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Total number of authors: 11

Published in: Biomaterials

Link to article, DOI: 10.1016/j.biomaterials.2020.120106

Publication date: 2020

Document Version Peer reviewed version

Biodegradable Poly(γ-glutamic acid)@Glucose Oxidase@Carbon Dot Nanoparticles for Simultaneous Multimodal Imaging and Synergetic Cancer Therapy

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Abstract

It is known that tumor antigens could induce obvious anti-tumor immune responses for efficient cancer immunotherapy when combined with checkpoint blockade. However, the amount of tumor antigens is often limited due to the suppressive tumor microenvironment (TME). Here, a new type of nanomaterial was developed to improve tumor treatment by the combined action of starving therapy/photodynamic therapy (PDT)/photothermal therapy (PTT) and checkpoint-blockade immunotherapy. In detail, the immunoadjuvant nanoagents (γ-PGA@GOx@Mn,Cu-CDs) were fabricated by integrating the GGT enzyme-induced cellular uptake polymer - poly (γ-glutamic acid) (γ-PGA), a glucose-
metabolic reaction agent - glucose oxidase (GOx), Mn,Cu-doped carbon dots (CDs) as photosensitizer and self-supplied oxygenator nanodots. $\gamma$-PGA@GOx@Mn,Cu-CDs nanoparticles (NPs) showed long retention time at the tumor acidic microenvironment and could further target cancer cells. The NPs also displayed both photothermal and photodynamic effects under laser irradiation at 730 nm. Interestingly, the endogenous generation of hydrogen peroxide ($H_2O_2$) caused by the nanoreactors could significantly relieve tumor hypoxia and further enhance in vivo PDT. By synergistically combining the NPs-based starving-like therapy/PDT/PTT and check-point-blockade therapy, the treatment efficiency was significantly improved. More importantly, the systematic antitumor immune response would eliminate non-irradiated tumors as well, which is promising for metastasis inhibition.

**Keyword:** tumor microenvironment, checkpoint-blockade immunotherapy, singlet oxygen, hyperthermia, metastasis inhibition

1. **Introduction**

By training patients’ immunological systems to remove cancer cells, cancer immunotherapy has become a novel and effective strategy for cancer therapy. It has shown considerable promises in the last few years [1-3]. Among different strategies for cancer immunotherapy, checkpoint blockade through blocking the connection of ligand programmed death-ligand 1 (PD-L1) to programmed cell Death 1 (PD-1) has received the most attention in recent years [4,5]. As a cancer cell-overexpressed protein, PD-L1 can cause the suppression of the immune system and cancer immune evasion. As a specific binding receptor of PD-L1, PD-1 was discovered in activated T cells. Their connection causes the suppression of immune response and inhibites the function of cytotoxic T-cells, so that the
inactivated cytotoxic T cells cannot attack cancer cells [6-10]. To reverse the immunosuppression mediated by tumor, therapeutic antibodies of anti-PD-1/PD-L1 were prepared to hinder this interaction. PD-L1 blockade represents a promising therapeutic strategy in animal cancer models [11, 12]. However, in many clinical trials, it was observed that the immune response was not easily activated, [13]. As a result, combining immune checkpoint blockade therapy with other strategies that can activate the systematic immune responses and efficiently trigger the T-cell infiltration may enhance the antitumor response rates and promote the application of immunotherapy in metastatic tumors.

Photo-induced cancer therapies can apply non-toxic photosensitizers to efficiently promote photodynamic therapy (PDT) and photothermal therapy (PTT), so that it becomes a promising treatment for in vivo tumor ablation [14-17]. Recently, we and other researchers reported various carbon dots (CDs) as biocompatible agents for PDT and PTT, which satisfy both PDT and PTT performance due to their special surface structure, obvious near-infrared (NIR) absorbance, high capability of photothermal conversion, and strong photostability [18-21]. Interestingly, a number of studies discovered that PDT and PTT with carbon-based photosensitizers (for example, graphene quantum dots, carbon nanotubes, polymer nanospheres, polymer dots or graphene oxide) could show anti-tumor immunological effects by generating tumor-associated agents from ablated tumor cell residues [22-25]. This kind of effect has also been discovered in a preliminary clinical trial study. However, the combination of CDs-based PDT with immunotherapy has not yet been reported. In this work, we first discovered that the tumor-associated antigens prepared in situ after PDT and PTT with immune-adjuvant CDs could display the vaccine like function, which when
combined with checkpoint blockade could enhance anti-tumor immune responses for effective cancer immunotherapy.

Cancer starving therapy is known for blocking the nutrients supply to suppress tumor growth [26-28]. Compared to traditional therapies, this kind of treatment shows more effective tumor-ablation effects by simply stopping tumor cells from getting energy [29]. To ensure higher efficiency, some researchers have combined the use of glucose starvation with other treatments, such as phototherapy and immunotherapy. It has been found that the tumor associated antigens from dead cells caused by starving therapy could induce specific antitumor immune responses [30,31]. In the latest study, Xie et al. found that cancer cell membrane camouflaged nanoparticles (NPs) might show antigen-releasing functions to promote immune responses after starving therapy, which showed an abscopal therapeutic effect combined with anti-PD-L1 blockade [32]. Although the combined starving therapy and immunotherapy exhibited a better antitumor therapeutic effect than single therapy, its efficacy still needs to be improved.

