US). The morphologies of G/T@L, G/T@L-E, and *E. coli* were examined under a high-resolution transmission electron microscope (FEI-Talos F200S, US). The absorbance spectra of G/T@L were measured via a ultraviolet–visible–near-infrared (UV-vis-NIR) spectrometer (Thermo Fisher, China).

Catalytic ability measurement

To evaluate the catalytic performance of G/T@L, a set of experiments was conducted. Specifically, 10 mL of glucose solution (1 mg/mL) was treated with 100 μL of PBS solutions containing G@L (1 mg/mL), G/T@L (1 mg/mL), or free GOx at various concentrations. The reactions were carried out at room temperature under ambient air conditions. The pH of the reaction mixture was monitored at different time points (0–120 min) using a pH meter (METTLER TOLEDO, China). The concentration of H₂O₂ generated was determined using a Hydrogen Peroxide Assay Kit (Bestio, China). Additionally, the oxygen content in the mixture was measured using a dissolved oxygen meter equipped with an oxygen probe (Shanghai REX Instrument Factory, China).

Growth characteristics of *E. coli* and G/T@L-E *in vitro*

E. coli DH5α was grown overnight at 37 °C as described above, and the resulting culture was used to prepare G/T@L-E. Both the original *E. coli* DH5α and G/T@L-E cultures were then diluted into 50 mL LB medium at 1:50 and cultured for 24 h at 37 °C. At each designated time point (0, 2, 4, 6, 8, 10, 12, and 24 h), OD₆₀₀ was recorded and viable counts were determined by plating serial dilutions on solid LB agar.

Cell culture

Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum (Gibco, US) and 1% (v/v) penicillin–streptomycin (Epizyme, China) was used to cultivate 4T1 tumor cells. The cells were kept at 37 °C in an incubator with 5% CO₂. All of the cells were obtained from the Cell Bank of the Chinese Academy of Science (Shanghai, China).

In vitro cell viability and live/dead staining

To detect cell viability, a standard Cell Counting Kit-8 (CCK-8, Bestbio) assay was performed. 4T1 cells were incubated with *E. coli* alone, L-E (blank liposomes attached to *E. coli* DH5α), G@L-E (liposomes loaded with GOx attached to *E. coli* DH5α), T@L-E (liposomes loaded with TPZ attached to *E. coli* DH5α), and G/T@L-E. The cytotoxicity was then assessed using the CCK-8 technique.

For the live/dead fluorescence staining assay, 4T1 cells were treated with the different treatments (the groups were set as: PBS, L-E, G@L-E, T@L-E, G/T@L, and G/T@L-E). After 24 h, the cells were rinsed twice with PBS and then stained for fluorescence imaging.

The cytotoxicity under normoxic and hypoxic conditions was evaluated using the CCK-8 assay. For normoxic conditions, cells were cultured in an incubator with 21% O₂ and 5% CO₂; for hypoxic conditions, cells were transferred to a sealed container containing AnaeroGen (Thermo Scientific) to achieve a controlled atmosphere of 1% O₂ and 5% CO₂. After pre-conditioning under respective oxygen conditions, cells were applied with various treatments (the groups were set as: G@L-E, T@L-E, and G/T@L-E, at a concentration of 1 × 10⁵ CFU/mL *E. coli* DH5α). After 24 h, the cytotoxicity was then assessed using the CCK-8 technique. The cytotoxicity was then assessed using the CCK-8 technique. To evaluate the glucose-dependent cytotoxicity, the culture medium was replaced with fresh medium containing either 25 mM glucose (with glucose) or glucose-free medium (without glucose) with different treatments. After 24 h, the cytotoxicity was then assessed using the CCK-8 technique.

Cellular uptake assay

To evaluate the internalization of G/T@L-E, a fluorescence-based cellular uptake assay was conducted. 4T1 cells were treated with the different treatments: (1) Control: without any treatment; (2) *E. coli* [bare *E. coli* dyed with fluorescein isothiocyanate (FITC)]; (3) G/T@L (liposomes dyed with DiI); (4) G/T@L-E (FITC-dyed *E. coli* with DiI-dyed liposomes). All groups were incubated for 4 h in the dark to allow cellular uptake.

Intracellular ROS detection

Using 2,7-dichlorodihydrofluorescein diacetate (DCFH-DA) as a fluorescent probe, we monitored intracellular H₂O₂ in 4T1 cells after the indicated treatments; untreated cells served as controls. Following an 8-hour incubation period, the cells were stained with DCFH-DA (Bestbio) for 2 h. The signal of intracellular ROS was then visualized using confocal laser scanning microscopy (CLSM) (Nikon Ti2, Japan).

Hypoxia-inducible factor-1α immunostaining

A hypoxic environment was established by incubating the cells with AnaeroGen (Thermo Scientific) for 8 h. During a pre-incubation period of 4 h, 4T1 cells were exposed to several treatments. Subsequently, the cells were probed with a primary antibody against hypoxia-inducible factor-1α (HIF-1α, Abcam) and a secondary antibody, Goat Anti-Rabbit IgG (H+L) Rhodamine (TRITC) (Abways TECHNOLOGY W). F-actin was simultaneously labeled with Actin-Tracker Green-488 (Beyotime).

Intracellular O₂ evaluation

The efficiency of O₂ depletion within cells was assessed using an O₂-sensing probe, [(Ru(dpp)₃)]Cl₂ (Sigma-Aldrich, Co. Ltd.), whose fluorescence is strongly quenched by O₂. The cells were incubated with the

$[(Ru(dpp)_3)]Cl_2$ for 2 h, followed by treatment with Control, L-E, G@L-E, T@L-E, G/T@L-E (each containing 1×10^4 CFU of *E. coli*), and hypoxia for an additional 4 h. Cells were subjected to hypoxia using an AnaeroGen (Thermo Scientific) for 4 h, followed by imaging on an inverted fluorescence microscope to capture $[(Ru(dpp)_3)]Cl_2$ fluorescence.

In vitro immunogenic cell death expression assay

Immunogenic cell death (ICD) in tumor cells was evaluated by immunofluorescence quantification of surface calreticulin (CRT), extracellular high mobility group box 1 (HMGB1), and intracellular heat shock protein 70 (HSP70). After 24 h of treatment with control, L-E, T@L-E, G@L-E, G/T@L, or G/T@L-E, cells were incubated with rabbit monoclonal antibodies against HMGB1 (Abcam), HSP70 (Proteintech), and CRT (Proteintech) overnight at 4 °C, and then stained with Goat Anti-Rabbit IgG (H+L) Rhodamine (TRITC) (Abways TECHNOLOGY W) for 1 h at 37 °C. The cells were also stained with 4',6-diamidino-2-phenylindole (DAPI, Beyotime) or Actin-Tracker Green-488 (Beyotime) for 20 min and observed using CLSM.

Animal model

Female BALB/c mice, aged 5 weeks and weighing 20–24 g, were obtained from the Animal Center of Southern Medical University. The mice were housed under specific pathogen-free (SPF) conditions with free access to food and water in a 22–24 °C, 30%–70% humidity, and a 12 h light/dark cycle environment. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanfang Hospital (Permit number: IACUC-LAC-20240820-002). All experiments were performed in the SPF facility of the Laboratory Animal Center, Nanfang Hospital. 4T1 tumors were induced by subcutaneously injecting 100 μ L of 4T1 cells (1×10^6 cells in PBS) into the second mammary fat pad of each mouse.

In vivo anticancer effect and immune activation exploration

By using a computer-generated random number table, 4T1 tumor-bearing mice were randomly divided into six groups ($n = 6$ per group): (1) PBS, (2) *E. coli* (10^6 CFU), (3) G@L-E (10^6 CFU), (4) T@L-E (10^6 CFU), (5) G/T@L (5 mg/kg), and (6) G/T@L-E (10^6 CFU). When the tumor volumes reached 50 mm³, the corresponding treatments were administered via intravenous injection. Mice were intravenously injected via the tail vein at 2-day intervals. Tumor volumes and body weights were recorded every 2 days over the 14-day experimental period. Tumor volumes were calculated via the formula: width² $\times$ length/2. Upon treatment completion, tumors were harvested and processed for H&E staining, Ki-67 immunostaining, and TUNEL assays.

For ICD immunofluorescence staining, 4T1 tumor-bearing mice were randomly divided into six groups ($n = 3$ per group). After 24 h of respective treatments, tumors were harvested for immunofluorescence staining of CRT, HSP70, and HMGB1.

The investigator responsible for tumor measurement and data analysis was blinded to the treatment allocation. Mice were excluded if they showed signs of severe distress (e.g., > 20% body weight loss, severe infection, or tumor ulceration) or if tumor volumes exceeded 2,000 mm³ prior to the scheduled endpoint. Animals reaching these endpoints were humanely euthanized by CO₂ asphyxiation followed by cervical dislocation. All surviving animals were euthanized with the same protocol at the termination of the experiment.

G/T@L-E colonization *in vivo*

To investigate the distribution of G/T@L-E in 4T1 tumor-bearing mice, we administered G/T@L-E (10^6 CFU) via injection. At various time points post-injection (12 h, 24 h, and 7 days), we harvested the major organs (heart, liver, spleen, lung, kidney, and tumor) from the mice. These organs were then excised, weighed, and homogenized in sterile PBS. Serial dilutions of the homogenates (10^{-1} , 10^{-2} , 10^{-3} , and 10^{-4}) were prepared and cultured on LB agar. Following 24 h of incubation, colonies were recorded, and bacterial titers were calculated as CFU per gram of tissue.

