Hypotoxic amphiphilic polymers with high fluoride content as oxygen carriers enhance photodynamic therapy against hypoxic tumors

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Photodynamic therapy (PDT) eradicates cancer cells by transforming tumor oxygen into highly reactive singlet oxygen (1O2) through a photosensitizer. However, pre-existing hypoxia within tumors and oxygen consumption during PDT can cause insufficient oxygen supply, hence hindering the effectiveness of this treatment modality. Fluorides, renowned for their excellent biocompatibility and oxygen affinity, have been widely applied in medical environments as carriers. In this study, a hypotoxic amphiphilic fluorinated polymer (PEGAF) was synthesized using polyethylene glycol methyl ether acrylate (PEGA) and perfluorooctyl acrylate (PFOEA) as monomers through the atomic transfer radical polymerization (ATRP) technology and used as carriers for oxygen and the photosensitizer chlorin e6 (Ce6). Experimental results demonstrated that PEGAF@Ce6-O2 substantially improved the hypoxic microenvironment of tumors and significantly inhibited tumor growth, markedly enhancing the outcome compared to sole PDT application. This study proposes a new strategy for enhancing the efficacy of PDT.

1. Introduction

Photodynamic therapy (PDT) is an emerging method for cancer treatment.1-3 The fundamental principle of PDT involves a photosensitizer (PS) transferring energy to the dissolved O2 within the tumor, generating cytotoxic singlet oxygen (1O2) for cancer treatment.4-6 Compared with conventional treatment methods, PDT boasts numerous advantages, including simple treatment equipment, non-invasiveness, selectivity, high safety, the absence of toxic side effects, a low likelihood of drug resistance, and no damage to critical organs such as the liver and kidneys.7-9 Currently, PDT is gradually gaining recognition and adoption due to its superior therapeutic benefits and high safety levels.10-12 Liu loaded indocyanine green (ICG) into liposomes (ICG-lipo), transforming in situ tumor cells into therapeutic vaccines through ER-targeted PDT.13 Chen synthesized a novel type of organic semiconductor material, C3N2, intended for tumor PDT treatment.14 Huang developed a multifunctional nanodrug containing the photosensitizer Ce6, triggering robust immunity through photodynamic therapy (PDT) and hypoxia alleviation.15 However, the PDT process necessitates a large volume of oxygen, exacerbating the hypoxic conditions in tumors, thereby reducing the therapeutic effect.16-19 To overcome this issue, researchers have developed various materials to alleviate tumor hypoxia, mainly falling into two categories. One is endogenous oxygen production, where compounds such as MnO2, hydrogen peroxide enzymes, Fe, and Pt can catalyze intra-tumoral H2O2 decomposition to generate O2.19-25 However, this method is limited by the low concentration of H2O2 within cells. The second approach involves directly delivering oxygen to the tumor using perfluorocarbons (PFCs). PFCs are an efficient oxygen supply system widely used in medical fields, known for their potent oxygen-carrying capacity, high biocompatibility and bio-inertia, low volatility, and minimal influence on surrounding tissues.26-30 Therefore, they can serve as oxygen carriers to enhance the efficacy of PDT in cancer treatment. Li31 fabricated a fluorinated, functional polysaccharide-based nanocomposite for oxygen delivery in hypoxic regions, and the results showed that the efficacy of treating solid tumors with fluorinated compounds was three times greater than that without fluorination. Tang32 developed
fluorinated semiconductor organosilicon-based oxygen nanoparticles pHPFON-NO/O₂ to combat tumor hypoxia, demonstrating the fluorinated nanoparticles’ excellent oxygen-carrying capacity and tumor treatment results.

