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# Degradable Fe<sub>3</sub>O<sub>4</sub>-based nanocomposite for cascade reaction-enhanced anti-tumor therapy†

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Cascade catalytic therapy has been recognized as a promising cancer treatment strategy, which is due in part to the induced tumor apoptosis when converting intratumoral hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) into highly toxic hydroxyl radicals (·OH) based on the Fenton or Fenton-like reactions. Moreover this is driven by the efficient catalysis of glucose oxidization associated with starving therapy. The natural glucose oxidase (GO<sub>x</sub>), recognized as a "star" enzyme catalyst involved in cancer treatment, can specially and efficiently catalyze the glucose oxidization into gluconic acid and H<sub>2</sub>O<sub>2</sub>. Herein, pH-responsive biodegradable cascade therapeutic nanocomposites (Fe<sub>3</sub>O<sub>4</sub>/GO<sub>x</sub>-PLGA) with dual enzymatic catalytic features were designed to respond to the tumor microenvironment (TME) and to catalyze the cascade reaction (glucose oxidation and Fenton-like reaction) for inducing oxidative stress. The GO<sub>x</sub>-motivated oxidation reaction could effectively consume intratumoral glucose to produce H<sub>2</sub>O<sub>2</sub> for starvation therapy and the enriched H<sub>2</sub>O<sub>2</sub> was subsequently converted into highly toxic ·OH by a Fe<sub>3</sub>O<sub>4</sub>-mediated Fenton-like reaction for chemodynamic therapy (CDT). In addition, the acidity amplification owing to the generation of gluconic acid will in turn accelerate the degradation of the nanocomposite and initiate the Fe<sub>3</sub>O<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> reaction for enhancing CDT. The resultant cooperative cancer therapy was proven to provide highly efficient tumor inhibition on HeLa cells with minimal systemic toxicity. This cascade catalytic Fenton nanocomposite might provide a promising strategy for efficient cancer therapy.

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## 1 Introduction

Cancer, one of the most difficult diseases to overcome over the past decades, has seriously threatened human health.<sup>1</sup> As an emerging strategy of nanocatalytic medicine, chemodynamic therapy (CDT) can transform H<sub>2</sub>O<sub>2</sub> into highly reactive oxygen species (ROS) in a tumor microenvironment (TME) by employing the Fenton reaction or Fenton-like reaction with metal ion catalysts (*e.g.*, Fe<sup>2+</sup>, Cu<sup>+</sup>, Mn<sup>2+</sup> and V<sup>2+</sup>).<sup>2–6</sup> The hydroxyl radical (·OH), the most toxic reactive oxygen species (ROS), can cause apoptosis of tumor cells owing to their high oxidation capability.<sup>7</sup> Compared with chemotherapy, radiotherapy, photothermal therapy and photodynamic therapy, CDT holds unique advantages including low side effects, high selectivity, activation by endogenous stimulus, and low treatment cost.<sup>8</sup> Most importantly, this approach ensures normal tissue safety to a certain extent, because the Fenton reaction will be substantially suppressed under slight alkaline conditions or in a normal

microenvironment with insufficient H<sub>2</sub>O<sub>2</sub> levels.<sup>9</sup> Compared with other nano-Fenton catalysts, ferroferric oxide nanoparticles (Fe<sub>3</sub>O<sub>4</sub> NPs) have attracted tremendous attention for biomedical applications due to their superparamagnetism, biodegradability, low toxicity, and cost-effectiveness.<sup>10–12</sup> Mazuel *et al.* evidenced a near-complete intracellular degradation of Fe<sub>3</sub>O<sub>4</sub> NPs by using stem cell spheroids as a tissue model and global spheroid magnetism as a fingerprint of the degradation process.<sup>13</sup> However, because of the relatively low drug loading and iron leakage, Fe<sub>3</sub>O<sub>4</sub> NPs presents the weak antitumor efficacy when used as drug carriers and iron source for CDT.<sup>14</sup> In addition, the CDT efficacy and clinical translation are restricted by the low conversion efficiency from Fe<sup>2+</sup> to Fe<sup>3+</sup>, consumption of hydroxyl radicals by glutathione (GSH),<sup>15</sup> the limited endogenous supply of H<sub>2</sub>O<sub>2</sub>.<sup>16,17</sup>

Several unique characteristics including mild acidity, rich glucose, low catalase activity and overproduced H<sub>2</sub>O<sub>2</sub> owing to the complex biological microstructure of TME open the "gate" for selective and efficient tumor treatments.<sup>18–23</sup> Clinical practice and in-depth exploration indicates that monotherapy is incapable of eliminating tumor cells completely, thus, recent studies have gradually gained a focus on the synergistic therapy. Numerous new TME-responsive strategies have acquired the gratifying achievements in nanomedicine, such as hypoxia-responsive controlled release drug delivery systems,<sup>24</sup>

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starvation therapy for tumor growth inhibition by glucose depletion,<sup>25</sup> and so on. Glucose oxidase (GO<sub>x</sub>), inherently biocompatible and degradable, can lead to the consumption of glucose in tumors for starvation therapy by efficient catalytic oxidation.<sup>26</sup> Furthermore, the generated H<sub>2</sub>O<sub>2</sub> in cancer cells can be effectively converted by Fenton reagents into toxic reactive oxygen species for CDT. Thus, CDT is expected to combine with GO<sub>x</sub>-mediated starvation therapy to arouse more efficient tumor suppression.

Different from inorganic theranostics, nanomedicines, fabricated from biodegradable polymer, demonstrate their unparalleled advantages such as biocompatibility, high drug loading, controlled drug release and stimuli-responsiveness.<sup>27</sup> For instance, Zhang *et al.* constructed a dual-catalytic nano-reactor for synergistic chemodynamic-starvation therapy by encapsulating O<sub>2</sub> carrier perfluorohexane into the hole of HMSNs, simultaneously loading GO<sub>x</sub> and Fe<sub>3</sub>O<sub>4</sub> nanoparticles on the surface of HMSNs and then coating the obtained NPs with the cancer cell membrane.<sup>25</sup> As the dual-catalytic nano-reactor promoted cascade catalytic reactions, the sequential glucose depletion and ·OH aggregation synergistically suppressed tumor metastasis with negligible side effects. In addition, Ke *et al.* fabricated a degradable polymersome nanoreactor containing polyprodrug, ultrasmall Fe<sub>3</sub>O<sub>4</sub> NPs, and GO<sub>x</sub> was loaded to integrate starvation therapy, CDT, and camptothecin-induced chemotherapy together for cooperative cancer therapy.<sup>28</sup> The nanocomposite design inducing tumor-activable cascade reactions represents an insightful paradigm for precise cooperative cancer therapy. More importantly, the modification of Fe<sub>3</sub>O<sub>4</sub> NPs with biodegradable polymer could improve the biocompatibility and effectively slow down the ion leakage in normal tissues during the delivery.