Statistical analysis

All data are presented as mean $\pm$ standard deviation (SD). GraphPad Prism (version 9.5.1) was used to perform statistical analysis. Statistical differences were evaluated using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test, or two-way ANOVA followed by Tukey's multiple comparisons test. * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$.

RESULTS AND DISCUSSIONS

Fabrication and characterization

The fabrication of the biohybrid soft material started with the construction of a hypoxia-responsive liposomal nanocarrier, followed by its electrostatic self-assembly onto the surface of living *E. coli* DH5 α to form a bacterium-lipid hybrid soft interface. Water-soluble O₂ consumer GOx and hypoxia-activatable prodrug TPZ were first loaded into the hydrophilic cores of liposomes (G/T@L) through the thin-film hydration method^[20]. Liposomal G/T@L was prepared using a lipid mixture of DPPC, DSPE-mPEG2000, cholesterol, and octadecylamine in a 6:2:1:1 mass proportion. The morphology and size distributions of G/T@L were then characterized using transmission electron microscopy (TEM), scanning electron microscopy (SEM), and dynamic light scattering (DLS). According to the TEM image [Figure 1A], a quasi-spherical shape was clearly observed with an average particle size of approximately 200 nm. DLS measurement of G/T@L shared a similar particle size with a polydispersity index of 0.109 [Figure 1B]. Quantitative assays revealed that GOx and TPZ were loaded in the liposomes at an encapsulation efficiency of 63.09% and 57.14%, respectively, as measured by a bicinchoninic acid protein assay kit and UV-vis spectroscopy [Supplementary Figure 1]. The UV-vis spectrum of G/T@L displayed a distinct absorption peak at 475 nm arising from GOx and another at 275 nm attributable to TPZ, confirming the co-encapsulation of both agents [Figure 1C]. To exploit the tumor-homing property of *E. coli* for therapeutic delivery, we constructed a biohybrid system by immobilizing GOx/TPZ-loaded liposomes (G/T@L) onto the surface of *E. coli* (designated as G/T@L-E). G/T@L-E was created when G/T@L was anchored onto the surface of *E. coli* DH5 α through electrostatic interaction. The zeta potential of the bacteria shifted significantly from -29.4 to +11.72 mV after liposome anchoring [Figure 1D], which was another evidence for surface modification. *E. coli* DH5 α displayed a rod-shaped morphology with a length of approximately 1 μ m. After liposome anchoring, a rough layer of G/T@L-E was clearly observed [Figure 1E], thereby confirming the stable attachment of G/T@L. The lipophilic dye DiI was mixed with pre-prepared liposomes to generate DiI-labeled liposomes, and *E. coli* DH5 α was co-incubated with FITC dye via isothiocyanate-mediated coupling to primary amines on bacterial surface proteins to produce FITC-labeled bacteria. The co-localization of G/T@L with *E. coli* DH5 α was visualized using CLSM [Figure 1F]. DiI-labeled liposomes around the FITC-labeled bacteria indicated the successful G/T@L attachment on the surface of *E. coli* DH5 α . To further validate these confocal microscopy results, flow cytometry was also carried out, and the data indicated that after mixing G/T@L (200 μ g/mL) and *E. coli* DH5 α (1×10^6 CFU/mL), more than 95% of *E. coli* DH5 α were successfully associated with G/T@L [Figure 1G]. All these findings confirmed the successful fabrication of G/T@L-E.

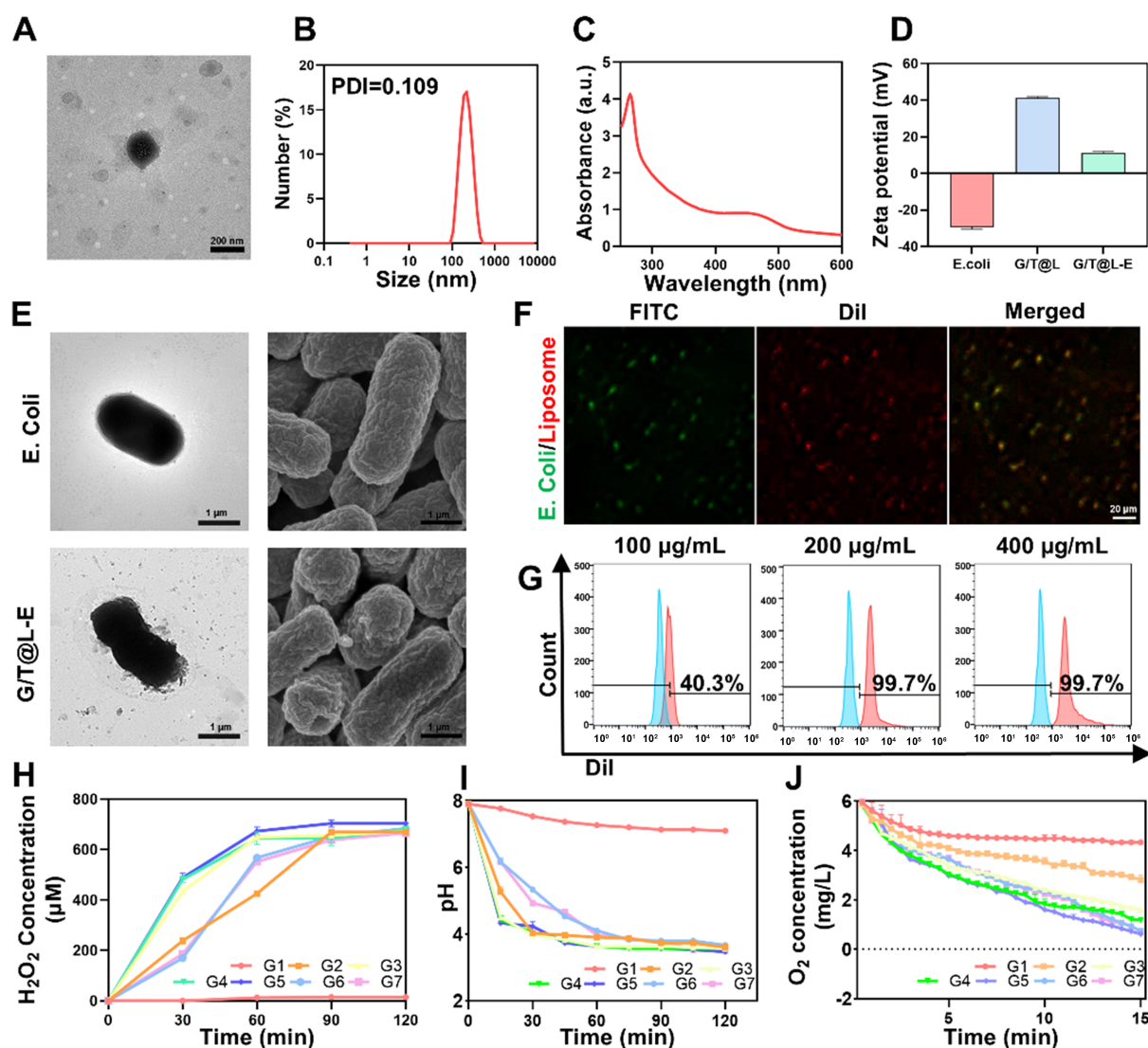


Figure 1. Characterization of G/T@L-E. (A) TEM image of G/T@L. Scale bar: 200 nm; (B) Size distribution of G/T@L via DLS; (C) UV-vis absorbance spectra of G/T@L; (D) Zeta potentials of *E. coli*, G/T@L, and G/T@L-E. $n = 3$; (E) TEM and SEM images of *E. coli* DH5 α and G/T@L-E. Scale bar: 1 μ m; (F) Typical CLSM images of G/T@L-E. The green and red channels indicate *E. coli* labeled with FITC and G/T@L labeled with Dil, respectively. Scale bar: 20 μ m; (G) Flow cytometric analysis of *E. coli* conjugated with different proportions of Dil-labeled liposome. Blue: bare *E. coli*; red: G/T@L-E; (H) H_2O_2 concentration in glucose solution (1 mg/mL, 10 mL) after addition of free GOx, G@L or G/T@L. $n = 3$; (I) pH decreases in glucose solution (1 mg/mL, 10 mL) with time after addition of free GOx, G@L or G/T@L. $n = 3$; (J) Time-dependent changes in dissolved O_2 in buffered saline with varied concentrations of free GOx, G@L and G/T@L in the glucose solution (1 mg/mL, 10 mL). $n = 3$. Data are presented as mean $\pm$ SD (The groups are defined as follows: G1: Control; G2: 0.2 mg/mL free GOx; G3: 0.4 mg/mL free GOx; G4: 0.6 mg/mL free GOx; G5: 0.8 mg/mL free GOx; G6: 1 mg/mL G@L; G7: 1 mg/mL G/T@L). TEM: Transmission electron microscopy; DLS: dynamic light scattering; UV: ultraviolet; *E. coli*: *Escherichia coli*; SEM: scanning electron microscopy; CLSM: confocal laser scanning microscopy; FITC: fluorescein isothiocyanate; GOx: glucose oxidase; SD: standard deviation; PDI: polydispersity index.