In this work, to apply NIR-triggered PDT/PTT and starving therapy to induce cancer immunotherapy with high efficiency, we fabricated multifunctional nanoagents (γ-PGA@GOx@Mn,Cu-CDs) by integrating the positive charge-induced cellular uptake polymer - Poly(γ-glutamic acid) (γ-PGA), a glucose-metabolic reaction agent - lucose oxidase (GOx), and Mn,Cu-doped CDs as photosensitizers and self-supplied oxygenator nanodots (denoted as Mn,Cu-CDs) for image-guided multimodal therapy (Scheme 1a). The NPs had many special characteristics to work as an innovative smart therapeutic agent, namely: (1) The coating of γ-PGA could significantly enhance tumor cell internalization and tumor retention via a speculated γ-glutamyl transpeptidase (GGT enzyme)-mediated endocytosis pathway [33]. (2) GOx could change glucose into gluconic acid and
H$_2$O$_2$, which is helpful to enhance cancer starving therapy [34]. (3) Meanwhile, catalase encapsulated within those NPs could work like nanoreactors to induce the decomposition of tumor endogenous and glucose-responsive sequential generation H$_2$O$_2$, which promotes the production of O$_2$ to relieve tumor hypoxia and further increase the efficiency of *in vivo* PDT [35-37]. (4) With pH/NIR-responsive degradation of the $\gamma$-PGA coating, the naked nanoparticles and GOx would be exposed to further improved PDT and starving therapy for killing cancer cells. (5) The obtained NPs exhibited high photothermal conversion efficiency up to 30.3%. (6) After the combination with checkpoint blockade immunotherapy with PD-L1 antibody, the promoted PDT therapy would effectively enhance the infiltration of cytotoxic T lymphocytes into distant tumors and hinder their growth, confirming a significant abscopal effect for metastasis inhibition (*Scheme 1b*).
Scheme 1. (a) Schematic illustration of starving and phototherapy mediated by \(\gamma\)-PGA@GOx@Mn,Cu-CDs NPs. (b) Schematic illustration of starving-like therapy, phototherapy, and immunotherapy mediated by \(\gamma\)-PGA@GOx@Mn,Cu-CDs NPs.

2. Experimental section

2.1 Materials

8-arm-PEG-NH\(_2\) (Mw = 20 kDa) and \(\gamma\)-PGA (Mw = 1000 kDa) were acquired from Nanjing Saitesi Co., Ltd (Nanjing, China). Sodium hydroxide (NaOH), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-Htetrazolium bromide (MTT), phenethyl
isothiocyanate, 1,3-diphenylisobenzofuran (DPBF), 4′,6-diamidino-2-phenylindole (DAPI), H2DCFHDA, 1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), and dioctyl sodium sulfosuccinate (AOT) were purchased from Aladdin Industrial Co., Ltd. (Shanghai, China). Dichloromethane (DCM) and succinic anhydride were purchased from Yuanye Biotechnology Co., Ltd. (Shanghai, China). All the chemicals were of analytical grade and used as received without further purification.

2.2 Instruments and characterization

The morphology of samples was studied by employing a transmission electron microscope (TEM, Tecnai G2 F30 S-TWIN) and SEM (JSM-6701F) measurement. The zeta potential of samples was measured through the dynamic laser light scattering technology (DLS, Malvern Nano-ZS 90 Nanosizer). The surface composition of the sample was measured through X-ray photoelectron spectroscopy (XPS, ESCALAB 250, Thermo Fisher). The electron spin resonance (ESR) spectrum of samples was measured by using Bruker EMXplus Spectrometer System. *In vitro* bright field and fluorescence images were performed with a confocal laser scanning microscope (CLSM, LTI-EA1R, Nikon, Japan). To monitor the temperature changes at the tumor site during irradiation, infrared thermal images were recorded with a PTT monitoring system MG33 (Shanghai Magnity Electronics Co. Ltd). The methods used for material characterization were displayed in the “Experimental Section” (Supplementary File)

3. Results and discuss

3.1 Synthesis and characterization of γ-PGA@GOx@Mn,Cu-CDs NPs
γ-PGA@GOx@Mn,Cu-CDs NPs were prepared by integrating natural γ-PGA, GOx, and Mn,Cu-CDs through a mild solution method. In the first attempt, we produced magnetofluorescent Mn,Cu-CDs with MnO2 and Cu(II). To enhance the water solubility and biocompatibility, cooperative self-assembly with γ-PGA and GOx was then applied. The designed γ-PGA@GOx@Mn,Cu-CDs NPs had acceptable physiological stability, obvious near-infrared (NIR) emission, good relaxivity, glucose-responsive sequential generation of H2O2 as well as effective 1O2 and heat production. More significantly, the γ-PGA@GOx@Mn,Cu-CDs NPs could effectively hydrolyze H2O2 to produce O2 and significantly ameliorate tumor hypoxia to increase PDT efficiency. In vitro and in vivo studies have demonstrated that this kind of magnetofluorescent γ-PGA@GOx@Mn,Cu-CDs NPs could be utilized as an H2O2-driven oxygenator in acidic conditions for multimodal imaging. It also effectively increased the efficacy of PTT, PDT, and starving treatment for hypoxic solid tumors by generating O2 in situ. The Mn,Cu-CDs were synthesized through a one-step cost-effective solvothermal reaction. Uniform Mn,Cu-CDs which had an average size of \( \sim 2.7 \) nm were prepared after solvothermal treatment (Figure 1a,b and Figure S1, based on the measurement of > 100 dots). The image (inset of Figure 1a) of high-resolution transmission electron microscopy (HRTEM) shows that the interplanar spacing of Mn,Cu-CDs was 0.21 nm, which corresponds to the (100) lattice spacing of graphene. X-ray photoelectron spectroscopy (XPS) of Mn,Cu-CDs shows seven peaks between 200 and 1000 eV (Figure 1c), assigned to C1s, P2p, S2p, N1s, O1s, Cu2p, and Mn2p, respectively. In the Cu 2p spectrum, obvious peaks of Cu2p\( ^{3/2} \) and Cu2p\( ^{1/2} \) were found at 931.9 and 952.4 eV, respectively (Figure 1d). This result confirmed the existence of Cu(II) in the Mn,Cu-CDs. Another two characteristic peaks in the Mn 2p spectrum (Figure 1e) at 653.3 and 640.9 eV represented the Mn(IV) \( 2p_{3/2} \) and Mn(IV) \( 2p_{1/2} \) spin-orbit peaks of MnO2, respectively, proving that Mn\(^{2+} \) was oxidized into MnO2 under the
high temperature and pressure. The stability of the Mn,Cu-CDs in the physiological medium is enhanced by surface modification with PEG. After surface modification, the zeta potential of the Mn,Cu-CDs changed from -18.2 to 12.9 mV (Figure S2).

Figure 1. (a) TEM image of Mn,Cu-CDs. Inset is HRTEM of Mn,Cu-CDs. (b) SEM image of Mn,Cu-CDs. (c) XPS spectrum of Mn,Cu-CDs. High-resolution XPS of Cu 2p (d) and Mn 2p (e) of Mn,Cu-CDs NPs. (f) TEM image of γ-PGA@GOx@Mn,Cu-CDs NPs. Inset is the of TEM of single γ-PGA@GOx@Mn,Cu-CDs NPs. (g) Element mapping images of γ-PGA@GOx@Mn,Cu-CDs NPs. (h) SEM image of γ-PGA@GOx@Mn,Cu-CDs NPs. (i) Coomassie blue-stained SDS-PAGE for the analysis of the GOx.