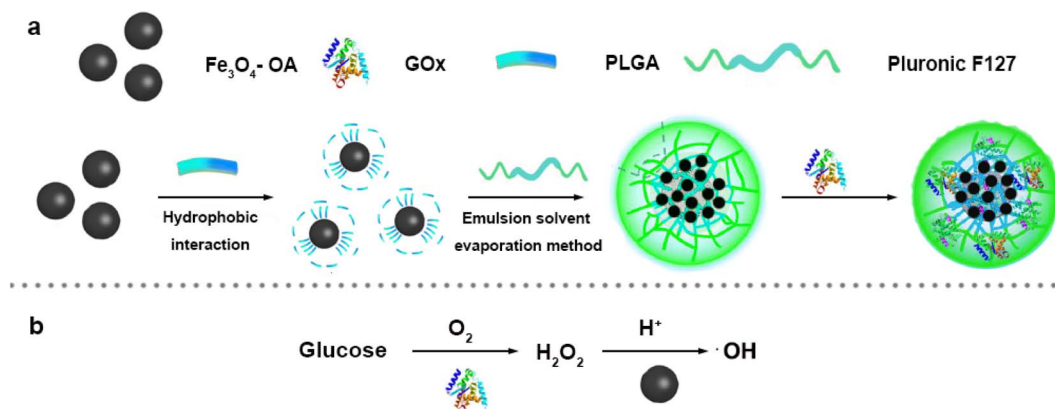
Toward the issues, herein, a biodegradable cascade catalytic nanocomposite (Fe<sub>3</sub>O<sub>4</sub>/GO<sub>x</sub>-PLGA) for cancer therapy was developed by encapsulating oleic acid (OA)-modified Fe<sub>3</sub>O<sub>4</sub> NPs and GO<sub>x</sub> into poly(lactic-co-glycolic acid) (PLGA) microspheres with emulsification-solvent evaporation technology (Scheme 1). Owing to better biocompatibility, higher drug loading and prolonged retention time, PLGA, approved by the U.S. Food and Drug Administration (FDA) as anticancer drug carrier, can be

hydrolyzed into lactic acid and glycolic acid and subsequently eliminated from body by metabolic pathways.<sup>29</sup> By co-loading GO<sub>x</sub> and Fe<sub>3</sub>O<sub>4</sub> NPs into biodegradable PLGA polymer NPs, GO<sub>x</sub> reacts with glucose and oxygen in the tumor cells to produce gluconic acid along with inexhaustible H<sub>2</sub>O<sub>2</sub>, which sustains Fe<sub>3</sub>O<sub>4</sub> NPs-mediated Fenton-like reaction to generate highly toxic ·OH. The excessive consumption of glucose in tumor cells, resulted in nutritional deficiencies and suppression of tumor growth. Moreover, because of the gluconic acid generation, the pH decrease would in turn accelerate the degradation of PLGA and promote the Fe<sub>3</sub>O<sub>4</sub>-H<sub>2</sub>O<sub>2</sub> reaction for an increasing generation of ·OH. Simultaneously, the intracellular oxygen was consumed during GO<sub>x</sub>-catalyzed oxidation of glucose, exacerbating the hypoxic state of tumors. Such orchestrated designed cascade catalytic nanocomposite integrates advantages of CDT, starvation therapy and hypoxia therapy. Therefore, the cascade reaction will dramatically improve the efficacy of tumor treatment and significantly reduce the side effects on normal tissues or organs.

## 2 Experimental section

### 2.1 Materials

3,3',5,5'-Tetramethylbenzidine (TMB, 99%, CAS 54827-17-7) was purchased from Alfa Company. β-D-Glucose (β-D-Glu, 99%, CAS 28905-12-6) was purchased from TCI Company. Phosphate-buffered saline (PBS), trypsin (1:250, CAS 9002-07-7), Dulbecco's modified eagle medium (DMEM), high-glucose DMEM (4500 mg L<sup>-1</sup>) and fetal bovine serum albumin (FBSA, 98%, CAS 9048-46-8) were purchased from Gibco, USA. HeLa cells were purchased from Beyotime Biotechnology Co. Ltd and Cell Counting Kit-8 (CCK8) was obtained from Beyotime Biotechnology Co. Ltd. Calcein acetoxymethyl ester (Calcein-AM, 90%, CAS 148504-34-1)/Propidium iodide (PI, 95%, CAS 25535-16-4) staining reagents were purchased from Yeasen Biotechnology (Shanghai) Co. Ltd. 5,5-Dimethyl-1-pyrroline N-oxide (DMPO, 97%, CAS 3317-61-1) was bought from J&K Scientific Company. Ferric chloride hexahydrate (FeCl<sub>3</sub>·6H<sub>2</sub>O, 98%, CAS 7705-08-0), ferrous chloride tetrahydrate (FeCl<sub>2</sub>·4H<sub>2</sub>O, 99%, CAS 13478-10-9), oleic acid (OA, 90%,



**Scheme 1** (a) Synthetic scheme of Fe<sub>3</sub>O<sub>4</sub>/GO<sub>x</sub>-PLGA. (b) The mechanism schematic of ·OH generation by cascade catalytic reaction.



CAS 112-80-1), polyoxyethylene oxypolypropylene ether block copolymer (Pluronic F127,  $M_w$  12600, CAS 9003-11-6), poly(D,L-lactic acid-co-glycolide) (PLGA (50 : 50),  $M_w$  7000–17000, CAS 26780-50-7) were purchased from Sigma-Aldrich Company. Glucose oxidase ( $GO_x$ , 100–250 U  $mg^{-1}$ , CAS 9001-37-0) was purchased from Shanghai Yuanye Biotechnology Co. Ltd. Ammonia ( $NH_3 \cdot H_2O$ , 25%, CAS 1336-21-6), acetone ( $C_3H_6O$ , 97%, CAS 67-64-1), *n*-octane ( $n-C_8H_{18}$ , 96%, CAS 111-65-9), chloroform ( $CHCl_3$ , 97%, CAS 67-66-3), hydrogen peroxide ( $H_2O_2$ , 30%, CAS 7722-84-1), terephthalic acid (TA, 99%, CAS 100-21-0), citric acid monohydrate ( $CA \cdot H_2O$ , 99%, CAS 5949-29-1), potassium dihydrogen phosphate ( $KH_2PO_4$ , 99%, CAS 7778-77-0), disodium hydrogen phosphate ( $Na_2HPO_4$ , 99%, CAS 7558-79-4), ethanol ( $C_2H_5OH$ , 99.5%, CAS 64-17-5) and methanol ( $CH_3OH$ , 99.5%, CAS 67-56-1), were analytically pure and obtained from Sinopharm reagent company. All reagents were analytical grade and were used directly without further purification. Throughout, Milli-Q ultrapure water was used in all needed experiments.

## 2.2 Characterization

Hydrodynamic diameter and zeta potential of the particles were determined by a dynamic light scattering (DLS) particle size analyzer (Malvern Nano-ZS90). Fourier transform infrared (FT-IR) spectra were measured on a FT-IR spectrometer (Vertex 80V, Bruker Germany) in the range of 4000–400  $cm^{-1}$  to confirm the chemical composition and structure information. Crystal structure of  $Fe_3O_4$ -PLGA was analyzed using X-ray diffraction (XRD, D8ADVANCE, Bruker Germany). The morphology microstructure of the nanocomposites was observed by transmission electron microscopy (TEM) (JEM-2100F STEM, JEOL Japan) and atomic force microscope (AFM) (Multimode 8, Bruker Germany). UV-vis spectra were obtained using a PerkinElmer Lambda 35 spectrophotometer (UV-2550, SHIMADZU, Japan) to analyze the catalytic oxidation activity. The hysteresis loops (300 K) of different  $Fe_3O_4$  NPs were tested on a vibrating sample magnetometer (VSM) (model 7404, LakeShore USA) to characterize their saturation magnetization. Fluorescence spectra were obtained using a fluorescence spectrum analyzer (LS55, PerkinElmer USA). Electron paramagnetic resonance (EPR) spectra were measured by JEOL JES-FA200 spectrometer (JEOL, Japan). The cytotoxicity of HeLa cell was observed by a fluorescence microplate reader (Synergy TM MX, Berton Corporation USA), and the ROS was observed on fluorescence microscope (Olympus IX71) and confocal laser scanning microscope (Leica TCS SP5 II).