In this study, a high-fluorinated polymer (PEGAF) was synthesized using PEGA and PFOEA as organic segments through the ATRP method, demonstrating its ability to alleviate tumor hypoxia and promote effective PDT in tumors (Scheme 1). The hydrophobic inner core of PEGAF is capable of not only loading the photosensitizer Ce6 but also, due to the high degree of fluorination in PFOEA, binding to a significant amount of oxygen, serving as a reliable oxygen carrier to alleviate the hypoxic microenvironment of tumors. Under the irradiation of a 660 nm laser, Ce6 can effectively generate cytotoxic ¹⁰₂. The oxygen-carrying capacity, photosensitizer-loading ability, and ROS generation capabilities of PEGAF were evaluated. Moreover, the antitumor capacity of PEGAF, loaded with O₂ and Ce6, was assessed.

2. Experimental

2.1. Materials
Poly(ethylene glycol) methyl ether acrylate (PEGA, Mn 480), ethyl 2-bromoisobutyrate (EBIB, 98%) and tris[2-dimethylamino ethyl] amine (Me₆Tren, 97%) were purchased from Sigma-Aldrich. CuBr₂ (99.9%), (perfluorooctyl)ethyl acrylate (PFOEA, 98%), 2,2,2-trifluoroethanol (THFE, >99.8%), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, >99%) and chlorin e6 (Ce6) were purchased from Every Green. Dulbecco’s modified Eagle’s medium (DMEM), phosphate buffered saline (PBS) and trypsin were purchased from Solarbio. 2′,7′-Dichlorodihydrofluorescein diacetate (DCFH-DA), singlet oxygen sensor green (SOSG) and Hoechst 33342 were obtained from Beyotime. All of the chemicals were used as supplied without further purification.

2.2. Cell lines and animals
4T1, MCF-7 and HeLa cell lines were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Science (Shanghai, China). Female nude mice were obtained from the Guangdong Medical Laboratory Animal Center (Guangdong, China) and all experiments were approved by the Institutional Ethical Committee of Animal Experimentation of Hainan Medical University in China.

2.3. Characterization
The chemical compositions of PEGAF were characterized by nuclear magnetic resonance (NMR, Bruker AV-400 MHz, Switzerland), with CDCl₃ as the solvent and tetramethylsilane (TMS) as the internal reference. FT-IR spectra of various samples were recorded using a Nicolet AVATAR 360 FTIR

Scheme 1  Synthesis of the amphiphilic fluorinated polymer PEGA-PFOEA (PEGAF) and its application in enhancing photodynamic therapy.
spectrometer. The molecular weights (MWs) and dispersity (D) were determined by gel permeation chromatography (GPC, Shimadzu RID-20A, Japan) and tetrahydrofuran was used as the eluent with a flow rate of 1.0 mL min\(^{-1}\) at 40 °C. Elemental mapping was performed using energy dispersive spectroscopy (EDS, JEOL JSM-7100F, Japan) with an acceleration voltage of 20 kV. A Marvin laser particle size analyzer was used to characterize the particle size of PEGAF. A 1 mg mL\(^{-1}\) PEGAF solution was added to a four-sided transparent colorimetric dish up to the scale range of the instrument, the test temperature was set at 25 °C, the average value was scanned 20 times, and the measurement was repeated three times (DLS, Malvern Zetasizer Nano ZS90, UK). Meanwhile, the specific morphology of the nanoparticles was observed using transmission electron microscopy (TEM, FEI, Tecnai 12, USA). 20 μL of a 1 mg mL\(^{-1}\) sample was dropped onto a copper net for 10 min, excess sample was removed with filter paper, then 20 μL of dyeing solution was dropped onto the copper net for 2 min, excess dye was removed with filter paper, and the net was placed under a drying lamp to dry. Finally, the test was carried out under an acceleration voltage of 100 kV. A thermal gravimetric analyzer (TGA) was used to test the thermal stability of PEGAF nanoparticles (NPs). 10 mg of PEGAF was placed in a small crucible and heated to 100 °C at a rate of 6 k min\(^{-1}\) to monitor the mass change of PEGAF. The oxygen content in the aqueous solution was determined using a portable dissolved oxygen meter (YSI 550A, USA). The 660 nm laser was obtained from Beijing Honglan Photoelectric Technology Co., Ltd (Beijing, China). The singlet oxygen production capability was measured using a fluorescence spectrophotometer (F-7100, HITACHI, Japan). Cell imaging was performed using a confocal laser scanning microscope (CLSM, Nikon A1 + T, Japan). The methyl thiazolyl tetrazolium (MTT) assay was conducted using a microplate reader (BioTek, SynergyHT, USA).