GOx-polymer NPs-synthesized for nanomedicine applications can be responsive to glucose and has been used as nanotherapeutics for melanoma starving and oxidation treatment through maintaining GOx in the tumor. However,
there are major obstacles remain, such as the low cellular uptake ratio, random (diffusion-induced) GOx release, and undesired GOx release from carriers, because of the resultant diverse side-effects and low-therapeutic drug concentration levels. Based on these considerations, γ-PGA could be a promising candidate to remain on the surface of NPs-based delivery system to improve cellular uptake. Here, we reported a multifunctional γ-PGA@GOx@Mn,Cu-CDs NPs with self-degradation and enhanced cellular uptake properties via simple and green chemistry methods (Figure S3). As shown in Figure 1f, the γ-PGA@GOx@Mn,Cu-CDs NPs were about 80 nm, and uniformly dispersed. Elemental mapping revealed the uniform distribution of C, Mn, and Cu elements, demonstrating successful Mn,Cu-CDs loading on those NPs (Figure 1g). Small Mn,Cu-CDs (inset of Figure 1f and Figure 1h) were located on the surface of polymer NPs. γ-PGA@GOx@Mn,Cu-CDs NPs had a negative Zeta potential of $-18.7$ mV (Figure S2), which demonstrated that γ-PGA were successfully coated on the NPs surface. Moreover, GOx specific band showed up in the SDS-polyacrylamide gel, evidencing efficient GOx loading within the γ-PGA@GOx@Mn,Cu-CDs NPs (Figure 1i). The loading amount of GOx in the NPs was 3.2 wt% by a UV-vis spectrometer.

Agents which have PTT ability can display strong absorption in the NIR region. As a result, the UV-Vis-NIR spectrum was used to measure the absorbance of γ-PGA@GOx@Mn,Cu-CDs NPs in the NIR region. Figure 2a displays that UV-Vis-NIR spectra of γ-PGA@GOx@Mn,Cu-CDs NPs had a broad absorption between 400-800 nm. Further investigation confirmed that the emission spectra of γ-PGA@GOx@Mn,Cu-CDs NPs depended on their various activation (Figure 2b). The photograph of γ-PGA@GOx@Mn,Cu-CDs NP dispersions displayed obvious red emission (inset of Figure 2b) under the excitation wavelength of 500 nm. To evaluate the photostability of γ-PGA@GOx@Mn,Cu-CDs NPs, the cell image
displays a strong red fluorescence of $\gamma$-PGA@GOx@Mn,Cu-CDs NPs-labeled 4T1 cells, which were spectrally resolved after irradiation for 120 min (Figure 2c). On the contrary, fluorescein isothiocyanate (FITC), a widely used fluorescent probe, lost its fluorescence intensity rapidly to background levels after irradiation for 30 min. To obtain biological applications, it is crucial to maintain stability in the physiological environment. As illustrated in the inset of Figure 2a, the $\gamma$-PGA@GOx@Mn,Cu-CDs NPs exhibited high stability in physiological mediums, including in water, phosphate-buffered solution (PBS), and Dulbecco’s modified Eagle medium (DMEM).
Figure 2. (a) UV-vis-NIR absorbance spectra of \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs. Inset is the photographs of \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs in water, PBS, and DEME. (b) Emission spectra of \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs. Inset is the fluorescent images of \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs solution (\(\lambda_{ex} = 500\) nm). (c) CLSM images showing time-dependent fluorescence of 4T1 cells incubated with \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs and FITC. Scale bar = 50 \(\mu\)m. (d) Dissolved oxygen concentrations of the \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs in different reaction systems. Inset is the oxygen production in acid H\(_2\)O\(_2\) solution (pH = 6.0). (e) ESR spectra of the \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs + TEMP in different reaction systems (The pH of the NPs solution is 6.0). (f) ESR spectra of the \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs + DMPO in different reaction systems (The pH of the NPs solution is 6.0). (g) FL images of SOSG stained 4T1 cells in a N\(_2\) atmosphere. Scale bar: 50 \(\mu\)m. (h) The generated H\(_2\)O\(_2\) concentrations and (i) pH values at different time points arising from the \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs catalyzed decomposition reaction of glucose (1.0 mg/mL).

3.2 The photodynamic activity of \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs in vitro

According to the previous reports [38,39], the MnO\(_2\) can effectively trigger the decomposition of H\(_2\)O\(_2\) to produce O\(_2\). To study the ability of \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs to generate O\(_2\), an O\(_2\) probe was used to test the dissolved O\(_2\) in \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs/acidic H\(_2\)O\(_2\) mixed solution. Figure 2d shows that O\(_2\) was produced when the \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs and acidic H\(_2\)O\(_2\) were present together, but no O\(_2\) was observed when the \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs or H\(_2\)O\(_2\) was not present. Also, O\(_2\) effervescence was observed in the acidic H\(_2\)O\(_2\) solution after the addition of the \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs (inset of Figure 2d). This result suggested that \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs were able to induce H\(_2\)O\(_2\) for decomposition. Since Mn-CDS could trigger H\(_2\)O\(_2\) for effective O\(_2\) generation, the \(^1\)O\(_2\)-production ability of the \(\gamma\)-PGA@GOx@Mn,Cu-CDS NPs with acidic H\(_2\)O\(_2\) was also studied by the electron spin resonance (ESR) technique with 2,2,6,6-Tetramethylpiperidine (TEMP) and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and with \(^1\)O\(_2\) utilized as trappers. In an N\(_2\) atmosphere, the \(\gamma\)-PGA@GOx@Mn,Cu-CDS NP solution showed a relatively low value of \(^1\)O\(_2\)-induced characteristic signal during laser irradiation (730 nm, 50 mW/cm\(^2\)) for 10 min. It was due to the low supply of O\(_2\)
(Figure 2e). By contrast, a significant signal was seen in the γ-PGA@GOx@Mn,Cu-CDs NPs solution after adding acidic H₂O₂ solution. Nevertheless, no obvious ESR signals for DMPOOH or DMPOOOH adducts were found for γ-PGA@GOx@Mn,Cu-CDs NPs under the irradiation of 730 nm (Figure 2f). The above results demonstrated the generation of ¹O₂ in the γ-PGA@GOx@Mn,Cu-CDs NP solution under laser irradiation of 730 nm, rather than O₂ or ·OH. As ¹O₂ could cause irreversible decrease for the absorbance of DPBF at 416 nm, 1,3-diphenylisobenzofuran (DPBF), working as the trapping agent, was used to assess the ¹O₂ generation efficiency of the γ-PGA@GOx@Mn,Cu-CDs NPs. With the existence of the γ-PGA@GOx@Mn,Cu-CDs NPs and 730 nm laser irradiation, the absorbance of DPBF decreased gradually with an increase of irradiation time (Figure S4). Additionally, the intracellular production of ¹O₂ by the γ-PGA@GOx@Mn,Cu-CDs NPs was studied through a singlet oxygen sensor green (SOSG) probe. The probe could generate an endoperoxide product with green fluorescence by reaction with ¹O₂ in PDT (Figure 2g).