## 2.3 Synthesis of $Fe_3O_4/GO_x$ -PLGA

**2.3.1  $Fe_3O_4$  NPs modified with oleic acid ( $Fe_3O_4$ -OA).**  $Fe_3O_4$ -OA were prepared by the chemical co-precipitation. In a typical procedure, 30 mL of 5.1%  $FeCl_2$  solution was slowly dropped into the 20 mL of 5.5%  $FeCl_3$  solution under mechanical stirring and nitrogen atmosphere. After adding 50 mL of concentrated ammonia aqueous drop by drop within 30 min, the reaction mixture was heated to 80 °C, and 4 mL of oleic acid was quickly injected into the mixture subsequently.

After 3 h, the reaction system was heated to 85 °C and remained for 30 min for complete volatilization of  $NH_3$ . pH was adjusted to neutral, and the residual ions were removed by rinsing with acetone and ultrapure water for 3–4 times in magnetic separation principle. Finally, hydrophobic  $Fe_3O_4$ -OA NPs were collected as  $Fe_3O_4$ -OA magnetic fluid with a solid content of 5  $mg\ mL^{-1}$ , *via* dispersing in an appropriate volume of *n*-octane and eliminating the acetone *via* mechanical stirring at 80 °C.

**2.3.2  $Fe_3O_4/GO_x$ -PLGA.** 1 mL of the obtained  $Fe_3O_4$ -OA magnetic fluid was first precipitated with methanol, and then dispersed in 1 mL chloroform for further use. 1 mL of 1% PLGA chloroform solution was mixed with the above  $Fe_3O_4$ -OA dispersion uniformly under ultrasound. The mixture was added into 25 mL of 10% Pluronic F127 aqueous solution and then emulsified with ultrasound in ice bath for 5–10 min to form brown milky tea-like emulsion of  $Fe_3O_4$ -PLGA nanocomposite. Afterwards, 2 mL of the pre-prepared 2.5  $mg\ mL^{-1}$   $GO_x$  solution was added into continuously emulsified  $Fe_3O_4$ -PLGA solution under mechanical stirring for 30 min at room temperature. The reaction system was stirred under the atmosphere at room temperature for 3 h to remove chloroform and obtain a brown-yellow colloidal solution. The products were centrifuged at 1000 rpm to remove the large particle agglomerates. After centrifugation at 9000 rpm for 10 min, the resultant  $Fe_3O_4/GO_x$ -PLGA nanoparticles were dispersed in 20 mL of ultrapure water and stored in 4 °C refrigerators for further use.

## 2.4 Fenton-like reaction activity of $Fe_3O_4$ -PLGA

Terephthalic acid (TA), adopted as a fluorescent probe, can react with  $\cdot OH$  to form highly fluorescent 2-hydroxyterephthalic acid with an emission peak at 430 nm upon exposure to the excitation wavelength of 325 nm. Thus, TA was used to detect the  $\cdot OH$  production to verify  $Fe_3O_4$ -PLGA-induced Fenton reaction. Typically, 0.4 mL of  $Fe_3O_4$ -PLGA aqueous, disodium hydrogen phosphate-citrate buffer (pH 3.5, 5 mL), TA solution (5 mM, 1 mL), and different concentrations (0, 2, 4, 6, 8, 10 mM) of  $H_2O_2$  solution were mixed uniformly. After incubation for 30 min in the dark at room temperature, NaOH (1 M, 1 mL) was added to terminate the reaction, and the remained  $Fe_3O_4$ -PLGA nanocomposites were removed by magnetic separation, then 3 mL of the supernatant was taken for fluorescence spectrum detection ( $\lambda_{ex} = 325\ nm$ ,  $\lambda_{em} = 430\ nm$ ).

The catalytic activity of  $Fe_3O_4$ -PLGA was evaluated by chromogenic substrate 3,3',5,5'-tetramethylbenzidine (TMB). Under acidic conditions, Fenton-like reaction catalyzed  $H_2O_2$  to generate  $\cdot OH$ , and further oxidized colorless TMB to form a blue mixture with maximum absorbance at 652 nm. Typically, TMB (5 mM),  $H_2O_2$  (0–10 mM) and  $Fe_3O_4$ -PLGA (0.05–0.20  $mg\ mL^{-1}$ ) were mixed evenly at different pH (3.0–7.4). After incubation for 30 min in the dark at room temperature, the reaction was terminated by removing the catalyst  $Fe_3O_4$ -PLGA with magnetic separation, and the UV spectra were detected on the microplate reader. Steady-state kinetics of  $Fe_3O_4$ -PLGA was investigated at pH 4.0 with 5 mM TMB and 0.20  $mg\ mL^{-1}$   $Fe_3O_4$ -PLGA while changing the concentration of  $H_2O_2$ . The apparent kinetic parameters were calculated in the light of



Lineweaver–Burk plots derived from Michaelis–Menten equation. The molar absorptivity of TMB was  $39\,000\text{ M}^{-1}\text{ cm}^{-1}$ .<sup>30</sup>

## 2.5 Cascade catalytic activity of $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$

TA (0.5 mM),  $\beta$ -D-glucose solution (0–5 mM) and  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  (0–5  $\text{mg mL}^{-1}$ ) were mixed uniformly in the buffer solution of pH 4.0. After incubation for 1 h in the dark at room temperature, 1 M NaOH solution was added to terminate reaction. The  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  were removed with the assistance of a magnet, and 3 mL of the supernatant was collected for fluorescence spectroscopic detection ( $\lambda_{\text{ex}} = 325\text{ nm}$ ,  $\lambda_{\text{em}} = 430\text{ nm}$ ).

TMB was used to evaluate the cascade catalytic performance of  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  in the presence of glucose. The typical procedure was as follows:  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  dispersion (0.4 mL, 5  $\text{mg mL}^{-1}$ ), buffer solutions at different pH (5 mL, pH 3.0–7.4), TMB solution (1 mL, 5 mM) and  $\beta$ -D-glucose solution (1 mL, 5 mM) were added in turn into a PE tube, then ultrapure water was added to make the total volume reach 10 mL. After 1 h incubation, the  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  was removed to terminate reaction with the aid of a magnet.

## 2.6 Evaluation of $\cdot\text{OH}$ generation ability of $\text{Fe}_3\text{O}_4\text{-PLGA}$ and $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$

Electron paramagnetic resonance (EPR) was used to measure the production of  $\cdot\text{OH}$  by using 50 mM of DMPO as the spin trapper.<sup>31</sup> The samples were prepared as following:  $\text{Fe}_3\text{O}_4\text{-PLGA}$  (20  $\mu\text{g mL}^{-1}$ ) with 10 mM  $\text{H}_2\text{O}_2$  in pH 5.0 buffer,  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  (20  $\mu\text{g mL}^{-1}$ ) in pH 5.0 buffer with 5 mM  $\beta$ -D-glucose, 10 mM  $\text{H}_2\text{O}_2$  in pH 5.0 buffer for control. The spectra of DMPO/ $\cdot\text{OH}$  were collected with an interval of 10 min.

## 2.7 Cytotoxicity experiment

**2.7.1 Cell culture.** The PC-12 cells were incubated in DMEM with 10% FBS and 1% penicillin–streptomycin at 37 °C and 5%  $\text{CO}_2$  in the incubator.

**2.7.2 Cytotoxicity.** *In vitro* CCK-8 assay was used to detect cytotoxicity of  $\text{Fe}_3\text{O}_4\text{-PLGA}$  and  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$ . PC-12 cells were placed into 96-well plate with  $5 \times 10^3$  cells per well and cultured for 24 h. Cell culture medium was extracted and fresh culture medium containing various concentrations of  $\text{Fe}_3\text{O}_4\text{-PLGA}$  and  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  was added respectively. After incubation for 24 h, the medium was treated with fresh medium (100  $\mu\text{L}$ ) and CCK-8 solution (10  $\mu\text{L}$ ), and then incubated at 37 °C for another 4 h. The absorbance of each well OD 490 was determined on the microplate reader.