In this biohybrid soft material system, the loaded GOx can consume O_2 and glucose to generate gluconic acid and H_2O_2 . To explore the enzymatic activity of GOx in G/T@L-E, the reaction products, including H_2O_2 and oxygen content, and the corresponding pH change were then monitored. Upon addition of GOx-loaded liposomes (G/T@L or G@L), the resulting H_2O_2 concentration was monitored. G@L and G/T@L (1 mg/mL) generated slightly less H_2O_2 compared to free GOx (0.8 mg/mL) within 2 h, which implied that after liposomal encapsulation, GOx could still effectively catalyze glucose into H_2O_2 , showing no detectable loss in catalytic ability compared to free GOx [Figure 1H]. Meanwhile, GOx can consume O_2 and glucose to

generate gluconic acid, leading to a decrease in pH value. Significant decreases in pH were continuously observed. The pH of the G@L and G/T@L groups decreased more slowly than that of the free GOx, but it reached the same value as the free GOx group at 120 min [Figure 1I]. Most critically, we expect G/T@L-E to kill tumor cells by catalyzing the conversion of glucose to H_2O_2 while simultaneously amplifying intracellular oxygen depletion via GOx-mediated consumption. This intensified hypoxia activates TPZ, synergistically amplifying their combined antitumor activity. To quantify the O_2 -depleting capacity of G/T@L, we measured dissolved oxygen using a portable dissolved oxygen meter. A rapid depletion of O_2 was observed for all groups, and the dissolved oxygen content of G@L and G/T@L exhibited no significant difference from that of the free GOx group [Figure 1J]. We can infer that the pronounced oxygen-consuming capacity exhibited by G/T@L may be conducive to the hypoxic activation of TPZ. Overall, the encapsulated GOx within liposomes retained comparable oxygen-consuming and catalytic abilities compared to free GOx, with no significant loss of function.

Intracellular behavior of G/T@L-E

After confirming the successful fabrication of G/T@L-E, we investigated the intracellular behavior of the engineered bacterium. To determine whether the fabrication procedure and the conjugated liposomes exert any toxic effects on bacterial viability, we evaluated the growth of G/T@L-E. As shown in Figure 2A, G/T@L-E displayed a similar growth curve to normal *E. coli* DH5 α , indicating the surface liposome coating did not impact the vitality of *E. coli* DH5 α . Meanwhile, the growth of both *E. coli* DH5 α and G/T@L-E on LB solid medium was also investigated, which further confirmed the aforementioned view [Supplementary Figure 2]. Subsequently, the *in vitro* behavior of G/T@L-E was investigated through a comprehensive set of cell experiments. As controls, we established five groups: (i) *E. coli* DH5 α alone; (ii) L-E: blank liposomes attaching to *E. coli* DH5 α ; (iii) G@L-E: liposomes loaded with GOx attaching to *E. coli* DH5 α ; (iv) T@L-E: liposomes loaded with TPZ attaching to *E. coli* DH5 α ; and (v) G/T@L-E. The cytotoxicity of G/T@L-E was first measured based on the CCK-8 cell viability assay. It was found that G/T@L-E exhibited significant concentration-dependent toxicity against 4T1 tumor cells [Figure 2B]. The CCK-8 experimental findings revealed that, at a concentration of 1×10^5 CFU/mL *E. coli* DH5 α , the viability of 4T1 cells in the G/T@L-E group was markedly lower than that in other groups, while the G@L-E and T@L-E group exhibited no appreciable difference compared with *E. coli* DH5 α and L-E group. This indicates that the cytotoxicity of GOx or TPZ used individually is not satisfactory under normoxic conditions, but the combination of GOx and TPZ can exert greater toxicity towards tumor cells. As shown in Supplementary Figure 3, G/T@L-E exhibited significantly higher cytotoxicity than G/T@L, indicating that the *E. coli* carrier substantially potentiates the antitumor efficacy of the liposome. Furthermore, live/death cell staining images of these groups further support the CCK-8 results that the G/T@L-E group showed the strongest toxicity towards 4T1 cells among the groups [Figure 2C]. The minor difference between live/dead staining and the CCK-8 assay for the T@L-E group likely reflects the divergent endpoints of the two assays, as TPZ may compromise membrane integrity while transiently preserving metabolic activity.

To investigate the endocytosis of G/T@L-E by tumor cells, 4T1 cells were incubated with G/T@L-E, which was composed of FITC-labeled *E. coli* DH5 α and DiI-labeled liposomes. Subsequently, the cells were visualized using CLSM. Compared to the control group, the CLSM images revealed significant green (FITC) and red (DiI) fluorescence surrounding the nuclei of 4T1 cells [Figure 2D]. This observation indicates that G/T@L-E can be efficiently internalized by 4T1 cells because of the efficient cellular internalization of *E. coli*. This result indicates that *E. coli* can serve as a biological carrier capable of delivering payloads directly into tumor cells. After G/T@L-E entered 4T1 cells, we observed that the bacteria mainly surrounded the nucleus while the liposomes were scattered in the cytoplasm. We also found that G@L-E and G/T@L-E had limited toxicity against 4T1 cells within a glucose-free cell culture medium but much stronger toxicity within a glucose cell culture medium [Figure 2E], which may imply that they exerted a better killing effect in

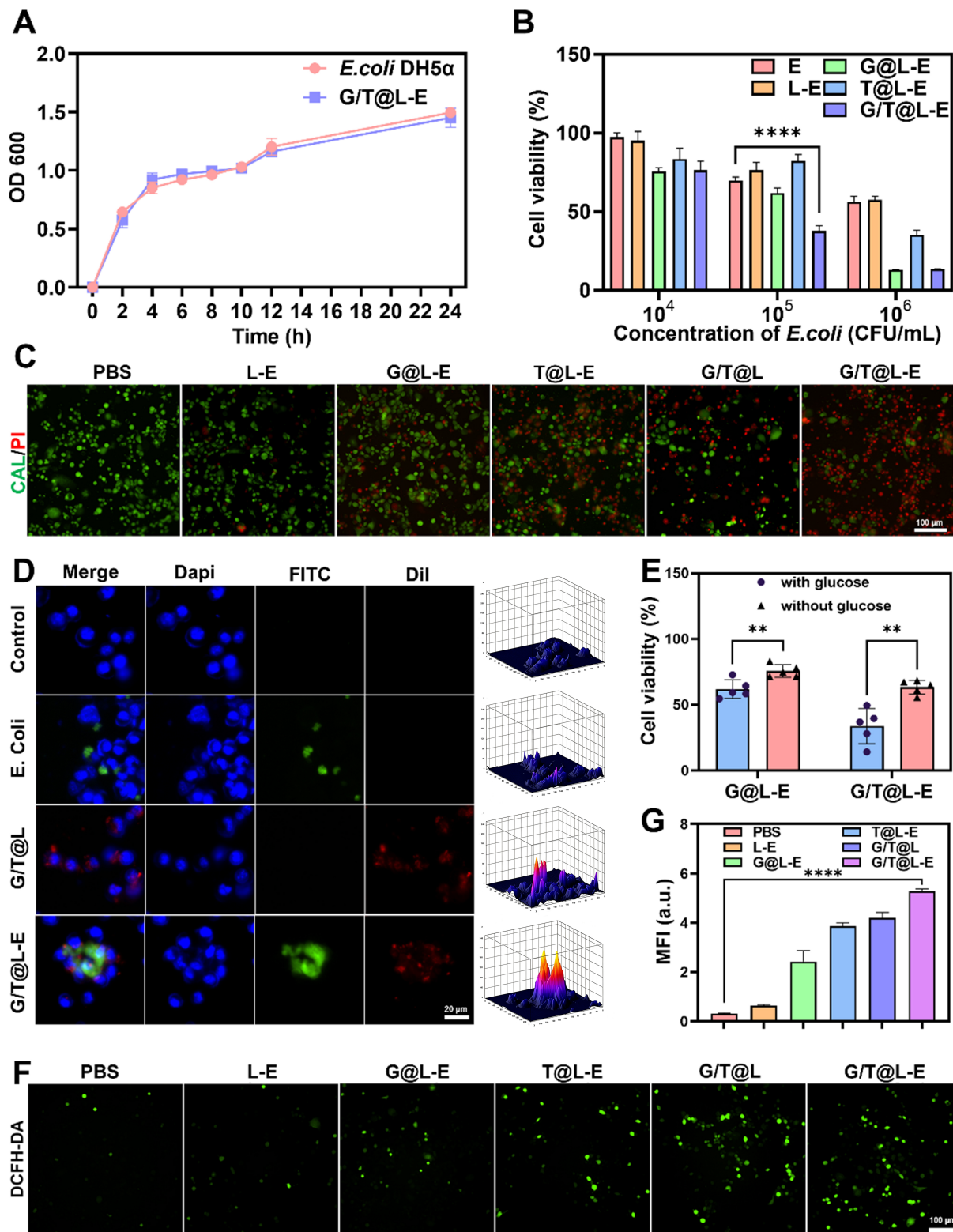


Figure 2. Intracellular behavior of G/T@L-E. (A) Growth curves of *E. coli* DH5α and G/T@L-E. $n = 3$; (B) Cell viability of 4T1 cells following different treatments. $n = 5$; (C) CLSM images of 4T1 cells stained with live/dead after various treatments. Scale bar: 100 μm; (D) CLSM images of 4T1 cells incubated with different treatments for 4 h. Scale bar: 20 μm; (E) Relative viabilities of 4T1 cells after 24 h incubation with different treatments with or without glucose. $n = 5$; (F and G) CLSM images and the corresponding fluorescence intensity of DCFH-DA-stained 4T1 cells. $n = 3$. Scale bar: 100 μm. Data are presented as mean $\pm$ SD. Significance was compared by one-way ANOVA followed by Dunnett's multiple comparisons test or two-way ANOVA followed by Tukey's multiple comparisons test. ** $P < 0.01$, **** $P < 0.0001$. *E. coli*: *Escherichia coli*; CLSM: confocal laser scanning microscopy; DCFH-DA: 2,7-dichlorodihydrofluorescein diacetate; SD: standard deviation; ANOVA: analysis of variance; CFU: colony-forming unit; CAL: calcein acetoxymethyl; PI: propidium iodide; PBS: phosphate-buffered saline; FITC: fluorescein isothiocyanate.