3.3 The catalytic hydrolysis capacity of γ-PGA@GOx@Mn,Cu-CDs NPs in vitro

As for glucose oxidation, the variations of H₂O₂ and pH were monitored over time to confirm the activities of γ-PGA@GOx@Mn,Cu-CDs NPs (Figure 2h,i) and free GOx (Figure S5). It was observed that the pH decreased and H₂O₂ concentration increased quickly in the combined solutions, when free GOx and γ-PGA@GOx@Mn,Cu-CDs NPs were incubated with glucose solution under enough supply of O₂. The result demonstrated that GOx coming from γ-PGA@GOx@Mn,Cu-CDs NPs could significantly promote glucose, H₂O, and O₂ converting into gluconic acid and H₂O₂. Compared to free GOx, there was no obvious loss in catalytic ability.
3.4 The photothermal effect of γ-PGA@GOx@Mn,Cu-CDs NPs in vitro

γ-PGA@GOx@Mn,Cu-CDs NPs showed an obvious absorption at the NIR wavelength, thereby exhibiting an expected PTT performance under laser irradiation of 730 nm even the concentration of γ-PGA@GOx@Mn,Cu-CDs NPs was relatively low (Figure S6a). To study the photothermal effect under NIR laser irradiation, dispersion of γ-PGA@GOx@Mn,Cu-CDs NPs in aqueous conditions was tested by monitoring the temperature elevation under laser irradiation of different power. As shown in Figure 3a and Figure 3b, γ-PGA@GOx@Mn,Cu-CDs NPs showed obvious increases in temperatures under different power laser irradiation. The temperature decreased little (Figure S6b) with an increase time because of minor degradation of γ-PGA. Furthermore, photothermal conversion efficiency (η) is an important parameter to assess the photothermal ability. By calculation using a formula from previous reports [40,41], τ can be calculated through the linear relationship between cooling time and –ln(θ) (Figure 3c and Figure S7) and the efficiency of photothermal conversion was confirmed to be 30.3%.
Figure 3. (a) The laser power dose and irradiation time-dependent temperature elevation of γ-PGA@GOx@Mn,Cu-CDs NPs solutions under 730 nm laser irradiation for 10 min. (b) IR images of γ-PGA@GOx@Mn,Cu-CDs NPs solutions under 730 nm laser at different power dose irradiation for 10 min. (c) The photothermal effect of γ-PGA@GOx@Mn,Cu-CDs NPs solution exposed to 730 nm laser irradiation (1.5 W/cm²) for 10 min. (d) Scheme of the degradation procedure for γ-PGA@GOx@Mn,Cu-CDs NPs. (e) The TEM image of γ-PGA@GOx@Mn,Cu-CDs NPs cultured with pH 7.4 buffer for 2 days. Inset is the size distribution of γ-PGA@GOx@Mn,Cu-CDs NPs. (f) TEM image of γ-PGA@GOx@Mn,Cu-CDs NPs cultured with pH 6.0 buffer with NIR laser irradiation for 10 min. Inset is the size distribution of γ-PGA@GOx@Mn,Cu-CDs NPs. (g) SEM image of γ-PGA@GOx@Mn,Cu-CDs NPs cultured with pH 6.0 buffer with NIR laser irradiation for 10 min. (h) T1 and T2-weighted MR images of γ-PGA@GOx@Mn,Cu-CDs NPs solutions with different Mn²⁺ and Cu²⁺ concentrations. The T1 relaxation rates (i) and T2 relaxation rates (j) of γ-PGA@GOx@Mn,Cu-CDs NPs solutions with different Mn²⁺ and Cu²⁺ concentrations.

3.5 The biodegradation properties of γ-PGA@GOx@Mn,Cu-CDs NPs

To evaluate the biodegradation properties of γ-PGA@GOx@Mn,Cu-CDs NPs in vitro, the biodegradation rate was investigated under various physiological
conditions (Figure 3d). Firstly, the morphology evolution of $\gamma$-PGA@GOx@Mn,Cu-CDs NPs incubated in PBS (pH=6.0 or pH=7.4) at 37 °C was directly observed. The partially biodegraded $\gamma$-PGA@GOx@Mn,Cu-CDs NPs were observed by TEM. After incubation for 3 days, the $\gamma$-PGA@GOx@Mn,Cu-CDs NPs partly collapsed in pH 6.0 buffer (Figure S8a), but the morphology of $\gamma$-PGA@GOx@Mn,Cu-CDs NPs had no obvious change in pH 7.4 buffer (Figure 3e). Then the $\gamma$-PGA@GOx@Mn,Cu-CDs NPs solution (disperse in a pH 6.0 or 7.4 buffer) was exposed to NIR laser irradiation for 10 min per day. We observed that the $\gamma$-PGA@GOx@Mn,Cu-CDs NPs (disperse in a pH 7.4 buffer) disassembled to smaller particles after 3 days (Figure S8b). However, all of the $\gamma$-PGA@GOx@Mn,Cu-CDs NPs (disperse in a pH 6.0 buffer) completely degraded and NPs that had a size of ~5 nm were found by TEM (Figure 3f) and SEM (Figure 3g) images after 1 day of incubation. This result was confirmed by previous reports [42]. Based on the above results, the in vitro release profiles of $\gamma$-PGA@GOx@Mn,Cu-CDs NPs were evaluated. From Figure S9, $\gamma$-PGA@GOx@Mn,Cu-CDs NPs remained relatively steady at neutral pH environment, and released GOx under an acidic environment. After laser irradiation, the GOx release of the irradiated group was markedly increased. Thus, the GOx release from $\gamma$-PGA@GOx@Mn,Cu-CDs NPs could be controlled by NIR laser and different pH conditions.