## 2.8 $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$ -induced oxidative stress in HeLa cells

HeLa Cells were placed into 96-well plate with  $8 \times 10^3$  cells per well and cultured for 24 h in DMEM medium to allow the attachment of cells. The medium of the 96-well plate was discarded followed by rinsing with PBS twice. Subsequently,  $\text{Fe}_3\text{O}_4\text{-PLGA}$  and  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  were separately dispersed into the 10% FBS containing high-glucose DMEM medium at the concentration of 4500  $\text{mg L}^{-1}$ , and then incubated into the 96-well plate. The pH value was respectively adjusted to 6.0 and

7.4 by the addition of HCl. After incubation for 24 h, the medium was then treated with 100  $\mu\text{L}$  of fresh medium containing 10  $\mu\text{L}$  of CCK-8 solution, then incubated at 37 °C for another 4 h. The absorbance of each well OD 490 was determined on the microplate reader.

For ROS observations by fluorescence microscope,  $5 \times 10^4$  of HeLa cancer cells were digested and resuspended into 1 mL 10% FBS containing high-glucose DMEM medium and subcultured into culture disk for another 6 h incubation. Subsequently, the medium was discarded and the disks were rinsed by PBS twice before 1 mL of high-glucose DMEM (pH 7.4 and 6.0) containing 1.0–10  $\mu\text{g mL}^{-1}$  of  $\text{Fe}_3\text{O}_4\text{-PLGA}$  or  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  was replaced. Finally, the above medium was removed completely by PBS rinsing followed by the fluorescence probe addition.

For viable and dead cells observations after 6 h cytotoxicity, the Calcein acetoxymethyl ester (Calcein-AM)/Propidium iodide (PI) staining reagents were respectively applied to stain the viable cells as green fluorescence and dead cells as red fluorescence under 490 nm excitation. Specifically, 100  $\mu\text{L}$  of 5  $\mu\text{M}$  Calcein-AM solution and 100  $\mu\text{L}$  of 10  $\mu\text{M}$  PI solution were added after the removal of the culture medium and rinsing of the disks. After 15 min of incubation, staining solution were removed and rinsed by PBS twice and the samples were subsequently visualized on fluorescence microscope and confocal laser scanning microscope.

# 3 Results and discussion

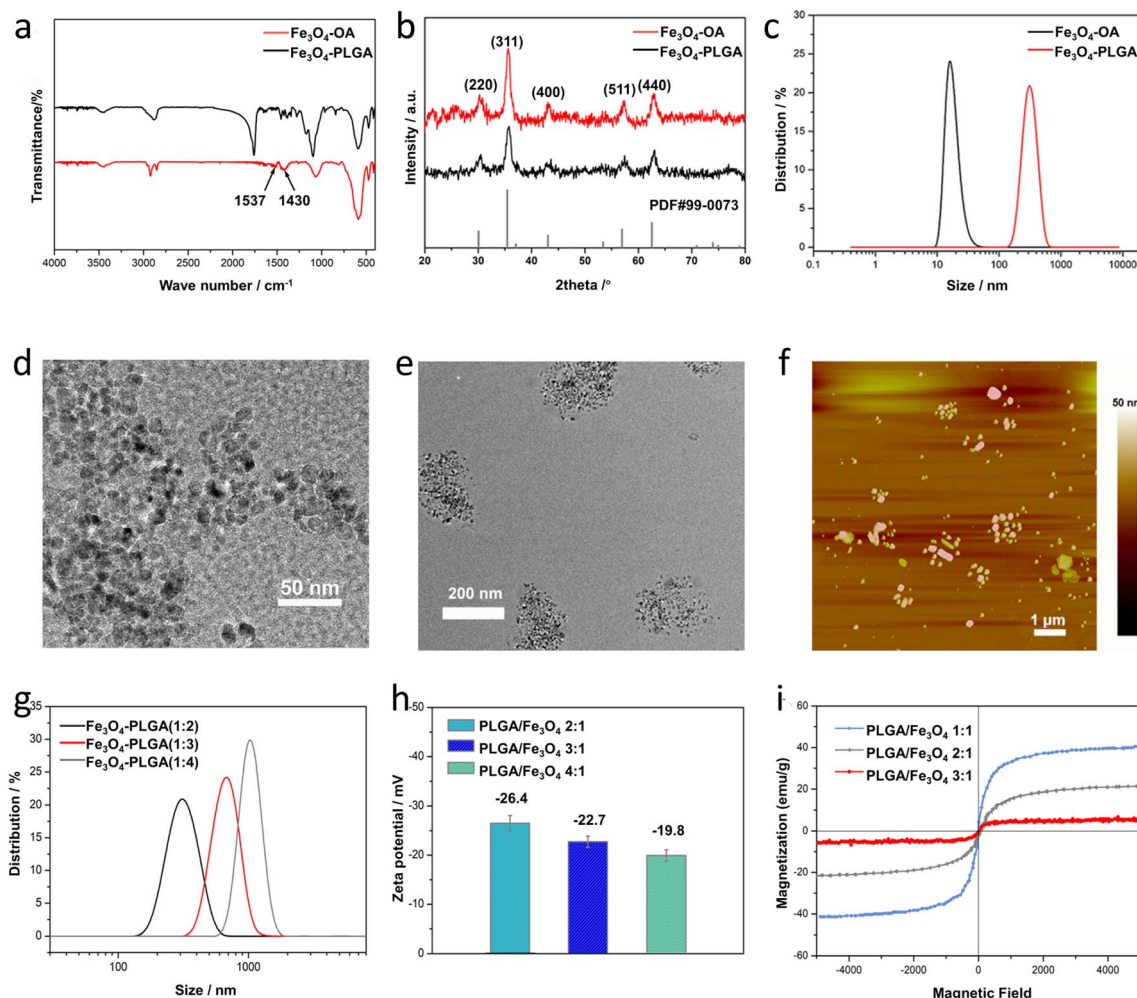
## 3.1 Preparation and characterization of $\text{Fe}_3\text{O}_4\text{-PLGA}$ and $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$

$\text{Fe}_3\text{O}_4\text{-PLGA}$  were prepared by a two-step chemical synthesis process. First, oleic acid was used to modify  $\text{Fe}_3\text{O}_4$  NPs synthesized by coprecipitation method<sup>32</sup> to form hydrophobic  $\text{Fe}_3\text{O}_4\text{-OA}$  NPs with excellent stability and monodispersity. Next, the hydrophobic  $\text{Fe}_3\text{O}_4\text{-OA}$  NPs were encapsulated into biodegradable PLGA with emulsification-solvent evaporation method by using Pluronic F127 as an emulsifier to generate hydrophilic  $\text{Fe}_3\text{O}_4\text{-PLGA}$  nanocomposite.<sup>33</sup>  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  were synthesized by further loading  $\text{GO}_x$  into  $\text{Fe}_3\text{O}_4\text{-PLGA}$  to selectively convert intratumoral abundant glucose into highly oxidative  $\cdot\text{OH}$ .