glucose-rich tumor cells than in normal tissue cells. A key hypothesis of this study is that GOx catalyzes O_2 consumption to exacerbate hypoxia levels in tumor cells, thereby amplifying the combinatory efficacy of TPZ and GOx^[21]. GOx catalyzes glucose oxidation and consumes O_2 to produce gluconic acid and ROS. Among these, H_2O_2 is a well-known ROS that has been widely utilized in antitumor strategies due to its proven ability to cause oxidative harm to tumor cells^[22]. To visualize intracellular H_2O_2 production, DCFH-DA was employed. Upon oxidation by H_2O_2 , DCFH-DA emits bright green fluorescence, allowing for the detection of H_2O_2 generation within cells. The fluorescence images revealed that the G/T@L-E group displayed the most intense fluorescence, thereby confirming the highest generation of H_2O_2 [Figure 2F and G]. This indicated that the GOx loaded in the system maintained good enzyme activity after being internalized by tumor cells and was able to catalyze glucose to generate H_2O_2 while consuming O_2 . The O_2 consumption made by GOx created a hypoxic microenvironment that activates TPZ, generating additional radical species. The synergistic interplay between GOX and TPZ amplified the overall ROS output in the G/T@L-E group.

***In vitro* hypoxia amplification of G/T@L-E**

We further evaluated the capacity of G/T@L-E to actively modulate the local oxygen microenvironment using the luminescent oxygen sensor $Ru(dpp)_3Cl_2$. Among all groups, G/T@L-E induced the strongest red fluorescence [Figure 3A]. The fluorescence quantification analysis also revealed that the level of hypoxia induced by G/T@L-E, comparable to that observed in 4T1 cells cultured under hypoxic conditions, was even slightly more pronounced, which was 111% for the hypoxia group [Figure 3B]. This can be inferred that GOx in the system consumes O_2 effectively, thereby amplifying the tensor hypoxia level in tumor cells in normoxia environment. Robust hypoxia can effectively trigger the activation of the hypoxia-sensitive prodrug TPZ, thus facilitating its application in anticancer chemotherapy^[23]. Due to the catalytic properties of GOx, we also observed that, in the presence of glucose, G/T@L-E could induce a more pronounced cellular hypoxia compared to the glucose-free condition [Figure 3C and D]. This finding may suggest that G/T@L-E can effectively deplete glucose at the tumor site, thereby demonstrating enhanced antitumor ability. In light of the fact that HIF-1 α is the primary transcriptional regulator governing cellular hypoxia responses^[24], HIF-1 α levels were quantified through immunofluorescence staining. Imaging by CLSM demonstrated that the nuclei of cells treated with G/T@L-E exhibited elevated levels of HIF-1 α [Figure 3E]. Furthermore, fluorescence quantification analysis provided additional confirmation, showing that the fluorescence intensity in the G/T@L-E group was 168% of that observed in the hypoxia group, thereby demonstrating that G/T@L-E effectively induced severe hypoxia in tumor cells [Supplementary Figure 4].

Next, 4T1 cells were incubated with G@L-E, T@L-E, or G/T@L-E in normoxic or hypoxic environments for 24 h. The viability of 4T1 cells treated with T@L-E exhibited a marked decrease under hypoxic conditions when compared to normoxic conditions, thereby confirming that the cytotoxicity of TPZ is activated by hypoxic stress. Notably, G/T@L-E induced a cell growth inhibition in a normoxic environment that was similar to that observed under hypoxic conditions [Figure 3F]. This suggests that G/T@L-E can exert the same effective cytotoxicity in well-oxygenated cells in tumor tissues as in hypoxic cells, guaranteeing the significant anti-tumor effect in the whole tumor area, including hypoxic cells and well-oxygenated cells. As a result, the hypoxia-responsive functionality is no longer restricted to pre-existing hypoxic regions; instead, the soft material G/T@L-E itself created the required low-oxygen condition on demand, thus amplifying antitumor therapy.

***In vitro* ICD induction of G/T@L-E**

Such extensive tumor cell death caused by G/T@L-E is expected to trigger a series of immune responses within the tumor microenvironment (TME). Dendritic cells (DCs) function as essential antigen-presenting cells, connecting innate and adaptive immune responses. In their immature state, DCs eliminate antigens released by dying tumor cells, a process that drives their own maturation. After maturation, the DCs migrate

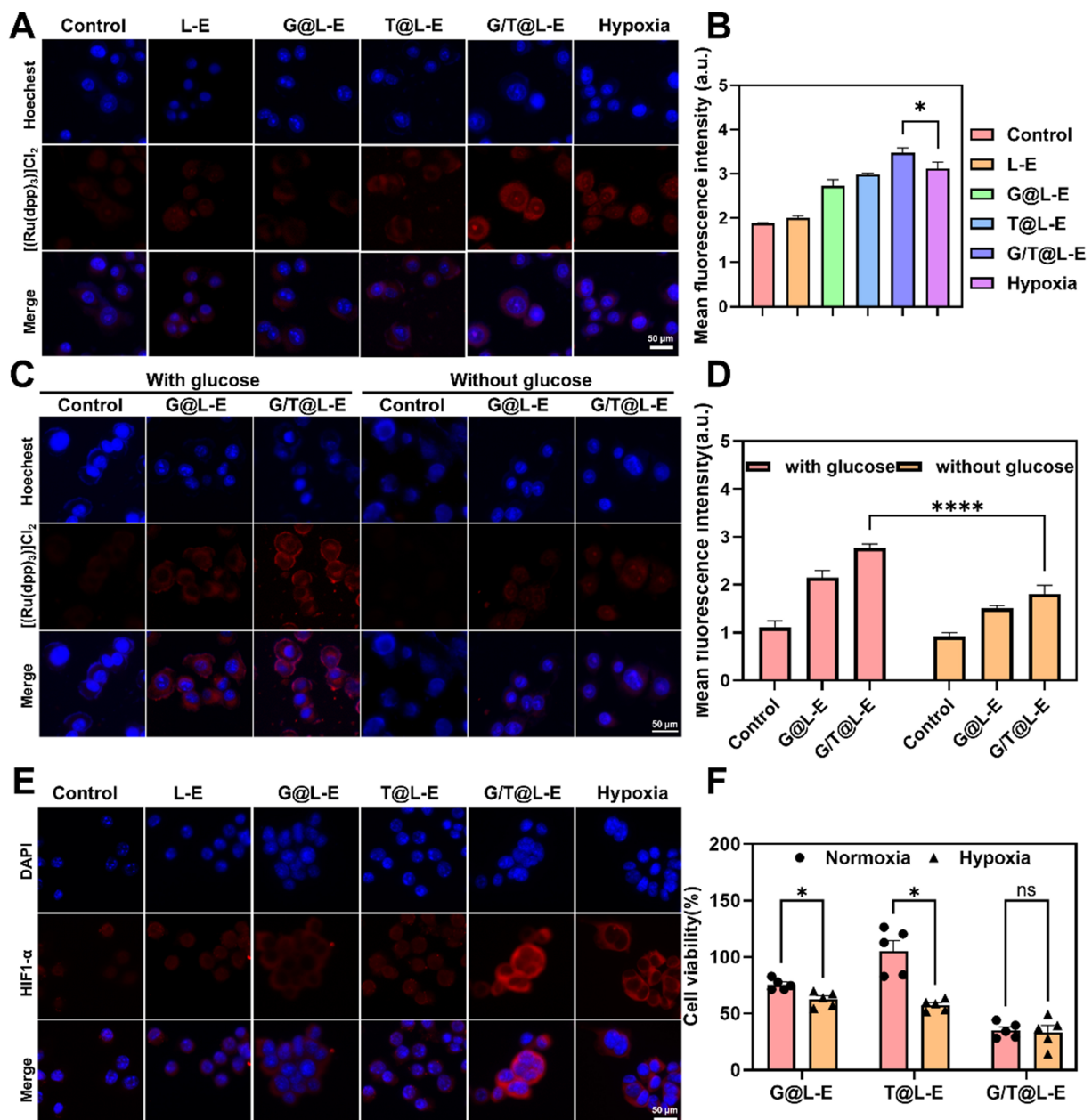


Figure 3. *In vitro* hypoxia amplification of G/T@L-E against 4T1 cells. (A) Confocal fluorescence images of the hypoxia level in 4T1 cells with various treatments. Scale bar = 50 μ m; (B) The corresponding fluorescence intensities of the hypoxia level in 4T1 cells with various treatments quantified by ImageJ software. $n = 3$; (C) Confocal fluorescence images of the hypoxia level in 4T1 cells cocultured with or without glucose. Scale bar = 50 μ m; (D) The corresponding fluorescence intensities of the hypoxia level in 4T1 cells cocultured with various treatments quantified by ImageJ software. $n = 3$; (E) Immunofluorescence images of HIF-1 α in 4T1 cells after different treatments. Scale bar = 50 μ m; (F) Cell viability of 4T1 cells after different treatments in normoxia or hypoxic conditions. $n = 5$. Data are presented as mean $\pm$ SD. Significance was compared by one-way ANOVA followed by Dunnett's multiple comparisons test or two-way ANOVA followed by Tukey's multiple comparisons test. * $P < 0.05$, **** $P < 0.0001$, ns: (not significant) $P \geq 0.05$. HIF-1 α : Hypoxia-inducible factor-1 α ; SD: standard deviation; ANOVA: analysis of variance.