### 2.4. Synthesis of PEGAF

With CuBr\(_2\) (1.85 mg, 0.008 mmol) and Me6Tren (11.5 mg, 0.05 mmol) as catalysts, 3 mL of 2,2,2-trifluoroethanol (THFE) was added to a sample vial, and the mixture was sonicated for 15 minutes to dissolve the CuBr\(_2\). Subsequently, the hydrophilic monomer PEGA (2.0 g, 4.15 mmol) and the initiator EBIB (81 mg, 0.415 mmol) were added to the solution. The vial was then covered with a rubber plug, and nitrogen was continuously blown into the vial through a long needle inlet while being expelled through a short needle outlet to maintain an inert atmosphere. The reaction was allowed to proceed at room temperature for 6 hours, resulting in a 99% yield. Subsequently, the hydrophobic block PFOEA (2.14 g, 4.14 mmol) containing fluorine was treated with nitrogen and added to the oxygen-free mixture using a syringe. The reaction continued at room temperature for an additional 16 hours, yielding 97.6% of the desired fluorinated polymer. To purify the product, the reaction mixture was poured into 500 mL of deionized water and sonicated for 15 minutes to achieve dispersion. The dispersed product was then transferred to a dialysis bag (MWCO = 3500) and dialyzed for 72 hours in a 5 L beaker, with the water being changed every 12 hours. After dialysis, the solution was freeze-dried for 24 hours to obtain a transparent gel-like substance (3.65 g), with a final yield of 90.1%.

### 2.5. Physicochemical characterization of PEGAF NPs

DLS was used to characterize the size of the PEGAF NPs, where 30 mg of PEGAF NPs were sonicated and dispersed in 5 mL of deionized water for 10 minutes, and then the size of the PEGAF NPs was measured from the supernatant using DLS. The detailed structure and morphology of the PEGAF NPs were examined using TEM. The critical micelle concentrations (CMC) of the PEGAF NPs were measured by observing changes in particle size. We prepared PEGAF solutions at different concentrations using the double dilution method, and after forming micelles, PEGAF showed a significant change in particle size.

### 2.6. C6 loading capacity of PEGAF NPs

500 μg of C6 and 500 μg of PEGAF were dissolved in 100 μL of DMSO, and the resulting solution was then slowly dropped into 5 mL of distilled water and subjected to sonication for 15 minutes. The formed PEGAF NPs were subsequently transferred into a dialysis bag with a molecular weight cut-off (MWCO = 3500 Da) and dialyzed for 24 hours in 4 L of physiological saline to remove any residual organic solvent and free C6. Lastly, the C6 loaded NPs were collected in a centrifuge tube and stored at 4 °C for future use.

To determine the loading capacity of PEGAF, several samples of C6 at known concentrations were initially prepared to establish a standard curve. Then, the post-dialysis PEGAF@C6 solution was diluted 100-fold to a range measurable by UV-visible spectroscopy, and the concentration of C6 was measured at a wavelength of 400 nm. Its loading capacity was then calculated based on the standard curve.

### 2.7. C6 release trend of PEGAF NPs

In order to simulate the C6 loaded NPs and the release trend of C6 in the acidic tumor microenvironment, 5 mL of C6 loaded NPs at a concentration of 300 μg mL\(^{-1}\) was dialyzed in acidic physiological saline with a pH of 5 and PBS buffer solution with a pH of 7.4. Samples were taken at specific time intervals and the changes in concentration were measured using UV-visible spectroscopy to determine the release trend of the PEGAF NPs in the tumor microenvironment.