3.6 T1- and T2-weighted MR imaging in vitro

Since Mn(II) and Cu(II) have high paramagnetism and low biotoxicity, $\gamma$-PGA@GOx@Mn,Cu-CDs NPs were tested by both T1 and T2-weighted MR imaging [43,44]. Figure 3h exhibited that the MR images of $\gamma$-PGA@GOx@Mn,Cu-CDs NPs with different concentrations displayed an obvious concentration-dependent brightening and darkening effect. The value of tested
relaxivity ($r_1$ and $r_2$) of the $\gamma$-PGA@GOx@Mn,Cu-CDs NPs was 9.85 (Figure 3i) and 34.89 mM$^{-1}$ s$^{-1}$ respectively (Figure 3j), which were comparable to other Mn$^{2+}$ or Cu$^{2+}$-containing MR imaging agents reported previously. Therefore, our $\gamma$-PGA@GOx@Mn,Cu-CDs NPs could serve as contrast agents when T1- and T2-weighted MR imaging are performed.

3.7 The hemocompatibility of $\gamma$-PGA@GOx@Mn,Cu-CDs NPs

It is vital to assess the hemocompatibility of the nanomaterials, when the bioapplication of NMs is eventually tested by intravenous injection. After the red blood cells (RBCs) interact with NMs together, the resultant morphological change of these cells is an essential parameter to assess the hemocompatibility of nanomaterials for further bioapplication. The micrographs of RBCs incubated with $\gamma$-PGA@GOx@Mn,Cu-CDs NPs at different concentrations (Figure 4a) retained the previous circular morphology, which indicated that the shape of RBCs was not influenced by the samples. Besides, the hemocompatibility of biomaterials could also be assessed by the hemolysis rate. Hemolysis caused the release of hemoglobin, which could promote thrombosis. The hemolysis rate changed from 0.37% to 0.97% (Figure S10) when $\gamma$-PGA@GOx@Mn,Cu-CDs NPs in different concentrations. The sample still showed a negligible effect on hemolysis even with a high concentration. The activated partial thromboplastin time (APTT) (Figure S11a) and prothrombin time (PT) (Figure S11b) data of $\gamma$-PGA@GOx@Mn,Cu-CDs NPs were in the concentration range of 50-250 $\mu$g/mL, which was similar to the time of the control group, confirming the satisfying hemocompatibility of $\gamma$-PGA@GOx@Mn,Cu-CDs NPs. These results demonstrated that $\gamma$-PGA@GOx@Mn,Cu-CDs NPs could be injected via the vein.

3.8 Localization of $\gamma$-PGA@GOx@Mn,Cu-CDs NPs in 4T1 cells
The analysis of colocalization was performed to investigate the location of γ-PGA@GOx@Mn,Cu-CDs NPs in cells. The 4T1 cells treated with GOx@Mn,Cu-CDs NPs and γ-PGA@GOx@Mn,Cu-CDs NPs after 3 h was shown by the fluorescence confocal images; the γ-PGA@GOx@Mn,Cu-CDs NPs is consistent with the red fluorescence, and the 4′,6-diamidino-2-phenylindole (DAPI) is represented by the blue fluorescence. Under a confocal laser scanning microscope (CLSM), the area of red fluorescence of γ-PGA@GOx@Mn,Cu-CD NPs indicated the amount of γ-PGA@GOx@Mn,Cu-CD NPs in lysosomes of 4T1 cells (Figure 4b). As expected, the fluorescent intensity of γ-PGA@GOx@Mn,Cu-CD NPs in the 4T1 cells was higher than that of GOx@Mn,Cu-CD NPs (Figure S12). Furthermore, Z-scan measurements further supported the fact that the fluorescence came from the intracellular region of 4T1 cells (Figure 4c and Figure S12). This result demonstrated that the arginine in γ-PGA@GOx@Mn,Cu-CD NPs had higher cellular uptake ability. We speculated that their ability to target 4T1 cells may be due to the coating of γ-PGA which may promote the cell uptake of γ-PGA@GOx@Mn,Cu-CDs NPs via a speculated endocytosis pathway that is mediated by GGT (Figure 4d).
Figure 4. (a) Morphology images of RBCs incubated with γ-PGA@GOx@Mn,Cu-CDs NPs at different concentrations. Scale bar = 10 μm. (b) Confocal fluorescence microscopic images of 4T1 cells incubated with γ-PGA@GOx@Mn,Cu-CDs NPs for 3 h. Scale bar = 20 μm. (c) Z-stack FL image of the red square in b. (d) Schematic illustrations on the mechanism of cellular uptake of γ-PGA@GOx@Mn,Cu-CDs NPs via the GGT-mediated pathway. (e) Viabilities of 4T1 cells incubated with γ-PGA@GOx@Mn,Cu-CDs NPs with 730 nm laser irradiation at different power dose (*P < 0.05, **P < 0.01, and ***P < 0.001). (f) Viabilities of 4T1 cells incubated with γ-PGA@GOx@Mn,Cu-CDs NPs after different treatments in the presence of 100 mg/mL glucose (*P < 0.05, **P < 0.01, and ***P < 0.001). (g) Live/dead cell staining assays of 4T1 cells incubated with γ-PGA@GOx@Mn,Cu-CDs NPs (0-100 μg/mL) with 730 nm laser irradiation (1.5 W/cm²) at in the presence of 100 mg/mL glucose. Scale bar = 100 μm.