to the lymph nodes, where they present antigens to naive T cells. Leveraging this process, we next examined how different treatments affected the immunogenicity of 4T1 cells. As depicted in Figure 4A, pretreated 4T1 cells were seeded in the upper compartment, with immature DCs placed in the lower compartment of a transwell system. Following a 24 h incubation with 4T1 cells pretreated with G/T@L-E, numerous synapse-like structures were observed on the surfaces of DCs [Figure 4B]. This observation suggested that DCs

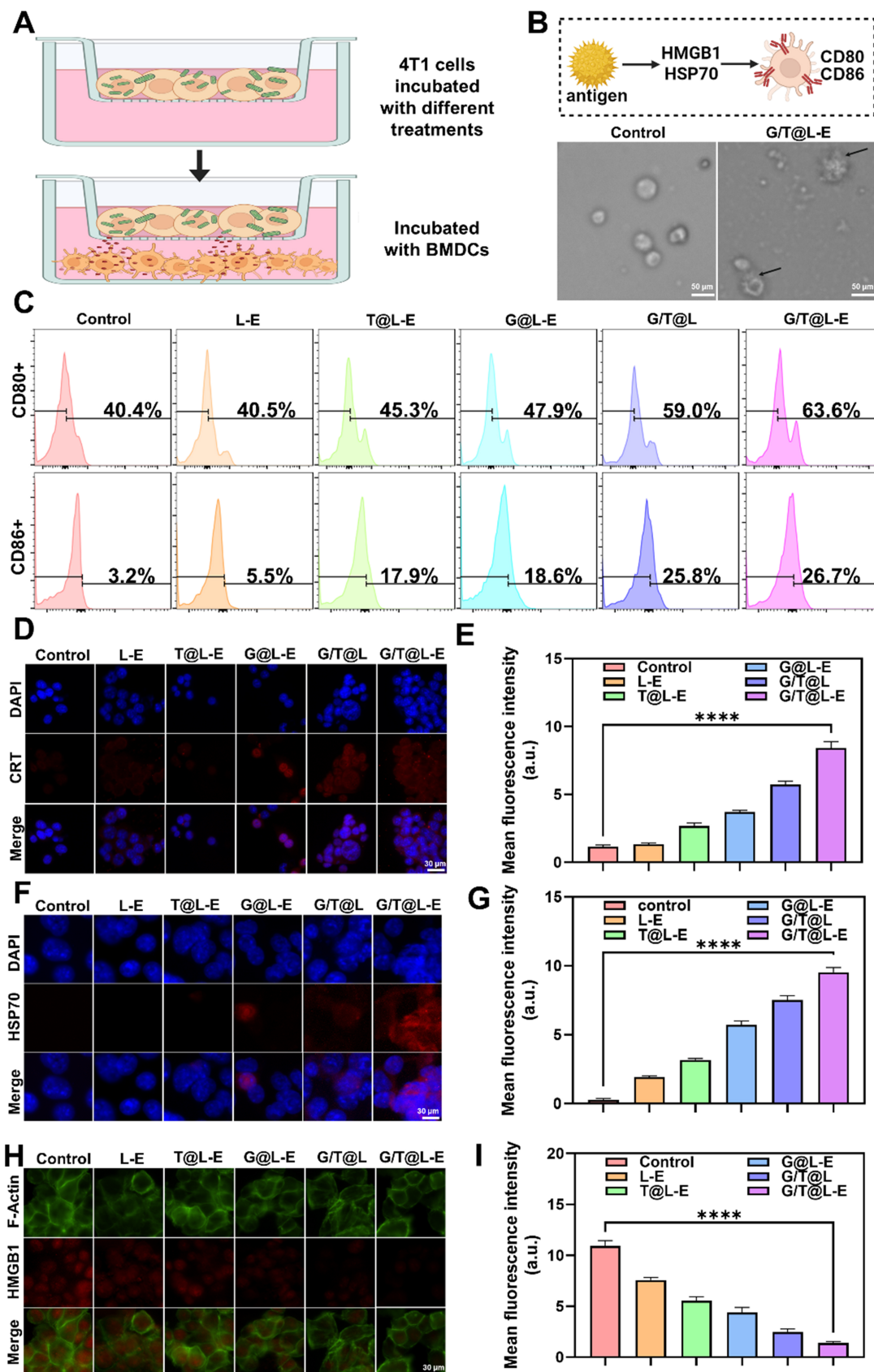


Figure 4. *In vitro* ICD induction of G/T@L-E against 4T1 cells. (A) Schematic diagram of the experiment to explore DC maturation *in vitro*; (B) Optical microscopic images of DCs following incubation. Scale bar: 50 μ m; (C) FCM analysis of CD80 and CD86 expression on CD11c+DCs following various treatments; (D) Immunofluorescence images of CRT of 4T1 cells after each treatment. Scale bar: 30 μ m; (E) The corresponding fluorescence intensities of CRT quantified by ImageJ software. $n = 3$; (F) Immunofluorescence images of HSP70 in 4T1 cells after each treatment. Scale bar: 30 μ m; (G) The corresponding fluorescence intensities of HSP70 quantified by ImageJ software. $n =$

3; (H) Immunofluorescence images of HMGB1 in 4T1 cells after each treatment. Scale bar: 30 μm ; (I) The corresponding fluorescence intensities of HMGB1 quantified by ImageJ software. $n = 3$. Data are presented as mean $\pm$ SD. Significance was compared by one-way ANOVA followed by Dunnett's multiple comparisons test or two-way ANOVA followed by Tukey's multiple comparisons test. **** $P < 0.0001$. Some elements in (A and B) were created with BioRender.com. ICD: Immunogenic cell death; DC: dendritic cell; FCM: flow cytometry; CRT: calreticulin; HSP70: heat shock protein 70; HMGB1: high mobility group box 1; SD: standard deviation; ANOVA: analysis of variance; BMDCs: bone marrow-derived dendritic cells; DAPI: 4',6-diamidino-2-phenylindole.

incubated with G/T@L-E had undergone significant maturation. We assessed DC maturation by quantifying increased surface expression of CD80 and CD86, key indicators of mature DCs [Figure 4C]. G/T@L-E can effectively induce DC maturation. The presence rates of CD80 and CD86 in the G/T@L-E group were 63.6% and 26.7%, respectively, which were higher than those in the control group and elicited the maximal DC maturation. To distinguish ICD-mediated DC maturation from direct bacterial pathogen-associated molecular patterns (PAMPs) driven activation, we performed a series of control experiments [Supplementary Figure 5]. As expected, *E. coli* alone induced substantial CD80 and CD86 upregulation on DCs, confirming that bacterial PAMPs are potent DC activators. Most importantly, when live *E. coli* was eliminated by antibiotic treatment prior to DCs co-culture, DCs still exhibited significant maturation compared to untreated controls. This residual DC activation can be unequivocally attributed to ICD-associated molecular patterns released from G/T@L-E-treated tumor cells. Collectively, these data indicate that DC maturation in our system is driven by synergistic contributions from both bacterial PAMPs and ICD-associated molecular patterns. To further evaluate the ability of G/T@L-E to induce ICD in 4T1 cells, the expression levels of key ICD markers, including CRT, HSP70, as well as HMGB1, were measured in 4T1 cells^[25,26]. CLSM results revealed that G/T@L-E induced a significantly higher level of CRT protein translocation to the cell membrane compared to other groups [Figure 4D and E]. Flow cytometry analysis revealed much higher CRT expression on the surface of 4T1 cells in the G/T@L-E groups [Supplementary Figure 6]. Moreover, the fluorescence signal for HSP70 was markedly stronger in the G/T@L-E group than in any other groups [Figure 4F and G]. Furthermore, CLSM results indicated that G/T@L-E significantly enhanced the release of HMGB1 protein from tumor cells [Figure 4H and I]. Enzyme-linked immunosorbent assay (ELISA) was then used to measure secreted HMGB1, a late-phase ICD marker. In 4T1 cells, the G/T@L-E group showed the greatest increase in HMGB1 expression [Supplementary Figure 7]. The robust immunogenic cell death triggered by G/T@L-E can be attributed to the synergistic interplay among ROS generation by GOx, TPZ chemotherapy activation driven by cellular hypoxia, and the inherent immunogenicity of *E. coli*.