**3.1.1 Fourier transform infrared spectroscopy (FT-IR) analysis.** As shown in Fig. 1a, the vibrational absorption peaks at  $585\text{ cm}^{-1}$  from  $\text{Fe}_3\text{O}_4\text{-OA}$  and  $\text{Fe}_3\text{O}_4\text{-PLGA}$  were assigned to the stretching vibration of the Fe–O in the crystalline lattice of  $\text{Fe}_3\text{O}_4$ .<sup>34</sup> The broad peaks of ( $\text{COO}^-$ ) stretch vibration band at  $1527$  and  $1430\text{ cm}^{-1}$  revealed the existence of chemisorbed OA as a carboxylate on the nanoparticles' surface.<sup>35</sup> The complexation between iron oxide and oleic acid can be proven from the appearance of characteristic symmetric ( $\nu_{\text{vs}}$ ) and asymmetric ( $\nu_{\text{as}}$ ) of  $\text{COO}^-$  stretches by FTIR analysis.<sup>36</sup> Zhang *et al.* proposed the complex interactions between carboxylate head ( $\text{COO}^-$ ) and Fe atoms in iron oxides could easily be determined by simply calculating the wave number separation ( $\Delta\nu$ ) between  $\nu_{\text{vs}}$  and  $\nu_{\text{as}}$  of  $\text{COO}^-$ , where  $\Delta\nu < 110\text{ cm}^{-1}$  for chelating bidentate,  $\Delta\nu = 140\text{--}190\text{ cm}^{-1}$  for bridging bidentate, and  $\Delta\nu = 200\text{--}300\text{ cm}^{-1}$







**Fig. 1** (a) FT-IR spectra of  $\text{Fe}_3\text{O}_4$ -OA NPs and  $\text{Fe}_3\text{O}_4$ -PLGA. (b) XRD spectra of  $\text{Fe}_3\text{O}_4$ -OA NPs and  $\text{Fe}_3\text{O}_4$ -PLGA. (c) Hydrodynamic diameter distribution of OA-capped  $\text{Fe}_3\text{O}_4$  NPs and  $\text{Fe}_3\text{O}_4$ -PLGA nanocomposite. (d) TEM images of  $\text{Fe}_3\text{O}_4$ -OA NPs. (e) TEM images of  $\text{Fe}_3\text{O}_4$ -PLGA nanocomposite. (f) AFM images of  $\text{Fe}_3\text{O}_4$ -PLGA nanocomposite. (g) Hydrodynamic diameter distribution with different  $\text{Fe}_3\text{O}_4$ /PLGA mass ratio. (h) Zeta potential of  $\text{Fe}_3\text{O}_4$ -PLGA with different PLGA/ $\text{Fe}_3\text{O}_4$  mass ratio. (i) VSM for  $\text{Fe}_3\text{O}_4$ -PLGA with different PLGA/ $\text{Fe}_3\text{O}_4$  mass ratio.

for monodentate interaction.<sup>37</sup> Based on the FTIR results, the calculated  $\Delta\nu$  for  $\text{Fe}_3\text{O}_4$ -OA NPs were found to be  $97\text{ cm}^{-1}$ , which revealed that the coordination mode between oleic acid and Fe atoms in  $\text{Fe}_3\text{O}_4$  NPs could be classified as chelating bidentate. Besides, the peaks at  $2920\text{ cm}^{-1}$  and  $2848\text{ cm}^{-1}$  from  $\text{Fe}_3\text{O}_4$ -OA NPs were attributed to the symmetric and asymmetric  $-\text{CH}_2$  stretches in oleic acid, respectively.<sup>38</sup> The peaks in the spectra of  $\text{Fe}_3\text{O}_4$ -PLGA at  $2920\text{ cm}^{-1}$  and  $2848\text{ cm}^{-1}$  disappeared, proving that  $\text{Fe}_3\text{O}_4$ -OA NPs were successfully encapsulated into PLGA NPs. And the new strong peak at  $1757\text{ cm}^{-1}$  appeared, which was observed for  $\text{C}=\text{O}$  stretching of PLGA,<sup>5</sup> further indicating that the absence of chemical changes in PLGA during the encapsulation process. The peaks at  $1270\text{ cm}^{-1}$  and  $1344\text{ cm}^{-1}$  from  $\text{Fe}_3\text{O}_4$ -PLGA attributed to the bending vibrations of  $\text{C}-\text{H}$ , and the peaks at  $840\text{ cm}^{-1}$  and  $1087\text{ cm}^{-1}$  ascribed to stretching vibrations of  $\text{C}-\text{O}-\text{C}$  all indicated the presence of PEO on the surface of  $\text{Fe}_3\text{O}_4$ -PLGA,<sup>39</sup> proving the successful modification of Pluronic F127 (Fig. S1†).

**3.1.2 Structure analysis.** The crystal structure of  $\text{Fe}_3\text{O}_4$  NPs before and after encapsulation inside PLGA was investigated through powder X-ray diffraction (XRD) (Fig. 1b). The dominant peaks at  $2\theta = 30.1^\circ$ ,  $35.5^\circ$ ,  $43.1^\circ$ ,  $57.0^\circ$  and  $62.6^\circ$ , which could be assigned to the (220), (311), (400), (511) and (440) lattice planes of  $\text{Fe}_3\text{O}_4$ -OA NPs and  $\text{Fe}_3\text{O}_4$ -PLGA NPs, were consistent with the position and relative intensity of the characteristic peaks from the standard  $\text{Fe}_3\text{O}_4$  PDF card (JCPDS 99-0073). It indicated successful preparation of  $\text{Fe}_3\text{O}_4$ -OA NPs. And compared with standard  $\text{Fe}_3\text{O}_4$ , the characteristic peak width of  $\text{Fe}_3\text{O}_4$ -OA NPs was relatively larger, possibly related to the surface lattice defects and shrinkage. Besides, the characteristic peak position and relative intensity of  $\text{Fe}_3\text{O}_4$ -PLGA did not change after encapsulation, suggesting physical coating but not chemical reaction between  $\text{Fe}_3\text{O}_4$ -OA NPs and PLGA.

**3.1.3 Morphology analysis.** The average hydrodynamic diameters of  $\text{Fe}_3\text{O}_4$ -OA NPs and  $\text{Fe}_3\text{O}_4$ -PLGA nanocomposites were respectively 16.97 and 315.06 nm with relatively uniform size distribution (Fig. 1c). The larger hydrodynamic diameters



of Fe<sub>3</sub>O<sub>4</sub>-PLGA demonstrated that the Fe<sub>3</sub>O<sub>4</sub> NPs were successfully encapsulated inside PLGA. TEM images showed Fe<sub>3</sub>O<sub>4</sub>-OA NPs presented uniform spherical morphology, with an average diameter of 15 nm in the dry state (Fig. 1d). After encapsulation inside PLGA, TEM images of Fe<sub>3</sub>O<sub>4</sub>-PLGA also presented a homogeneous distribution with an average dimension of  $\approx 270$  nm in regular spherical morphology (Fig. 1e), where the internal Fe<sub>3</sub>O<sub>4</sub> NPs exhibited a similar regular arrangement. Likewise, AFM images indicated that the Fe<sub>3</sub>O<sub>4</sub> NPs in Fe<sub>3</sub>O<sub>4</sub>-PLGA presented a similar regular arrangement (Fig. 1f). Obviously, owing to the swelling of Pluronic F127 on the outer layer of Fe<sub>3</sub>O<sub>4</sub>-PLGA in aqueous solution, the average hydrodynamic diameters of Fe<sub>3</sub>O<sub>4</sub>-PLGA were significantly larger than the result of TEM. The above results indicated that Fe<sub>3</sub>O<sub>4</sub>-OA NPs had been successfully encapsulated into the PLGA nanospheres modified with Pluronic F127 and dispersed uniformly inside without large aggregated precipitates.