3.9 Cytotoxicity assay

For γ-PGA@GOx@Mn,Cu-CDs NPs to be used as a therapeutic agent, their cytotoxicity should be considered carefully. The standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) protocol was employed to investigate cell viability. As shown in Figure S13, 4T1 and NH3T3 cells were greater than
95% viable when the concentration of γ-PGA@GOx@Mn,Cu-CDs NPs was 0-250 μg/mL. Therefore, γ-PGA@GOx@Mn,Cu-CDs NPs could be considered to have a relatively low cytotoxicity. Afterwards, the effect of synergistic therapy on 4T1 cells was investigated. Firstly, 4T1 cells were incubated with γ-PGA@GOx@Mn,Cu-CDs NPs of different concentrations (dispersed in glucose-free DMEM medium) for 4 h followed by irradiation with a laser at 730 nm and a power density of 0.1 or 1.5 W/cm² for 10 min (Figure 4e). The γ-PGA@GOx@Mn,Cu-CDs NPs exhibited dose-dependent increase cytotoxicity against 4T1 cells. The relative cell viability was reduced to ∼18.2% and ∼8.9% after PDT and PDT/PTT with 150 μg/mL γ-PGA@GOx@Mn,Cu-CDs NPs, respectively. Subsequently, after incubation with the γ-PGA@GOx@Mn,Cu-CDs NPs, the cells were cultured using glucose DMEM medium, and then followed by 730 nm laser irradiation at a power density of 0, 0.1 or 1.5 W/cm² for 10 min (Figure 4f). No obvious cell death was found in the starving-like therapy (γ-PGA@GOx@Mn,Cu-CDs NPs + glucose) group, and the apoptosis rates were 26.5% when the concentration was 100 μg/mL. For the γ-PGA@GOx@Mn,Cu-CDs NPs + glucose/0.1 W/cm² group, the cell viability decreased to 9.1%, demonstrated a higher rate of apoptosis. This result indicated that the PDT efficiency was improved significantly, because GOx can oxidize the intracellular glucose to increase H₂O₂ concentrations. By contrast, upon 1.5 W/cm² of laser irradiation, a cell mortality rate of about 100% was obtained when the concentration was 100 μg/mL. The intracellular ATP content identification (Figure S14) revealed that starvation or other treatment could reduce the intracellular ATP level due to the consumption of glucose and inhibition of metabolisms. A significant lower ATP level was observed in the γ-PGA@GOx@Mn,Cu-CDs NPs + glucose/0.1 W/cm² group due to the combination inhibition effect.
Next, we conducted the cell viability irradiated by NIR laser under the hypoxic environment to confirm that γ-PGA@GOx@Mn,Cu-CDs NPs can generate O₂ to enhance PDT. The cell viability was detected after irradiation with a 730 nm laser for 10 min in N₂. As shown in Figure S15, significantly enhanced cancer cell destruction under PDT was observed for γ-PGA@GOx@Mn,Cu-CDs NPs, compared to that achieved without a 730 nm laser. To further investigate the efficiency of *in vitro* anticancer of the γ-PGA@GOx@Mn,Cu-CDs NPs under the *in vitro* tumor microenvironment, the co-staining assay of calcein AM and propidium iodide (PI) was applied. The result in Figure 4g suggested that the viability of cells decreased significantly with the increasing concentration of γ-PGA@GOx@Mn,Cu-CDs NPs, which consisted of the results of the MTT assay. The above results confirmed the potential of γ-PGA@GOx@Mn,Cu-CDs NPs to work as a desired therapeutic agent in tumor microenvironments that are hypoxic, mildly acidic, or glucose-rich with a high efficiency.

### 3.10 *In vivo* multimodal imaging

Due to the strong effects of *in vitro* synergistic therapy, the distribution of γ-PGA@GOx@Mn,Cu-CDs NPs *in vivo* was investigated by multimodal imaging. The fluorescence images were obtained at various times after injection (Figure 5a,b), which exhibited that the maximum accumulation amount of γ-PGA@GOx@Mn,Cu-CDs NPs in the tumor region was achieved after injection for 36 h. Additionally, it was found that the tumor had higher fluorescence intensity in *ex vivo* tissue analysis compared with that of the part organs (Figure 5c). Due to the macrophage uptake in the reticuloendothelial system, liver and kidney displayed strong fluorescence signal, but the amount of γ-PGA@GOx@Mn,Cu-CDs NPs decreased steadily over time. Next, we measured the Mn levels of the tumor and major organs at different time intervals after injection of the γ-
PGA@GOx@Mn,Cu-CDs NPs by inductively coupled plasma mass spectrometry (ICP-AES). As shown in Figure 5d, the trends of biodistribution and removal of the γ-PGA@GOx@Mn,Cu-CDs NPs in the tumor and major organs were consistent with the results of the FL imaging (Figure 5c). Besides, in vivo MR imaging was performed to further investigate the high rate of retention of the γ-PGA@GOx@Mn,Cu-CDs NPs in the tumor sites. Compared with the pre-injection group, the tumor region exhibited a brightening effect (Figure 5e) at 12 h post-injection. Furthermore, it was found that the tumor region had a stronger T1-weighted MRI signal after injection for 24 and 48 h. The signal intensities of T1 MRI of the tumor region were measured by the ImageJ, (Figure S16). The result further demonstrated that γ-PGA@GOx@Mn,Cu-CDs NPs greatly contributed to the increase of signal intensity in the tumor region.
Figure 5. (a) Real-time in vivo red FL images after injection of γ-PGA@GOx@Mn,Cu-CDs NPs in 4T1 tumor mice at different time points. (b) FL intensities from the tumor area at 48 h post-injection. (c) FL intensities from the tumor area at 48 h post-injection. Inset is ex vivo images of mice major organs and tumors. (d) Time-dependent concentrations of Mn in the major organs and tumors as measured by ICP-AES. (e) Real-time in vivo T1-weighted MR images after injection of γ-PGA@GOx@Mn,Cu-CDs NPs in 4T1 tumor-bearing mice at different time points. (f) PA images of tumors in mice after injection with γ-PGA@GOx@Mn,Cu-CDs NPs at different time points. (g) Real-time in vivo US images after injection of γ-PGA@GOx@Mn,Cu-CDs NPs in 4T1 tumor-bearing mice at different time points. (h) IR thermal images of 4T1 tumor-bearing mice injected with γ-PGA@GOx@Mn,Cu-CDs NPs under the 730 nm laser (0.1 or 1.5 W/cm²) irradiation.

Based on the effective absorption within the NIR region, the feasibility of γ-PGA@GOx@Mn,Cu-CDs NPs for in vivo photoacoustic imaging was subsequently investigated. After injection for 0, 24, and 48 h, the PA images of the tumors showed that γ-PGA@GOx@Mn,Cu-CDs NPs could accumulate in the tumor effectively. After prolonging the circulation time in blood vessels during the entire imaging process, the PA signals could still be relatively maintained (Figure 5f). Besides the satisfying tumor-targeting capability, the generation potency of O₂ in the tumor was also investigated by ultrasound imaging. Figure 5g exhibiting an increase in O₂ inside the tumor over time when γ-PGA@GOx@Mn,Cu-CDs NPs-after systematic administration. The amount of O₂ was quantified by the echo intensity in the tumor and the maximum O₂ was observed after injection for 36 h. Tumor cells have been confirmed to be able to produce abundant H₂O₂ with a stable rate, which becomes the log scale after the cell numbers more than a certain amount. Therefore, the γ-PGA@GOx@Mn,Cu-CDs NPs reaction with H₂O₂ was slow and time dependent. The result was also consistent with that found in other studies [42]. The amount of O₂ in the tumor after treated with γ-PGA@GOx@Mn,Cu-CDs NPs increased by 1.83 ± 0.11 fold compared to that of the tumor before injection (Figure S17).