### 2.8. Photothermal properties of PEGAF@C6 NPs

The photosensitizer C6 exhibits superior photothermal conversion efficiency. However, its aggregation within PEGAF could potentially impact the photothermal conversion efficiency of C6. To measure the heating curve of PEGAF@C6 under the rated power, 1 mL of PEGAF NPs containing 600 μg of C6 was placed in a quartz tube, and a group with the same concentration of free C6 was set as a control. The sample was then irradiated for 800 s under a 660 nm laser with a power density of 0.5 W cm\(^{-2}\), and the temperature was recorded every
30 seconds using a thermometer until it ceased to rise further. In order to further validate the thermal stability of the PEGAF NPs, PEGAF@Ce6 NPs were irradiated for 480 s using a near-infrared laser of 660 nm with a power density of 0.5 W cm\(^{-2}\) and then allowed to cool down to room temperature. This heating–cooling process was repeated four times to assess the thermal stability of the PEGAF@Ce6 NPs.

2.11. In vitro photo-toxicity efficacy

The vitality of cells after various treatments was evaluated using the MTT method. Different concentrations of PEGAF and PEGAF@Ce6 nanoparticles were prepared and added to the cells, followed by incubation overnight in a 5% CO\(_2\) incubator for 2 hours. After a single wash with oxygen-free PBS, the cells were incubated with 1.5 mL of the 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) probe for 30 minutes. Subsequently, the cells were washed with oxygen-free PBS two times and irradiated for 3 minutes per dish with a 660 nm laser at an intensity of 50 mW cm\(^{-2}\). 4T1 cell nuclei were stained using Hoechst 33342 (0.1 mM) for 10 minutes, followed by another 20-hour incubation. In the experiment, 10 \(\mu\)L of 5 mg mL\(^{-1}\) MTT solution was added to each group, followed by incubation at 37 °C in a 5% CO\(_2\) incubator for 4 hours. The supernatant was removed, and 150 \(\mu\)L of DMSO was added to each well. The absorbance at 570 nm was measured using a microplate reader, and cell viability was calculated from these measurements.

2.12. Tumor model

To investigate the in vivo anti-tumor effects of these treatment approaches, we established a subcutaneous 4T1 tumor model in female nude mice. A suspension of 1 \(\times\) 10\(^6\) 4T1 cells was prepared in 5 mL of serum and injected subcutaneously into each mouse under anesthesia. The tumor volume was calculated using the formula \(V = 0.5LW^2\), where \(L\) is the longitudinal diameter of the tumor and \(W\) is the transverse diameter of the tumor. All animal procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Hainan Medical University and approved by the Animal Ethics Committee of Hainan Medical University.

2.13. In vivo efficacy

When the tumor volume reached 80 mm\(^3\), the tumor-bearing mice were randomly divided into four groups: PBS, PEGAF@DOX, PEGAF@Ce6, and PEGAF@Ce6-O\(_2\) (2.5 mg kg\(^{-1}\) Ce6, 2.5 mg kg\(^{-1}\) DOX). Mice in each group received one intra-tumoral injection. After the injection, groups containing Ce6 were irradiated with a 660 nm laser at a power density of 500 mW cm\(^{-2}\) for 10 minutes at the tumor site. The tumor size and mouse weight were measured daily. On the 6th day, all animals were euthanized, tumors were excised, the tumor growth inhibition rate was calculated, and tumor apoptosis immunohistochemical analysis was performed.

2.14. Statistical analysis

The results are presented as mean ± standard deviation of three separate experiments. We applied Student’s t-test to examine significant differences between groups and \(p < 0.05\) was regarded as statistically significant.