The influence of the PLGA doping amount on the particle size and morphology of Fe<sub>3</sub>O<sub>4</sub>-PLGA was investigated by changing the PLGA/Fe<sub>3</sub>O<sub>4</sub> mass ratio from 2 : 1 to 4 : 1. Obviously, with the continuous increase of PLGA content, Fe<sub>3</sub>O<sub>4</sub>-PLGA gradually became larger, and the number of Fe<sub>3</sub>O<sub>4</sub> NPs encapsulated in a single polymer nanoparticle increased (Fig. S2†). The particle size of the synthesized Fe<sub>3</sub>O<sub>4</sub>-PLGA with the PLGA/Fe<sub>3</sub>O<sub>4</sub> mass ratio of 2 : 1 was about 270 nm (Fig. S2 a and d†) and rose to about 550 nm when the mass ratio increased to 3 : 1 (Fig. S2b and e†) with unchanged spherical morphology. And as the mass ratio of PLGA/Fe<sub>3</sub>O<sub>4</sub> reached 4 : 1, Fe<sub>3</sub>O<sub>4</sub>-PLGA nanocomposites were nearly spherical and the size was close to 1  $\mu$ m (Fig. S2c and f†). The larger size of the Fe<sub>3</sub>O<sub>4</sub>-PLGA nanocomposite with the increased PLGA/Fe<sub>3</sub>O<sub>4</sub> mass ratio suggested more Fe<sub>3</sub>O<sub>4</sub> NPs encapsulated in single PLGA NP. The more PLGA content brought about higher viscosity of the reaction system, resulting in a larger emulsion droplet where more Fe<sub>3</sub>O<sub>4</sub> NPs were encapsulated owing to the decreased diffusion rate of the Fe<sub>3</sub>O<sub>4</sub> NPs during emulsification.<sup>40</sup> Furthermore, the higher PLGA concentration facilitated aggregation of the hydrophobic Fe<sub>3</sub>O<sub>4</sub> NPs and PLGA inside the large emulsion droplets, also bringing about a larger particle size.

Similarly, the hydrodynamic diameters of Fe<sub>3</sub>O<sub>4</sub>-PLGA nanocomposites prepared with different PLGA/Fe<sub>3</sub>O<sub>4</sub> mass ratio (2 : 1, 3 : 1, and 4 : 1) were respectively  $324 \pm 15$  nm,  $679 \pm 21$  nm and  $1045 \pm 45$  nm (Fig. 1g), further proving that the increased amount of PLGA could enlarge the size of the Fe<sub>3</sub>O<sub>4</sub>-PLGA. In addition, the surface zeta potential of the Fe<sub>3</sub>O<sub>4</sub>-PLGA presented a downward trend and changed from  $-26.4$  eV to  $-22.7$  eV and  $-19.8$  eV (Fig. 1h). The PLGA shell became thicker with the increase of the PLGA amount, bringing about the part screening of the negative charges on the surface of Fe<sub>3</sub>O<sub>4</sub>-OA NPs. The increase of the PLGA/Fe<sub>3</sub>O<sub>4</sub> mass ratio lead to larger size and poorer stability of the Fe<sub>3</sub>O<sub>4</sub>-PLGA nanocomposite, also demonstrating that it was difficult to maintain spherical morphology and better stability for the prepared Fe<sub>3</sub>O<sub>4</sub>-PLGA if the PLGA/Fe<sub>3</sub>O<sub>4</sub> mass ratio exceeded 4 : 1. Therefore, considering the encapsulated number of Fe<sub>3</sub>O<sub>4</sub>-OA NPs in the nanocomposites and better biocompatibility, the optimal PLGA/

Fe<sub>3</sub>O<sub>4</sub> mass ratio was chosen as 4 : 1 in the following experiments.

**3.1.4 Magnetic analysis.** Magnetic saturation values of Fe<sub>3</sub>O<sub>4</sub>-OA NPs and Fe<sub>3</sub>O<sub>4</sub>-PLGA nanocomposites were 65.1 emu g<sup>-1</sup> and 40.5 emu g<sup>-1</sup>, respectively (Fig. S3†). Complementarily, as shown in Fig. 1i, with the growing of PLGA/Fe<sub>3</sub>O<sub>4</sub> mass ratio, magnetic saturation of Fe<sub>3</sub>O<sub>4</sub>-PLGA decreased from 40.5 emu g<sup>-1</sup> (1 : 1) to 21.9 emu g<sup>-1</sup> (2 : 1) and 5.1 emu g<sup>-1</sup> (3 : 1). In general, superparamagnetism is mainly relative to the particle size and surface-modified nonmagnetic materials.<sup>41</sup> The smaller grain size favours the higher value of *M<sub>s</sub>*, while the presence of non-magnetic coating materials can lead to a decrease in magnetic saturation due to the reduction of the effective weight fraction of the magnetic cores encapsulated in PLGA NPs.<sup>42</sup>

### 3.2 Fenton-like reaction of Fe<sub>3</sub>O<sub>4</sub>-PLGA

Terephthalic acid (TA) was severed as a probe to quantitatively analyze the content of  $\cdot$ OH, further verifying the catalyzing mechanism of Fe<sub>3</sub>O<sub>4</sub>-PLGA for Fenton-like reaction. It was based that in the presence of  $\cdot$ OH, TA would be oxidized into hydroxyterephthalic acid (TAOH) with strong fluorescence emission at 430 nm. And the fluorescence intensity of TAOH would enhance with the increased  $\cdot$ OH in the system. As shown in Fig. 2a, only when H<sub>2</sub>O<sub>2</sub> was introduced in the system of Fe<sub>3</sub>O<sub>4</sub>-PLGA-TA, the strong fluorescence emission signal appeared at  $\lambda_{em}$  430 nm. This result indicated that Fe<sub>3</sub>O<sub>4</sub>-PLGA catalytically oxidized H<sub>2</sub>O<sub>2</sub> to generate  $\cdot$ OH. As H<sub>2</sub>O<sub>2</sub> concentration increased from 0 mM to 10 mM, the fluorescence intensity of TAOH at  $\lambda_{em}$  430 nm increased almost linearly, indicating the increasing generation of  $\cdot$ OH by Fe<sub>3</sub>O<sub>4</sub>-PLGA-induced Fenton catalysis was associated with the growing H<sub>2</sub>O<sub>2</sub> concentration within a certain concentration range.

3,3',5,5'-Tetramethylbenzidine (TMB), one of the most sensitive dyes,<sup>30</sup> was used to optimize reaction conditions (pH, nanocomposite concentration) of Fenton-like reaction catalyzed by Fe<sub>3</sub>O<sub>4</sub>-PLGA. The produced  $\cdot$ OH from the disproportionation of H<sub>2</sub>O<sub>2</sub> under the catalysis by Fe<sub>3</sub>O<sub>4</sub>-PLGA in acidic environment would oxidize colorless TMB to chromogenic TMB cation-free radicals, which could be assayed at 652 nm ( $E = 3.9 \times 10^4$  M<sup>-1</sup> cm<sup>-1</sup>) in UV-Vis spectrometer. In Fig. 2b, the UV absorption intensity at 652 nm from TMB oxidate has a strong dependence on pH of the reaction system (Fig. 2b).<sup>41</sup> The results suggested that Fe<sub>3</sub>O<sub>4</sub>-PLGA showed the higher catalytic activity from pH 4.0 to 6.0 (Fig. 2c), which was related to high catalytic activity of Fe<sub>3</sub>O<sub>4</sub> under acidic condition and looser state of the nanocomposites due to the PLGA degradation under acidic condition. Moreover, the acid environment could prevent dissolved iron from precipitation, and higher concentration of dissolved iron contributed to an increased yield of  $\cdot$ OH generation. The highest catalytic relative activity at pH 4.0 was defined as 100%, and the catalytic relative activity remained about 30% even the system pH was adjusted to 6.5. The tumor acidity boosted the degradation of Fe<sub>3</sub>O<sub>4</sub>-PLGA and the released Fe<sub>3</sub>O<sub>4</sub> NPs further mediated the Fenton-like reactions for CDT use. Only 2.6% of relative activity at pH 7.4 was attributed to the