3.11 The therapeutic effect of γ-PGA@GOx@Mn,Cu-CDs NPs in vivo
The in vivo therapeutic effect of γ-PGA@GOx@Mn,Cu-CDs NPs was further investigated. Four groups (six mice each group) of 4T1 tumor-bearing nude mice were utilized in this study. In the treatment groups, mice were intravenously injected with γ-PGA@GOx@Mn,Cu-CDs NPs (0.25 mg/mL, 100 µL) and irradiated under laser at 730 nm and power density of 0, 0.1 or 1.5 W/cm² for 10 min. To monitor and record the tumor temperature, the NIR camera was applied (Figure 5h and Figure S18). After being treated with γ-PGA@GOx@Mn,Cu-CDs NPs, the tumor surface temperatures of mice significantly increased and maintained at around 50.3 °C under exposure to NIR light with a power of 1.5 W/cm². By contrast, the tumor region temperature of PBS (1.5 W/cm²) and γ-PGA@GOx@Mn,Cu-CDs NPs (0.1 W/cm²) group did not have a significant change under NIR laser irradiation. After different treatments, a digital caliper was used to measure the tumor sizes every 2 days (Figure 6a). The mice tumor sizes presented a slight reduction after being treated with γ-PGA@GOx@Mn,Cu-CDs NPs compared to the PBS control group. The result suggested the limitation of the antitumor effect of starving-like therapy on 4T1 tumor models. In γ-PGA@GOx@Mn,Cu-CDs NPs + 0.1 W/cm² laser group, an improved antitumor effect on the 4T1 tumor could be observed due to the enhanced PDT, compared to that of the γ-PGA@GOx@Mn,Cu-CDs NPs group. The triple synergistic treatment group, γ-PGA@GOx@Mn,Cu-CDs NPs + 1.5 W/cm² laser group, showed the best treatment effect. This was further confirmed by both the photographs and weights of tumors excised from mice (Figure 6b,c). Both H&E and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining of tumors after treatment were then carried out to further examine the synergistic treatment effects (Figure 6d,e). The results showed that nearly all tumor cells were dead in the group treated with γ-PGA@GOx@Mn,Cu-CDs NPs + 1.5 W/cm² laser. The body weights of mice were recorded during the treatment (Figure 6f), but there
were no remarkable bodyweight fluctuations or significant differences among various groups. The result demonstrated that the prepared nanoplatforms could improve the tumor inhibitory effect with negligible systematic toxicity. Additionally, H&E staining and excisional liver images (Figure 6g) also confirmed the weak systemic toxicity of γ-PGA@GOx@Mn,Cu-CDs NPs with laser. There were no pathological changes in the heart, spleen, liver, lung, and kidneys, compared to the control group. The above result demonstrated that the side effects of γ-PGA@GOx@Mn,Cu-CDs NPs to normal tissues were limited.

Figure 6. (a) Tumor volume growth curves with various treatments (*P < 0.05, **P < 0.01, and ***P < 0.001). (b) Images of tumors collected from different groups of mice 14 d after different treatment: group 1, control; group 2, γ-PGA@GOx@Mn,Cu-CDs NPs; group 3, γ-
PGA@GOx@Mn,Cu-CDs NPs + 730 nm laser at 0.1 W/cm²; group 4, γ-PGA@GOx@Mn,Cu-CDs NPs + 730 nm laser at 1.5 W/cm². (c) Tumor weight curves with various treatments (*P < 0.05, **P < 0.01, and ***P < 0.001). H&E (d) and TUNEL (e) stained sections of tumors after various treatments. (f) Bodyweight curves of mice in each group during 14 d treatment. (g) H&E stained sections of main organs and images of the excisional livers.

3.12 The checkpoint blockade process of our combined γ-PGA@GOx@Mn,Cu-CDs NPs plus aPD-L1 immunotherapy in vivo

As a novel and promising strategy for cancer immunotherapy, checkpoint blockade has obtained considerable attention in the last few years [45,46]. Increasing evidence from various research teams has evaluated that cancer immunotherapy could be combined with different treatment modalities (such as PDT, PTT, chemotherapy, RT, and starving-like therapy) to improve the result of cancer treatment [47-50]. In this work, we investigated whether the strong immunological responses induced by synergistic treatment with γ-PGA@GOx@Mn,Cu-CDs NPs could stop the growth of tumor cells with programmed cell death 1 ligand (PD-L1) checkpoint blockade.