3. Results and discussion

3.1. Synthesis of PEGAF

In this study, the hydrophilic block PEGA and the hydrophobic block PFOEA were used to synthesize a polymer with a high fluorine content (PEGAF). PEGAF was characterized using NMR, FTIR, and GPC. The \(^1\)H NMR results indicated that the polymerization of PEGA in the first step and the polymerization of PEGA-PFOEA in the second step were entirely reactive (Fig. 1A and B). Fig. 2B shows that the reaction yield was maintained at 97.6%, which was determined through the integration of the –CH\(_3\) hydrogen signal of the PEGA crude product with the C=–C hydrogen signals of the two monomers. As per \(^1\)H NMR (Fig. 1C), the methyl (f) and methylene (g and h) protons in PEGAf could be observed at \(\delta = 3.36\) ppm and \(\delta =
3.50–3.70 ppm, respectively. Meanwhile, methylene (e) and methine (d) could be seen at $\delta = 2.0$–2.50 ppm, resulting from the polymerization reaction leading to the disappearance of C–C double bonds, and a recognizable PFOEA fluoride signal was detected in the $^{19}$F NMR spectrum (Fig. 1D). In addition, FTIR analysis showed a noticeable reduction in the C–C double bonds around 1600–1680 cm$^{-1}$ (Fig. 1E). Concurrently, the number average molecular weight ($M_n$) of PEGA after the first step of polymerization was 5130 Da with a dispersity ($D$) of 1.037. After the second polymerization step, PEGAF maintained a narrow dispersity ($D$) of 1.055 with an average molecular weight ($M_n$) of 10633 Da (Fig. 1F). The observed increment in molecular weight at each reaction step provides evidence for the successful control of the polymerization process.

3.2. Physicochemical characterization of PEGAF NPs

PEGAF NPs were prepared using the nanoprecipitation method by introducing PEGAF dropwise into water. Due to the hydrophobicity of PFOEA and the hydrophilicity of the PEGA block, PEGAF demonstrated self-assembly behavior in water, leading to the formation of a hydrophobic inner core composed of PFOEA and a hydrophilic outer shell composed of PEGA.

The size of the nanoparticle affects its efficiency of transportation within the body, with smaller nanoparticles generally exhibiting better penetration into tumor tissues. Therefore, DLS and TEM were employed to characterize the size and morphology of the PEGAF NPs. Changes in the diameter of PEGAF before and after Ce6 loading were

![Fig. 1 Synthesis of PEGAF.](image-url)
measured in aqueous solutions. According to DLS results (Fig. 2A and B), the diameters of PEGAF and PEGAF@Ce6 were 91 nm and 125 nm, respectively. TEM images showed that the diameters of PEGAF and PEGAF@Ce6 were 31.7 nm and 38 nm, respectively (Fig. 2C and D). The diameters obtained via DLS were larger than those obtained via TEM, likely due to the swelling of the hydrophilic PEGA chains in the aqueous solution and their contraction under drying conditions. To achieve the desired circulation time, maintaining the colloidal stability of PEGAF NPs in vivo is imperative. Thus, the colloidal stability of PEGAF and PEGAF@Ce6 in PBS was investigated. As shown in Fig. 2E, the mean particle size remains unchanged in PBS after incubation for 7 days, demonstrating good colloidal stability under physiological conditions. Thermogravimetric analysis (TGA) indicated that the weight loss of PEGAF was within a human-compatible range, underscoring its excellent thermal stability (Fig. 2E). Furthermore, the CMC of PEGAF was measured by detecting particle size changes in solution (Fig. 2G), which were in the range of 15.7–31.5 μg mL⁻¹. Additionally, elemental analysis revealed even distributions and mass percentages of carbon, oxygen, and fluorine in PEGAF, with fluorine atoms having a high mass percentage of up to 28.58% (Fig. 2H). The collective results of particle size, morphology, solution stability, thermal stability and element distribution confirm the successful synthesis of the desired materials with excellent physicochemical properties.