In vivo distribution of G/T@L-E

To assess the specificity and colonization capacity of G/T@L-E *in vivo*, a biodistribution experiment was then carried out. When 4T1 tumors in Balb/c mice reached $\sim 50 \text{ mm}^3$, the animals received a single intravenous injection of G/T@L-E at 1×10^6 CFU. The mice were then sacrificed at 12 h, 24 h, and 7 days after injection of G/T@L-E. Next, the major organs and tumor tissues were harvested, homogenized, diluted, and cultured on LB agar for 24 h. CFU enumeration over time showed that *E. coli* was steadily cleared from the heart, liver, spleen, kidneys, and lungs within 24 h. However, the CFU count in tumor tissues remained substantially higher than in other major organs, even 623-fold higher than that in the liver group at 7 days [Figure 5A and B], highlighting the capacity of G/T@L-E to specifically target tumors and selectively colonize within the distinct TME due to *E. coli* DH5 α . This preferential tumor accumulation is likely attributable to the hypoxic and immunosuppressive tumor microenvironment, which provides a favorable niche for bacterial colonization. The intriguing targeted accumulation capability of G/T@L-E was further confirmed through *ex vivo* fluorescence imaging of dissected tumors and major organs at 12, 24, and 36 h [Figure 5C and D, Supplementary Figure 8], implying that the *E. coli* DH5 α of G/T@L-E had a stronger tumor biodistribution capacity than G/T@L. Meanwhile, *in vivo* whole body fluorescence imaging revealed that G/T@L-E could be retained in the tumor region within 8 h post injection [Supplementary Figure 9]. Hypoxia,

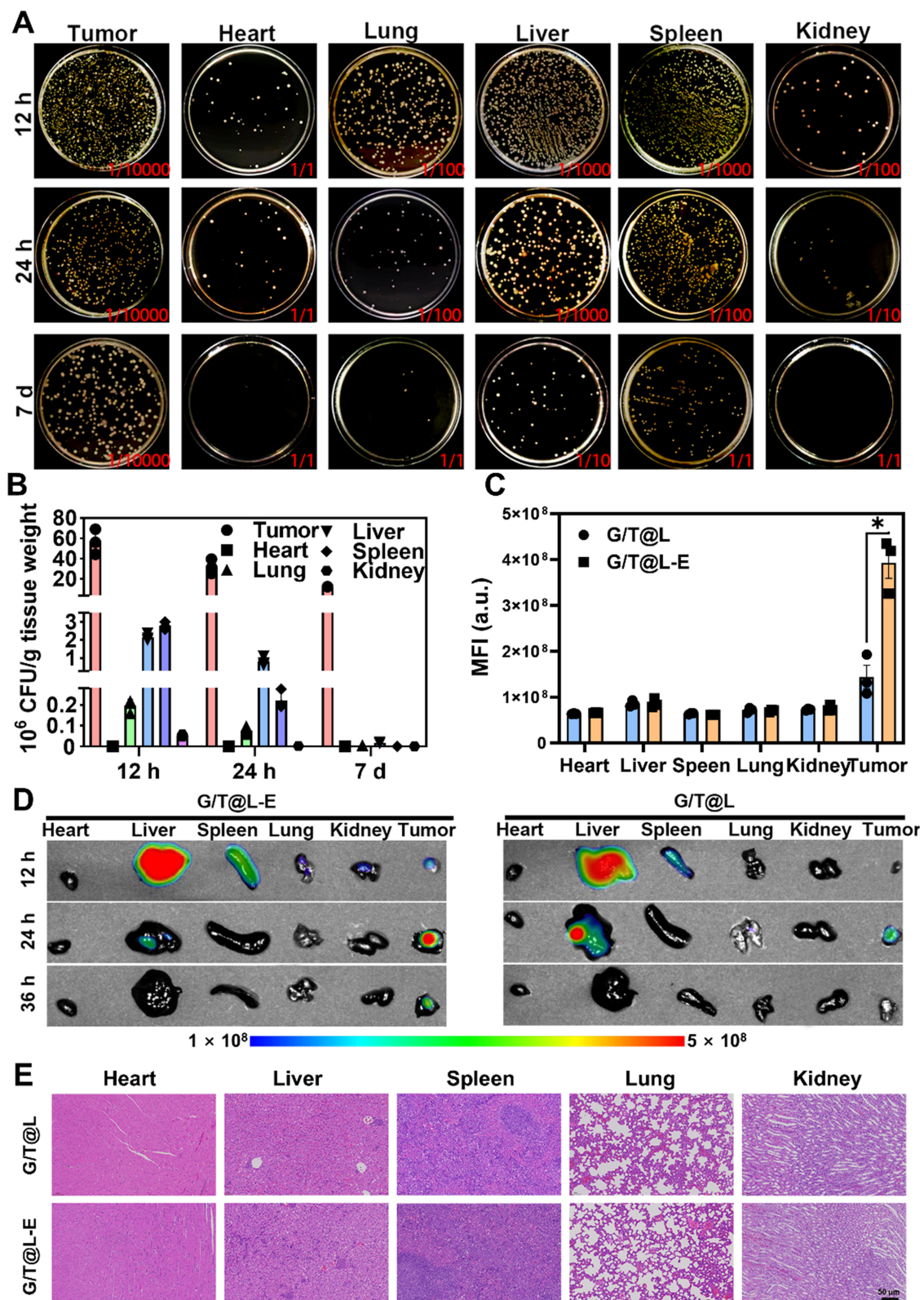


Figure 5. *In vivo* distribution of G/T@L-E. (A and B) Representative images and quantified CFU counts from chromogenic LB agar with serial dilutions of organs. $n = 3$; (C) Corresponding fluorescence intensity in 24 h and (D) *in vivo* fluorescence images of major organs and tumors post-injection after 12, 24, and 36 h. $n = 3$; (E) H&E-stained images obtained from major organs of 4T1-bearing Balb/c mice after injection of G/T@L or G/T@L-E in 24 h. Scale bar = 50 μ m. Data are presented as mean $\pm$ SD. The significances were compared by two-way ANOVA followed by Tukey's multiple comparisons test. $^*P < 0.05$. CFU: Colony-forming unit; LB: Luria-Bertani; SD: standard deviation; ANOVA: analysis of variance; MFI: mean fluorescence intensity.

acidity, and immune suppression within the tumor microenvironment collectively promote *E. coli* accumulation and persistence. In our G/T@L-E system, this stable colonization supports sustained GOx-mediated H₂O₂ generation and TPZ activation for antitumor effects. Colonization stability may additionally depend on vascular permeability, bacterial traits, and host immune equilibrium, for which future optimization is necessary. The cytotoxicity of G/T@L-E on major organs was subsequently assessed through H&E staining, revealing no histopathological abnormalities [Figure 5E]. Hence, G/T@L-E did not cause significant inflammation or other toxicity *in vivo*. A key design principle of our biohybrid system is the immobilization of G/T@L liposomes onto the surface of *E. coli*, exhibiting an intrinsic tropism for the hypoxic regions of solid tumors, which enables active and selective delivery of GOx and TPZ to the tumor site. Collectively, these mechanistic features underscore that immobilization goes beyond a physical attachment, serving as an integral feature of the therapeutic design that confers multifunctional benefits.

In vivo anti-tumor effect

To continue investigating the therapeutic potential, the *in vivo* antitumor efficacy was evaluated using a 4T1 tumor-bearing Balb/c mouse model [Figure 6A]. An *in-situ* breast cancer model was established using 4T1 cells. The resulting tumor-bearing mice were then randomly divided into six groups ($n = 6$ per group), each receiving different treatments: PBS, E, G@L-E, T@L-E, G/T@L, and G/T@L-E. Previous studies have indicated that a single intravenous injection of *E. coli*, at doses between 1×10^4 and 1×10^6 CFU per mouse, is generally well tolerated^[27,28]. Thus, each mouse in the *E. coli*, G@L-E, T@L-E, and G/T@L-E groups was intravenously injected with 1×10^6 CFU of bacteria every 2 days. As shown in Figure 6B, the released GOx catalyzes the conversion of glucose into H₂O₂ while depleting O₂. This process increases ROS levels and induces intracellular hypoxia. Within the resulting hypoxic TME, the minimally toxic prodrug TPZ is activated, becoming cytotoxic to tumor cells and thereby enabling effective chemotherapy. This dual action results in substantial apoptosis and ICD of tumor cells. G/T@L-E significantly suppressed tumor growth compared with the other groups, as indicated by tumor volume [Figure 6C] and representative tumor image [Figure 6D]. Notably, the tumor suppression rate achieved by G/T@L-E was as high as 86.76%. In contrast, the tumor suppression rates of G@L-E and T@L-E used individually were only 28.61% and 72.82%, respectively [Figure 6E]. This clearly demonstrates that G/T@L-E can achieve a highly significant synergistic therapeutic effect. This observation further substantiated that the O₂ depletion mediated by GOx can efficiently activate TPZ, resulting in a markedly enhanced therapeutic efficacy when GOx and TPZ are co-administered, as opposed to their individual application. Mice were weighed every other day throughout treatment, and there were no discernible variations in body weight across the groups [Figure 6F]. By inducing hypoxia and activating TPZ chemotherapy, G/T@L-E elicited substantial tumor cell apoptosis and effectively inhibited tumor cell proliferation [Figure 6G]. As previously described, immunohistochemical analysis revealed the induction of ICD by different treatments [Figure 6H]. This was evidenced by the high expression of HSP70 [Figure 6I and Supplementary Figure 10] and CRT [Figure 6J and Supplementary Figure 11], as well as the release of HMGB1 [Supplementary Figure 12]. These changes can promote ICD-associated immune responses and show potential for priming adaptive antitumor immunity. It was shown that G/T@L-E induced the severest ICD among the other groups. Overall, G/T@L-E has not only effectively inhibited tumor growth *in vivo* but also triggered significant ICD of tumors *in vivo*. These results demonstrate that G/T@L-E induces ICD and promotes immunostimulatory effects *in vivo*. Although direct evidence for *in vivo* antitumor immunity is not yet available, our findings highlight the potential of the G/T@L-E system to serve as an immunomodulatory platform for breast cancer therapy. Beyond the direct antitumor effects, it is important to consider the potential impact of the G/T@L-E system on the senescence status of surrounding normal cells. Cellular senescence plays a dual role in tissue homeostasis; while it serves as a tumor-suppressive mechanism, excessive or aberrant senescence can disrupt tissue architecture and contribute to chronic inflammation and aging-related pathologies^[29]. Next, blood biochemistry analysis and hematological assays of healthy Balb/c mice after injection of various treatments in 24 h were evaluated [