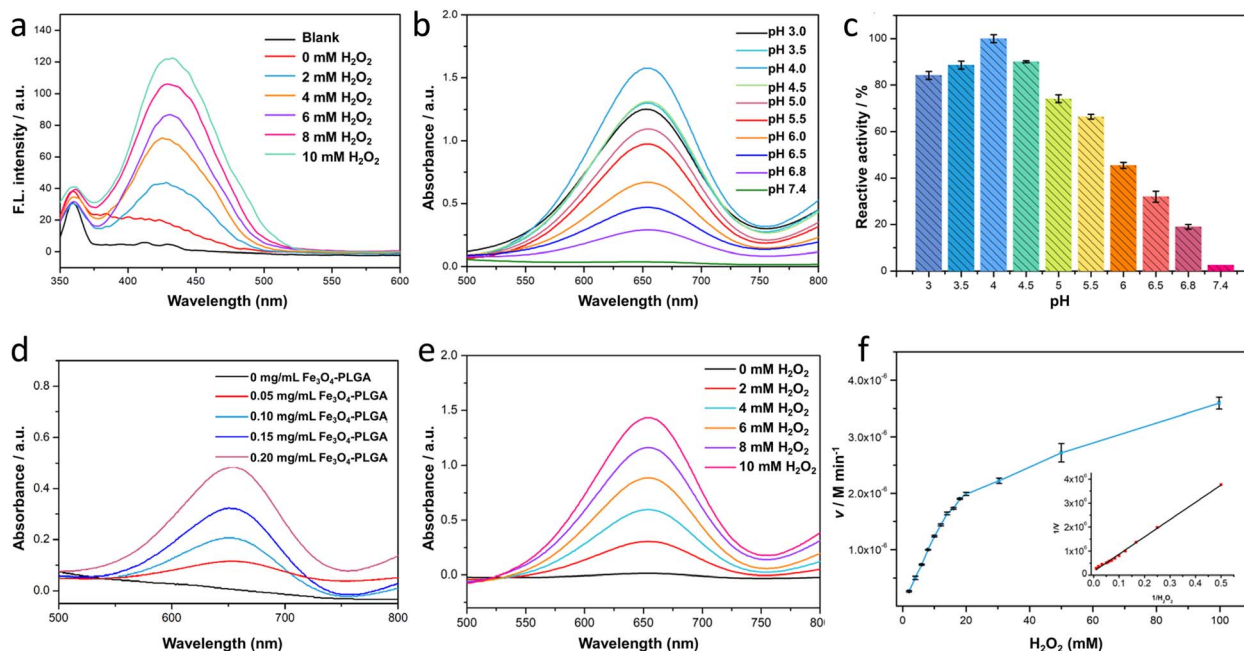


Fig. 2 (a) Fluorescence spectra of  $\text{Fe}_3\text{O}_4$ -PLGA- $\text{H}_2\text{O}_2$ -TA system. (b) The absorption curves of  $\text{Fe}_3\text{O}_4$ -PLGA- $\text{H}_2\text{O}_2$ -TMB systems at different pH. (c) The influence of pH on the catalytic relative activity of  $\text{Fe}_3\text{O}_4$ -PLGA nanocomposite. (d) The absorption curves of  $\text{Fe}_3\text{O}_4$ -PLGA- $\text{H}_2\text{O}_2$ -TMB systems with different concentrations of  $\text{Fe}_3\text{O}_4$ -PLGA nanocomposite. (e) The absorption curves of  $\text{Fe}_3\text{O}_4$ -PLGA- $\text{H}_2\text{O}_2$ -TMB systems upon varied concentrations of  $\text{H}_2\text{O}_2$ . (f) Michaelis-Menten kinetics and Lineweaver-Burk plotting (insert figure) of  $\text{Fe}_3\text{O}_4$ -PLGA nanocomposite.

slower degradation rate of PLGA and lower catalytic activity of  $\text{Fe}_3\text{O}_4$  NPs under neutral condition. Then, it was found that the absorbance of  $\text{Fe}_3\text{O}_4$ -PLGA-TMB- $\text{H}_2\text{O}_2$  increased gradually in the acidic environment when more  $\text{Fe}_3\text{O}_4$ -PLGA nanocomposites were added into the system within a certain concentration range (Fig. 2d), while no change was observed without the addition of  $\text{Fe}_3\text{O}_4$ -PLGA, further confirming excellent catalytic activity of nanocomposites for Fenton-like reactions. In the last, with the increased  $\text{H}_2\text{O}_2$  concentration, the absorbance intensity of  $\text{Fe}_3\text{O}_4$ -PLGA- $\text{H}_2\text{O}_2$ -TMB system increased linearly at 652 nm, consistent with the results of TA fluorescence reaction. It further proved the intrinsic biodegradability and catalytic capacity of  $\text{Fe}_3\text{O}_4$ -PLGA at acidic pH, and also indicated that the increased  $\text{H}_2\text{O}_2$  concentration contributed to the improvement of catalytic efficiency (Fig. 2e).

Based on the above optimal conditions, Fenton-like reaction kinetics of  $\text{Fe}_3\text{O}_4$ -PLGA was investigated by changing  $\text{H}_2\text{O}_2$  concentration, so as to further evaluate the enzymatic catalytic activity as peroxidase. It was found that  $\text{Fe}_3\text{O}_4$ -PLGA followed the typical Michaelis-Menten model toward  $\text{H}_2\text{O}_2$  by tracking change curves of absorbance (652 nm) in a real-time manner (Fig. 2f and S4†). The catalytic kinetic constant ( $K_m$ ) value of  $\text{Fe}_3\text{O}_4$ -PLGA with  $\text{H}_2\text{O}_2$  as the substrate was 48.76 mM, much lower than that of  $\text{Fe}_3\text{O}_4$  magnetic nanoparticles (154 mM)<sup>43</sup> and  $\text{Fe}_2\text{O}_3$  magnetic nanoparticles (324 mM),<sup>44</sup> revealing strong affinity to  $\text{H}_2\text{O}_2$ . The  $K_m$  of the catalyst, closely related with catalytic rate and substrate concentration, reflected the catalyst specificity to catalytic substrate.<sup>39</sup> The lower the  $K_m$ , the higher the physical affinity to substrate.<sup>45</sup> Subsequently, the  $V_{\max}$  was calculated to be  $1.12 \times 10^{-7} \text{ M s}^{-1}$  from Lineweaver-Burk plot.

Interestingly, the initial fast (burst) increase of absorbance (Fig. S4†) might be associated with a small amount of  $\text{Fe}_3\text{O}_4$  NPs through the external side of the  $\text{Fe}_3\text{O}_4$ -PLGA NPs. Then the gradually increased absorbance implied looser nanocomposites due to the acid degradation of PLGA and subsequent rapid release of  $\text{Fe}_3\text{O}_4$  to generate  $\cdot\text{OH}$ .