3.3. PEGAF Ce6 loading and release
The Ce6 loading and release capabilities of PEGAF were measured utilizing UV-visible absorption spectrophotometry. As a result of the limited water solubility of Ce6 and the constrained loading capacity of PEGAF, an excess of Ce6 was sonicated to load it into the hydrophobic core of PEGAF. After 24 hours of dialysis in saline, the concentration of Ce6 was determined using UV-visible spectroscopy, a standard curve was plotted, and its loading rate was calculated (Fig. 3A and B). As shown in Fig. 3B, following dialysis, the content of Ce6
Fig. 3  Capacity of Ce6 load, release and photothermal conversion efficiency. (A) Known concentrations of Ce6 and PEGAF@Ce6’s UV-visible absorption peak after dialysis. (B) Standard curve of PEGAF@Ce6 based on (A). (C) and (D) UV-visible absorption spectra showing Ce6 release at pH 5 and pH 7.4. (E) The release of Ce6 from PEGAF@Ce6 at pH 5 and pH 7.4 based on (C) and (D). (F) Heating and cooling cycles of PEGAF@Ce6. (G) and (H) Heating curves for PEGAF@Ce6 and free Ce6 solutions at different concentrations.
in PEGAF NPs was 5.82 μg mL\(^{-1}\) and the loading capacity of PEGAF was 36.8%. Subsequently, the dialyzed solution was placed in media with pH 5 and 7.4, respectively, simulating the weakly acidic environment within the tumor, to test the Ce6 release capability of PEGAF. The changes in the absorbance of the material were measured at different time intervals using UV-visible spectroscopy (Fig. 3C and D). The characteristic UV-visible peaks of Ce6 were located at 400 nm, and then, the Ce6 release curves of PEGAF@Ce6 were plotted and compared with the Ce6 release curve of a physiological saline solution at pH 7.4 (Fig. 3E). As observed from Fig. 3C and D, there is a notable discrepancy in the Ce6 release rate of PEGAF@Ce6 between acidic and neutral solutions. This difference is likely attributed to the solubility behavior of Ce6: at neutral pH, higher absorbance values indicate Ce6’s solubility, whereas lower values at acidic pH suggest decreased solubility. This phenomenon aligns with the reported pKa values of Ce6 (6.5–8.5). After 14 hours, the Ce6 release rate of PEGAF@Ce6 reached 73% in a saline solution of pH 5, significantly higher than the release rate of below 12% in a saline solution of pH 7.4, demonstrating the excellent pH responsiveness of PEGAF.

3.4. Photothermal properties of PEGAF@Ce6 NPs

The aggregation of loaded Ce6 could potentially affect the photothermal effects of Ce6, and excessive temperatures may influence the results of animal experiments. Consequently, the photothermal properties of PEGAF@Ce6 were investigated and compared with those of free Ce6 (Fig. 3G and H). Under irradiation with a laser of power density 0.5 W cm\(^{-2}\) for 800 seconds, the temperature of PEGAF@Ce6 NPs rapidly increased to 70 °C, and the temperature rise in free Ce6 was slightly higher, but the overall trend remained unchanged. Subsequently, upon performing four cycles of heating and cooling in a PEGAF@Ce6 solution with a Ce6 content of 600 μg, the PEGAF@Ce6 NPs did not exhibit significant photo-
bleaching (Fig. 3F), which supports the suitability of PEGAF@Ce6 NPs for photodynamic treatment within tumors.

3.5. Measurement of O₂ release

Similar to perfluorocarbons, fluorinated polymers have the ability to dissolve a significant amount of oxygen due to van der Waals interactions between oxygen and the fluorinated polymer. Therefore, we initially purged 10 mL of distilled water with nitrogen for 10 minutes to remove pre-dissolved oxygen. Then, 10 mg of PEGAF were added to the solution, and oxygen was loaded by flushing with oxygen for 15 min. Subsequently, we employed a portable dissolved oxygen meter to monitor the changes in oxygen concentration in various solutions,36 thereby studying the oxygen-carrying capacity and release behavior of PEGAF (Fig. 4A). It can be observed that even after 600 seconds, PEGAF still retained a concentration of 19 mg mL⁻¹ of oxygen, whereas the oxygen concentrations in the control groups of PBS and water were both below 15 mg mL⁻¹. The continuous decrease in oxygen concentration can be attributed to the reduced oxygen concentration in the surrounding environment, which leads to oxygen diffusion, as well as the consumption of oxygen by the electrodes of the portable dissolved oxygen meter. Importantly, compared to water and PBS, PEGAF maintained a relatively constant and higher level of oxygen concentration. Therefore, PEGAF NPs can store oxygen for an extended period, providing a reliable supply of oxygen to hypoxic tumors.