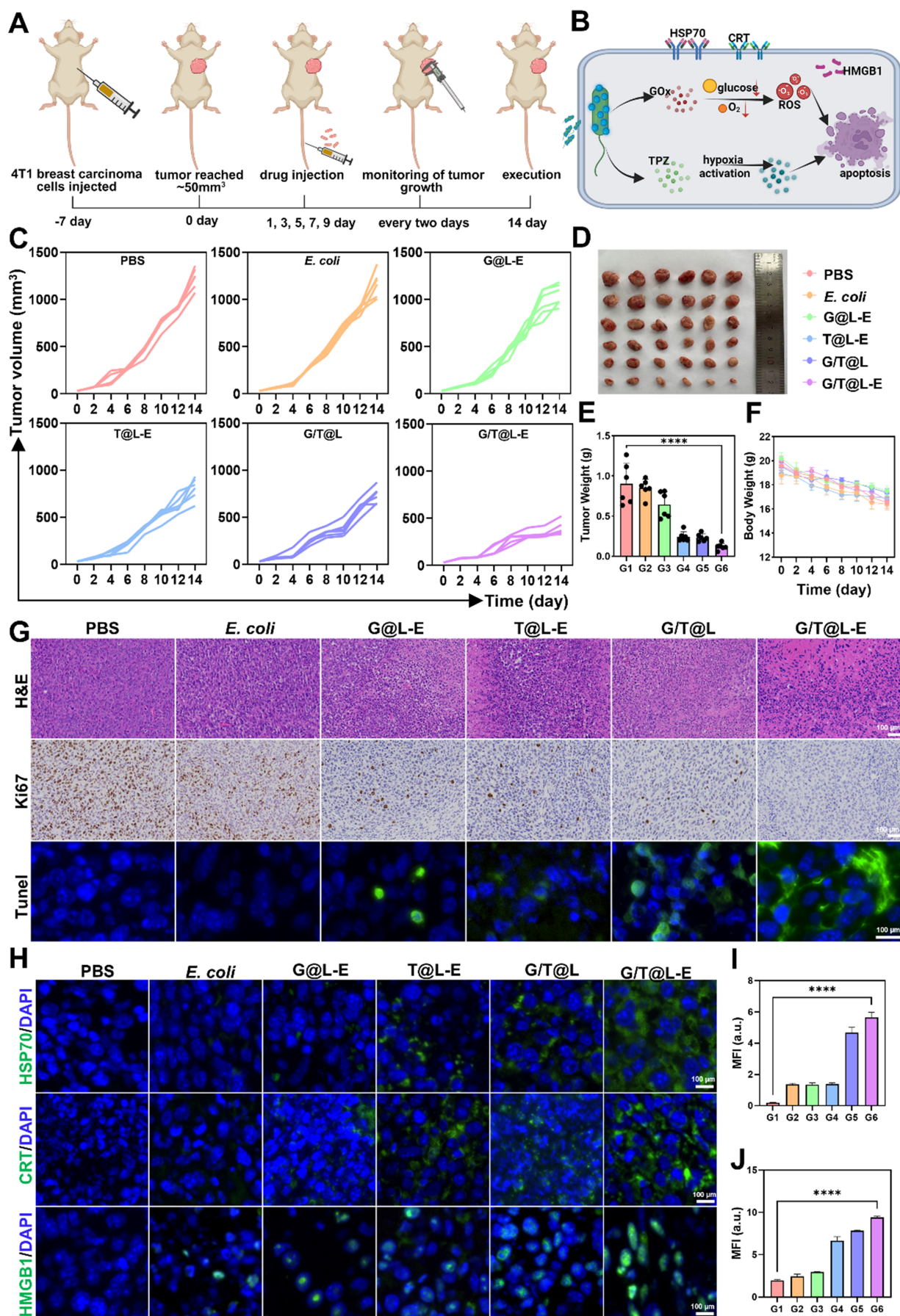


Figure 6. *In vivo* toxicity of various treatments against 4T1 tumor. (A) Scheme for establishing a 4T1 tumor-bearing mouse model and treatments *in vivo*; (B) Scheme of ICD caused by G/T@L-E; (C) Tumor volumes after different treatments. $n = 6$; (D) Representative tumor

image of the harvested 4T1 tumors following various treatments after 14 days; (E) Tumor weights after different treatments. $n = 6$; (F) Body weights after different treatments. $n = 6$; (G) H&E staining, Ki-67, and TUNEL of tumor tissues after different treatments. Scale bar: 100 μm ; (H) Immunofluorescence images of HSP70, CRT, and HMGB1 from tumor tissue following various treatments. Scale bar: 100 μm ; (I) The corresponding fluorescence intensities of HSP70 quantified by ImageJ software. $n = 3$; (J) The corresponding fluorescence intensities of CRT quantified by ImageJ software. $n = 3$ (The groups are defined as follows: G1: PBS; G2: *E. coli*; G3: G@L-E; G4: T@L-E; G5: G/T@L; G6: G/T@L-E). Data are presented as mean $\pm$ SD. Significance was compared by two-way ANOVA followed by Tukey's multiple comparisons test. **** $P < 0.0001$. Some elements in (A and B) were created with BioRender.com. ICD: Immunogenic cell death; HSP70: heat shock protein 70; CRT: calreticulin; HMGB1: high mobility group box 1; PBS: phosphate-buffered saline; SD: standard deviation; ANOVA: analysis of variance; GOx: glucose oxidase; ROS: reactive oxygen species; TPZ: Tirapazamine; *E. coli*: *Escherichia coli*; DAPI: 4',6-diamidino-2-phenylindole.

[Supplementary Figure 13](#)]. The results demonstrated that there were no significant variations in these biochemical indicators across all groups, thereby confirming the excellent biocompatibility and safety of G/T@L-E. We also measured the serum levels of TNF- α and IL-6 by ELISA at 24 h after different treatments [[Supplementary Figure 13J and K](#)]. No significant variations were observed in these systemic inflammatory cytokines across all groups, which means that the infection rapidly cleared in 24 h. Blood samples collected at 12 h, 24 h, and 7 days after injecting G/T@L-E were plated for CFU enumeration [[Supplementary Figure 14](#)]; no significant number of bacteria was found in the blood. The cytotoxicity of G/T@L-E on major organs was subsequently assessed through H&E staining, revealing no histopathological abnormalities [[Supplementary Figure 15](#)]. Hence, G/T@L-E did not cause significant inflammation or other toxicity *in vivo*.

Limitations

We acknowledge that the current study has several limitations. First, we did not directly measure immunosuppressive populations, such as regulatory T cells, or perform T cell depletion experiments to establish causality. Second, the long-term safety of the bacterial delivery system requires further evaluation. Future studies are warranted to address these aspects.

CONCLUSION

To summarize, we have devised a soft biohybrid bacterial delivery system, G/T@L-E, which integrated a living bacterial core (*E. coli* DH5 α) with a functionalized lipid shell, fabricated via supramolecular interfacial self-assembly. It combated breast cancer by utilizing and intensifying the intracellular hypoxia instead of inhibiting it. Electrostatic anchoring of these liposomes onto the bacterial surface yields a living soft construct that combines the active tumor-homing and penetrating capabilities of the microbe with the programmable cargo release functionality of the synthetic lipid interface. The intrinsic tumor-targeting capability of *E. coli* provides a biological foundation for selective payload delivery, while the liposomal cargo confers potent therapeutic activity. G/T@L-E system demonstrated remarkable efficacy in targeting and penetrating tumor tissues, thereby significantly boosting the accumulation of TPZ and GOx within the tumor. GOx-catalyzed glucose oxidation further exacerbated intracellular hypoxia, which subsequently activated the hypoxia-sensitive TPZ prodrug to exert its chemotherapeutic effects. More intriguingly, large-scale ICD of tumor cells was triggered by the natural immunogenicity of the bacteria, in conjunction with the combined effects of oxidative stress and TPZ activation. The findings validated that biohybrid soft materials with engineered living-synthetic interfaces offer a versatile and powerful platform for next-generation cancer therapy, which holds considerable promise in the intensifying tumor hypoxia and modulating the immunosuppressive microenvironment in breast cancer.