In this study, the left and right flanks of each Balb/c mice were inoculated with 4T1 tumor cells. The two tumors of both sides in mice were chosen to be primary tumors with the treatment of starving-like therapy/PDT/PTT, and distant tumors (2 cm away) were treated without direct light exposure. All 4T1 tumors mice were divided into seven different groups: 1) control; 2) γ-PGA@GOx@Mn,Cu-CDs NPs, 3) γ-PGA@GOx@Mn,Cu-CDs NPs + anti-PD-L1 (aPD-L1); 4) γ-PGA@GOx@Mn,Cu-CDs NPs with laser irradiation of 730 nm at a power of 0.1 W/cm²; 5) γ-PGA@GOx@Mn,Cu-CDs NPs with laser irradiation at 730 nm at a power of 0.1 W/cm² and anti-PD-L1; 6) γ-PGA@GOx@Mn,Cu-CDs NPs with laser irradiation at 730 nm at the power of 1.5 W/cm²; 7) γ-PGA@GOx@Mn,Cu-CDs NPs with laser irradiation of 730 nm at the power of 1.5 W/cm² and anti-PD-L1. After the intravenous injection with various therapeutic
agents for 24 h, the tumors of mice right side from group 4-7 were irradiated by laser at 730 nm at 1.5 mW/cm² for 10 min every 6 h. At days 1, 3, 5, mice in group 5-7 were injected intravenously with anti-PD-L1 antibody at 600 μg/kg after light irradiation (Figure 7a, Figure S19a, and Figure S20a). It was found that group 7 could hinder the growth of primary tumors more obviously than group 2-6, whereas group 3 and group 5 respectively showed more therapeutic efficiency than group 2 and 4 at applied anti-PD-L1 dose (Figure 7b,c, Figure S19b,d, Figure S20b,d, and Figure S21a), verifying combination treatment could enhance the immune therapy of anti-PD-L1. Interestingly, group 7 triggered CD8+ cytotoxic T lymphocytes (CTL) infiltration (over 2.5 folds than that of the control group) in the primary tumor (Figure 7d). Surprisingly, group 6, and group 7 were found to be able to hinder the progression of non-irradiated distant tumors remarkably (Figure 7e,f, Figure S20c,d, and Figure S21b), while Group 7 showed more therapeutic efficiency than group 6, verifying that combined treatments could bring an enhanced antitumor immune response. Interestingly, group 2, 3, and 4 revealed significantly greater tumor growth inhibition than group 1, indicating GOx-catalyzed decomposition of intratumoral glucose-induced starving-like therapeutic effects (Figure S19c,d and Figure S20c,d). TUNEL (Figure S22) and H&E (Figure 7h) staining of the tumor after treatment were then carried out to further examine the therapeutic effects. The results showed that nearly all tumor cells were dead in the right and left tumor treated with γ-PGA@GOx@Mn,Cu-CDs NPs with laser irradiation at 730 nm and a power of 1.5 W/cm² plus anti-PD-L1.
Figure 7. (a) Schematic illustration to show the experimental design of combining PDT/PTT/starving-like therapy with anti-PD-L1 therapy. The tumor growth curves (b), the photograph of tumors (c), and percentages of CTL infiltration (d) for primary tumors (right) after various treatments (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$). The tumor growth curves (e), the photograph of tumors (f), and percentages of CTL infiltration (g) for nonirradiated tumors (left) after various treatments (**$P < 0.01$ and ***$P < 0.001$). (h) H&E stained tumor slices of primary and nonirradiated tumors after various treatments. (i) The IFN-$\gamma$ levels in sera from mice detected at 7 days after various treatments (*$P < 0.05$ and ***$P < 0.001$).
Furthermore, CTL infiltration increased significantly in the distant tumors after the combined treatment (Figure 7f, Figure S19f, and Figure S20f), compared to other treatment groups. The combined treatment-induced DC maturation was further investigated. The expression of the co-stimulatory molecules CD80 and CD86 on DCs was determined in the inguinal lymph nodes. The combined treatment could significantly increased the expression of both CD80 and CD86 compared to that resulted from other groups (Figure S23). The effective generation of interferon-gamma (IFN-γ) in the mice serum samples was measured at day 7 post-irradiation (Figure 7i) after starving-like therapy/PDT/PTT with anti-PD-L1 treatment. This confirmed the effective cellular immune responses triggered by the combined treatment. No obvious bodyweight fluctuation nor remarkable changes were found between each group (Figure S24). Additionally, H&E staining (Figure S25) demonstrated the negligible systemic toxicity of γ-PGA@GOx@Mn,Cu-CDs NPs. There were no obvious pathological changes in the heart, liver, spleen, lung, kidney, nor intestine compared to that of the control group. All the above results indicated that the γ-PGA@GOx@Mn,Cu-CDs NPs treatment combined with anti-PD-L1 blockade could trigger effective antitumor immune responses synergistically, so that tumors can be destroyed with combined treatment directly and the progression of tumors can be halted without direct laser irradiation.

Based on the above inspiring findings, it was found that γ-PGA@GOx@Mn,Cu-CDs NPs could offer enhanced combination therapy owing to the tumor microenvironment that is hypoxic, mildly acidic, and glucose-rich (Scheme 1b). Furthermore, the tumor-related antigens produced in situ after combination therapy with γ-PGA@GOx@Mn,Cu-CDs NPs showed vaccine-like functions. After further combination with checkpoint blockade, obvious anti-tumor immune responses for effective immunotherapy were observed. Afterward, the
releasing tumor-associated antigens could be engulfed, processed, and presented by antigen-presenting cells, such as dendritic cells. These cells could activate CTLs, whose activity could be kept and promoted by anti-PD-L1 checkpoint blockade. At last, spreading tumor cells could be destroyed specifically due to the migration of CTLs into distant tumors and cellular immunity after mediation. Besides, the hematological assessment was performed by the standard serum biochemistry assay and complete blood panel test after injection with $\gamma$-PGA@GOx@Mn,Cu-CDs NPs for 1, 7, and 18 days (Figure S26). No remarkable difference was observed within all the measured results, which verified that $\gamma$-PGA@GOx@Mn,Cu-CDs NPs plus anti-PD-1 had satisfying biocompatibility and promising clinical applications.

4. Conclusion

This study designed a biodegradable, multifunctional NPs to trigger combined starving-like therapy/PDT/PTT and immunotherapy. The $\gamma$-PGA@GOx@Mn,Cu-CDs NPs with targeted polymer $\gamma$-PGA enhanced cellular internalization and tumor retention. Moreover, the $\gamma$-PGA@GOx@Mn,Cu-CDs NPs completely degraded into Mn,Cu-CDs in TME 2 days after laser exposure. The $\gamma$-PGA@GOx@Mn,Cu-CDs NPs exhibited excellent tumor inhibition due to the high tumor accumulation and starving-like therapy/PDT/PTT. In combination with anti-PD-L1 checkpoint blockade treatment, $\gamma$-PGA@GOx@Mn,Cu-CDs NPs-based combination therapy could ablate primary tumors directly and suppress distant tumors via activating strong anti-cancer immune responses. In summary, this study shows a novel strategy for cancer treatment, which could remove primary tumors as well as attack and destroy spreading metastatic tumors.

Conflicts of interest
The authors have no existing conflicts to declare.

Acknowledgments

This project was supported by the Foundation of Jiangsu Collaborative Innovation Center of Biomedical Functional Materials and Villum Fonden, Denmark, Project No. 13153. M.Z. and T.Z. thanks the China Scholarship Council (CSC) for generous support. We also would like to thank M.W. of the Nanjing University for their dedicated acquisition of the PA, MR, and fluorescence imaging data.

Appendix A. Supplementary data

Supplementary data to this article can be found online.

References


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