3.6. In vitro ROS generation

The generation of reactive oxygen species (ROS) plays a crucial role in PDT, and an increase in oxygen concentration promotes the production of ROS, ultimately leading to cellular damage.37,38 To validate this point, we utilized SOSG and DCFH-DA as probes to assess the ability of PEGAF to generate singlet oxygen (¹O₂) and ROS, respectively. By monitoring the fluorescence intensity, which serves as a measure of ¹O₂ content, it was observed that SOSG was oxidized by ¹O₂, resulting in an increase in fluorescence intensity (Fig. 4B). As depicted in Fig. 4B, after 10-minute irradiation with PEGAF@Ce6 NPs, the fluorescence intensity was three times higher compared to free Ce6, indicating an enhanced oxygen solubility of PEGAF. Conversely, the fluorescence intensity in the PBS group was nearly negligible.

Fig. 5  (A) Cell viability with different cell types and at various PEGAF concentrations for 24 h (n = 5). (B)–(D) Cell viability of different cell types and at various PEGAF@Ce6 concentrations for 24 h (n = 5) with laser irradiation (50 mW cm⁻²) and without laser irradiation.
After confirming the ability of PEGAF@Ce6 NPs to generate $^{1}$O$_2$ in solution, the production of ROS in 4T1 cells was further detected using DCFH-DA as the ROS indicator and CLSM imaging. The probe emits bright green fluorescence upon oxidation by ROS. CLSM images showed negligible green fluorescence in cells treated with free Ce6, PBS, and PEGAF@Ce6 without laser irradiation. However, cells treated with PEGAF@Ce6 + L exhibited significant green fluorescence, while cells treated with Ce6 alone showed negligible fluorescence, which may be attributed to insufficient oxygen, resulting in the inability to produce enough reactive oxygen species. Furthermore, quantitative analysis of fluorescence

![Graph A](image1.png)

![Graph B](image2.png)

![Graph C](image3.png)

![Graph D](image4.png)

![Graph E](image5.png)

**Fig. 6** In vivo PDT efficacy. (A) 4T1 tumor growth curves of different groups after injection treatments of 2.5 mg kg$^{-1}$ (n = 5). (B) The body weight of the mice in different groups (n = 5). (C) Digital images of dissected tumor tissues. (D) Tumor weight of mice in different groups. The PEGAF@Ce6-O$_2$ group shows $p < 0.001$ versus the PBS group. All of the data are presented as means ± SD (n = 5, Student’s t-test; statistical differences were considered significant at **p < 0.05 and very significant at ****p < 0.001). (E) H&E and HIF-1α staining of tumor sections from mice treated with various solutions (scale bar = 100 μm).
intensity was performed using CLSM (Fig. 4D), yielding consistent results with the previous findings. Importantly, the average green fluorescence intensity of the PEGAF@Ce6 + L treatment group reached 200.23, significantly higher than that of the PBS treatment group (23.17) and the free Ce6 treatment group (31.59), indicating the superior ROS generation ability of PEGAF@Ce6 NPs.