DECLARATIONS

Authors' contributions

Conceived and designed the experiments: Li, Y. (Yunshi Li); Tu, Y.

Wrote the first draft of the manuscript with contributions from all co-authors: Li, Y. (Yunshi Li)

Performed the experiments and analyzed the data: Li, Y. (Yunshi Li); Jiang, Y.; He, Y.; Wang, H.; Xu, C.; Lu, W.; Qin, H.; Tian, H.

Supervised the project: Tu, Y.; Li, Y. (Yingjia Li); Shen, L.

All authors have approved the final version of the manuscript.

Availability of data and materials

All data needed to evaluate the conclusions in the paper are present in the paper and/or the [Supplementary Materials](#). The datasets generated and analyzed during the current study are available from the corresponding author Tu, Y., upon reasonable request.

AI and AI-assisted tools statement

Not applicable.

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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanfang Hospital (Permit number: IACUC-LAC-20240820-002). All experiments were performed in the specific pathogen-free facility of the Laboratory Animal Center, Nanfang Hospital. All animal experimental procedures complied with the regulations of China and Nanfang Hospital regarding the health and welfare of laboratory animals.

Consent for publication

Not applicable.

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Supplementary Materials

[Supplementary Materials](#)

REFERENCES

1. Fan, X.; Chen, S.; Manshadi, F.; et al. Advances in soft mechanocaloric materials. *Adv. Funct. Mater.* **2026**, *36*, 2420997. [DOI PubMed PMC](#)
2. Ye, X.; Chen, Y.; Lv, C.; et al. *In situ* formed hydrogels for soft bioelectronics. *Mater. Horiz.* **2025**, *12*, 9537-55. [DOI PubMed](#)
3. Song, Y.; Huang, X.; Zhang, X.; Deng, M.; Aya, S.; Huang, M. Ferroelectric nematic liquid crystals showing high birefringence. *Adv. Sci.* **2025**, *12*, e2414317. [DOI PubMed PMC](#)
4. He, Z.; Chen, L.; You, W.; et al. Weaving-inspired asymmetric entangled nodes in multi-component polymer networks. *Nat. Mater.* **2026**, *25*, 107-16. [DOI PubMed PMC](#)
5. Atinafu, D. G.; Kim, Y. U.; Kim, S.; Kang, Y.; Kim, S. Advances in biocarbon and soft material assembly for enthalpy storage: fundamentals, mechanisms, and multimodal applications. *Small* **2024**, *20*, e2305418. [DOI PubMed](#)
6. Ye, M.; Zhou, Y.; Zhao, H.; Wang, Z.; Nelson, B. J.; Wang, X. A review of soft microrobots: material, fabrication, and actuation. *Adv. Intell. Syst.* **2023**, *5*, 2300311. [DOI](#)
7. Delgado-Pujol, E. J.; Martínez, G.; Casado-Jurado, D.; et al. Hydrogels and nanogels: pioneering the future of advanced drug delivery systems. *Pharmaceutics* **2025**, *17*, 215. [DOI PubMed PMC](#)
8. Liu, Y.; Zhang, J.; Wang, D. Preparation schemes and applications of multifunctional PVA composite hydrogels. *Chem. Eng. J.* **2025**, *508*, 160946. [DOI](#)
9. Boufidis, D.; Garg, R.; Angelopoulos, E.; Cullen, D. K.; Vitale, F. Bio-inspired electronics: soft, biohybrid, and “living” neural interfaces. *Nat. Commun.* **2025**, *16*, 1861. [DOI PubMed PMC](#)

10. Soliman, M.; Forbes, F.; Damian, D. D. Yeast-driven and bioimpedance-sensitive biohybrid soft robots. *Cyborg. Bionic. Syst.* **2025**, *6*, 0233. [DOI PubMed PMC](#)
11. Kinjo, R.; Morimoto, Y.; Jo, B.; Takeuchi, S. Biohybrid bipedal robot powered by skeletal muscle tissue. *Matter* **2024**, *7*, 948-62. [DOI](#)
12. Tang, S.; Davoudi, Z.; Wang, G.; et al. Soft materials as biological and artificial membranes. *Chem. Soc. Rev.* **2021**, *50*, 12679-701. [DOI PubMed](#)
13. Celi, N.; Cai, J.; Sun, H.; Feng, L.; Zhang, D.; Gong, D. Biohybrid flexible sperm-like microrobot for targeted chemo-photothermal therapy. *ACS. Appl. Mater. Interfaces.* **2024**, *16*, 24341-50. [DOI PubMed](#)
14. Ma, X.; Liang, X.; Li, Y.; et al. Modular-designed engineered bacteria for precision tumor immunotherapy via spatiotemporal manipulation by magnetic field. *Nat. Commun.* **2023**, *14*, 1606. [DOI PubMed PMC](#)
15. Yang, Y.; Zwijnenburg, M. A.; Gardner, A. M.; et al. Conjugated polymer/recombinant *Escherichia coli* biohybrid systems for photobiocatalytic hydrogen production. *ACS. Nano.* **2024**, *18*, 13484-95. [DOI PubMed PMC](#)
16. Kim, D.; Liu, A.; Diller, E.; Sitti, M. Chemotactic steering of bacteria propelled microbeads. *Biomed. Microdevices.* **2012**, *14*, 1009-17. [DOI PubMed](#)
17. Zhang, L.; Chen, Z.; Ran, H.; et al. Biomimetic magnetobacterial microrobots for active pneumonia therapy. *Nat. Commun.* **2025**, *16*, 7856. [DOI PubMed PMC](#)
18. Felfoul, O.; Mohammadi, M.; Taherkhani, S.; et al. Magneto-aerotactic bacteria deliver drug-containing nanoliposomes to tumour hypoxic regions. *Nat. Nanotechnol.* **2016**, *11*, 941-7. [DOI PubMed PMC](#)
19. Yang, S.; Sheffer, M.; Kaplan, I. E.; et al. Nonpathogenic *E. coli* engineered to surface display cytokines as a new platform for immunotherapy. *Res. Sq.* **2024**. [DOI PubMed PMC](#)
20. Zhang, B.; Zhang, J.; Li, Y.; et al. *In situ* STING-activating nanovaccination with TIGIT blockade for enhanced immunotherapy of anti-PD-1-resistant tumors. *Adv. Mater.* **2023**, *35*, e2300171. [DOI PubMed](#)
21. Luo, S.; Zhao, C.; Wang, R.; et al. Higher tumor/organ accumulation ratio of porous dual infinite coordination polymer nanocomposites for efficient tumor photothermal-starvation-dual hypoxia chemo synergistic therapy. *Small* **2025**, *21*, e2411188. [DOI PubMed](#)
22. Gu, P.; Liu, J.; Gao, T.; et al. Temperature-sensitive nano-GOx combined with downregulation of tumor stemness to initiate robust antitumor efficacy. *ACS. Nano.* **2025**, *19*, 11738-55. [DOI PubMed](#)
23. Shi, X.; Li, X.; Liu, B.; et al. Construction of a multifunctional nanodrug delivery system based on tirapazamine and glucose oxidase: its antitumor activity. *ChemistrySelect* **2025**, *10*, e202403165. [DOI](#)
24. Qannita, R. A.; Alalami, A. I.; Harb, A. A.; et al. Targeting hypoxia-inducible factor-1 (HIF-1) in cancer: emerging therapeutic strategies and pathway regulation. *Pharmaceuticals* **2024**, *17*, 195. [DOI PubMed PMC](#)
25. Galluzzi, L.; Guilbaud, E.; Schmidt, D.; Kroemer, G.; Marincola, F. M. Targeting immunogenic cell stress and death for cancer therapy. *Nat. Rev. Drug. Discov.* **2024**, *23*, 445-60. [DOI PubMed PMC](#)
26. Huang, J.; Duan, F.; Xie, C.; et al. Microbes mediated immunogenic cell death in cancer immunotherapy. *Immunol. Rev.* **2024**, *321*, 128-42. [DOI](#)
27. Xu, X.; Ding, Y.; Dong, Y.; et al. Nanobody-engineered biohybrid bacteria targeting gastrointestinal cancers induce robust STING-mediated anti-tumor immunity. *Adv. Sci.* **2024**, *11*, e2401905. [DOI PubMed PMC](#)
28. Zhao, Y.; Li, M.; Guo, Y.; et al. Neutrophil hitchhiking nanoparticles enhance bacteria-mediated cancer therapy via NETosis reprogramming. *J. Control. Release.* **2024**, *367*, 661-75. [DOI PubMed](#)
29. Liu, X.; Ye, Y.; Li, Z.; Liao, L.; Wei, Q. Mechanical rejuvenation of senescent stem cells and aged bone via chromatin remodeling. *Nat. Commun.* **2026**, *17*, 1684. [DOI PubMed PMC](#)

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