3.7. In vitro photo-toxicity efficacy

ROS can directly induce tumor cell death. Therefore, the MTT method was utilized to evaluate the therapeutic effects of different formulations.\(^{18,39}\) Initially, we examined the toxicity of PEGAF alone on three kinds of cells. Even at a concentration of 1000 \(\mu\)g mL\(^{-1}\), cell viability stayed above 80% (Fig. 5A). Subsequently, the toxicity of PEGAF@Ce6 on the three cells, both with and without laser irradiation, was tested. Without laser irradiation, Ce6 maintained a cell viability of above 80% within the tested concentration range, and a small amount of Ce6 even promoted cell growth in the absence of light (Fig. 5B–D). After 10 minutes of continuous laser irradiation, significant cellular toxicity was observed with PEGAF@Ce6 when the Ce6 concentration surpassed 2.5 \(\mu\)g mL\(^{-1}\). Within the tested concentration range, the toxicity in the non-laser group was notably lower than that in the laser group (Fig. 5D). The results also demonstrated no significant difference between various cell types in cell death mediated by ROS. These results indicate that PEGAF can effectively load oxygen and generate ROS under the action of a 660 nm laser, thereby enhancing the therapeutic effect on tumor cells.

3.8. In vivo PDT efficacy in tumor growth

Encouraged by the remarkable therapeutic effect of PEGAF NPs at the cellular level, we further investigated their influence on tumor growth in vivo. An antitumor study was carried out on 4T1 tumors in nude mice. PEGAF@Ce6, PEGAF@Ce6-O2, PEGAF@DOX, and PBS were injected intratumorally on the first and third days, followed by irradiation with a 660 nm laser, and tumor volume was then continuously monitored over 5 days (Fig. 6A). The tumor growth curve indicated a rapid increase in tumor volume in mice treated with PBS. After 660 nm laser irradiation, PEGAF@Ce6 and PEGAF@DOX had a mild inhibitory effect on tumor growth in mice. The most pronounced antitumor effect was observed with the application of PEGAF@Ce6-O2, leading to a tumor weight reduction of 63.42% on the final day, presumably owing to the enhancement of the PDT effect caused by a high oxygen load in PEGAF (Fig. 6B). The morphology of tumors after treatment with different formulations was consistent with the results mentioned earlier (Fig. 6C). It is also noteworthy that no significant decrease in the body weight of mice was observed in any of the treatment groups, indicating the excellent biocompatibility of the employed polymers (Fig. 6D). H&E staining of tumor slices on the final day revealed that PEGAF@Ce6-O2 induced the highest proportion of tumor cell apoptosis. Concurrently, HIF-1\(\alpha\) staining of the tumor also demonstrated PEGAF’s effective ability to ameliorate the hypoxic conditions of the tumor (Fig. 6E).

4. Conclusion

In this study, we have developed a highly fluorinated polymer that can effectively load O\(_2\) and Ce6 into its hydrophobic core due to the excellent oxygen affinity and self-assembly ability of fluorine. The self-assembled spheres formed have uniform sizes and can stably exist in aqueous solutions. The incorporation of O\(_2\) and Ce6 enables PEGAF to exhibit high levels of \(1^1\)O\(_2\)/ROS generation both in vitro and in vivo. Compared to the control group, PEGAF@Ce6-O2 significantly enhances the PDT effect and achieves satisfactory anticancer results. This study provides a new approach for utilizing fluorinated nanomaterials to alleviate tumor hypoxia and enhance PDT efficacy.

Author contributions

Jun-an Zhang: validation, data curation, visualization, investigation, formal analysis, and writing – original draft. Jiang-feng Sheng: validation, data curation, visualization, and investigation. David Haddleton: conceptualization, supervision, and writing – review & editing. Paul Wilson: validation, data curation, visualization, and investigation. Yong-jie Mo: validation, data curation, visualization, and investigation. Hong-li Li: conceptualization, supervision, and writing – review & editing. Lin-lu Zhao: validation, data curation, visualization, and investigation. Hong-lei Zhao: validation, data curation, visualization, and investigation. Lin-hua Zhu: conceptualization, supervision, writing – review & editing, and funding acquisition. Chun-yan Dai: conceptualization, supervision, writing – review & editing, and funding acquisition.

Data availability

The data are available from the corresponding author on reasonable request.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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