### 3.3 Cascade catalytic activity of $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA

The cascade reaction of  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA was expected in the order that the loading  $\text{GO}_x$  converted endogenous glucose into gluconic acid and  $\text{H}_2\text{O}_2$  with the help of intracellular oxygen, and subsequently  $\text{Fe}_3\text{O}_4$ -PLGA catalyzed the oxidation of  $\text{H}_2\text{O}_2$  into toxic  $\cdot\text{OH}$  via Fenton-like reaction under TME. The process of catalytic cascade reaction was verified by measuring the fluorescence spectra of the  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA-glucose-TA system without addition of  $\text{H}_2\text{O}_2$ . It was found in Fig. 3a and b that the TA-containing system showed a strong fluorescence emission peak at 435 nm in the presence of both  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA and  $\beta$ -D-glucose, indicating the cascade catalytic activity of  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA to  $\beta$ -D-glucose. It confirmed that  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA can efficiently boost the yield of  $\cdot\text{OH}$  in the presence of  $\beta$ -D-glucose. Moreover, it was also found that the increased concentrations of  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA led to a gradient ascent in the fluorescence intensity of  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA-glucose-TA system (Fig. 3a), which indicated that the cascade catalytic capacity of  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA was positively correlated with the concentration of  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA in a certain range. Similarly, the fluorescence intensity of  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA-glucose-TA system grew almost linearly with the ascent of the glucose concentration (0–5 mM) under the conditions of  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -





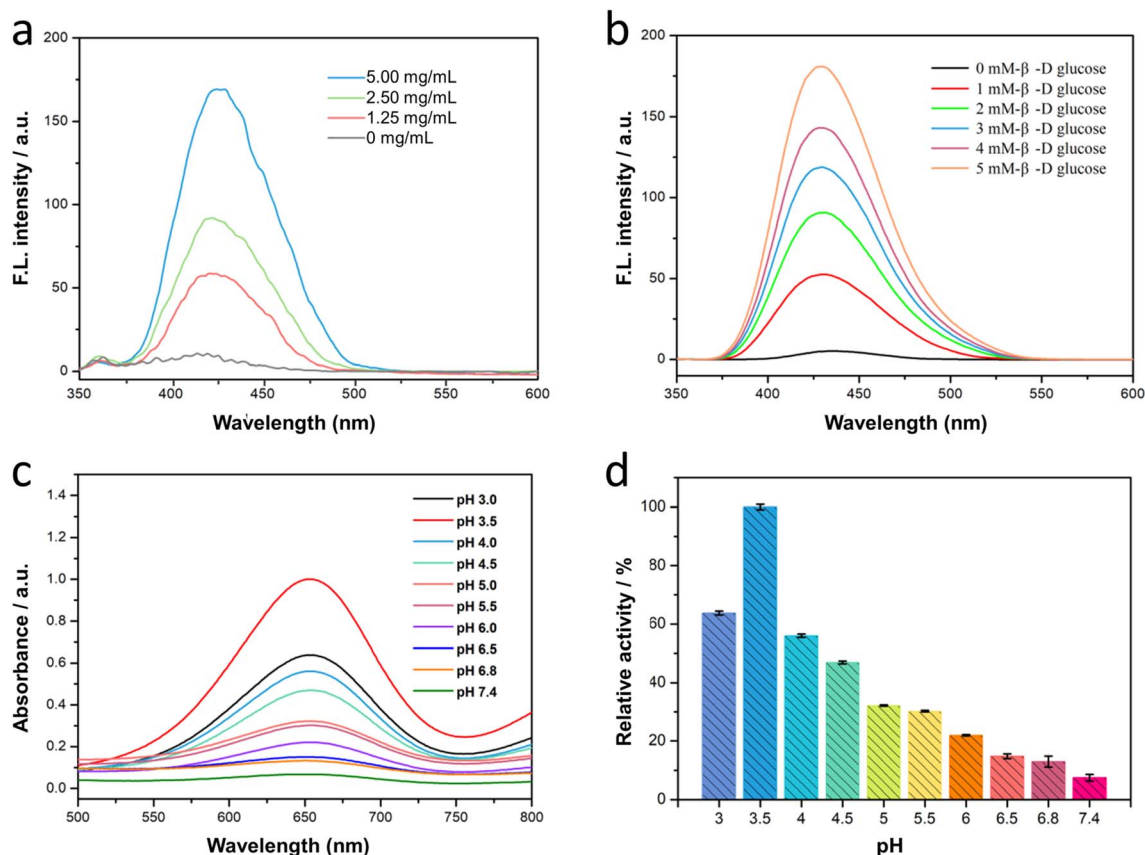


Fig. 3 (a) Effects of different  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  nanocomposite concentration and (b) different  $\beta\text{-D-glucose}$  concentration on the fluorescence intensity of  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$ -glucose-TA system. (c) The absorption curves of  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$ -glucose-TMB system under different pH conditions. (d) The influence of pH on the catalyzed activity of  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  nanocomposite.

PLGA ( $0.2 \text{ mg mL}^{-1}$ ), TA ( $0.5 \text{ mM}$ ), and pH 4.0. The cascade catalytic activity of  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  was related to the concentration of the substrate  $\beta\text{-D-glucose}$ , which conformed to the catalytic principle (Fig. 3b). The concentration dependence of the  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  NPs' cascade catalytic activity on  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  NPs and glucose was similar to that of  $\text{Fe}_3\text{O}_4\text{-PLGA}$  NPs' catalytic activity on  $\text{Fe}_3\text{O}_4\text{-PLGA}$  and  $\text{H}_2\text{O}_2$ . The above results indicated that the  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  will provide superior therapeutic efficiency for cancer by simultaneously depleting glucose and boosting cascade reaction.<sup>46</sup>

Furthermore, to clarify the influence of pH on the cascade catalytic activity of  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  nanocomposite, TMB was applied to monitor the radical production by colorimetric reaction. The absorbance of  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$ -glucose-TMB system was the highest at pH 3.5 (Fig. 3c), slightly different from the optimal pH of  $\text{Fe}_3\text{O}_4\text{-PLGA}$ . It might be due to the influence of  $\text{GO}_x$  catalytic reaction. It was also found that  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$ -glucose-TMB system presented different absorbance at 652 nm from pH 3 to pH 7.4. The relative activity of cascade catalysis at pH 3.5 was the highest and defined as 100% to quantify the influence on pH. Interestingly,  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  still remained more than 20% of reactive activity in a certain pH range from 3.0 to 6.0, while only 5.3% of reactive activity at pH 7.4 (Fig. 3d). The pH dependence of the cascade catalytic activity

of  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  nanocomposite was associated with the acid degradation of PLGA and high catalytic activity of  $\text{Fe}^{2+}$  for Fenton reaction at acidic pH. The above results further confirmed the huge potential of  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  for TME-specific CDT.

Electron paramagnetic resonance (EPR) spectroscopy using 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as the radical trapper also clearly validated the generation of  $\cdot\text{OH}$ . As shown in Fig. S5,<sup>†</sup> typical EPR spectrum with 1 : 2 : 2 : 1 intensity characteristic for  $\cdot\text{OH}$ ,<sup>47</sup> were obtained for  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  and  $\text{Fe}_3\text{O}_4\text{-PLGA}$  nanocomposites, while no signal was found for control. Moreover, the intensity of the  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  was higher than that of  $\text{Fe}_3\text{O}_4\text{-PLGA}$ , which further evidenced that  $\text{GO}_x$  consumed glucose into  $\text{H}_2\text{O}_2$  and the enriched  $\text{H}_2\text{O}_2$  was subsequently converted into abundant highly toxic  $\cdot\text{OH}$  by a  $\text{Fe}_3\text{O}_4$ -mediated Fenton-like reaction. Together, these results demonstrated that the  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  nanocomposite afforded a  $\text{H}_2\text{O}_2$  self-supplying CDT platform under acidic condition.

### 3.4 Cytotoxicity analysis

The excellent Fenton-like activity of  $\text{Fe}_3\text{O}_4\text{-PLGA}$  and  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  further inspired us to explore its potential to induce oxidative stress. To this end, the cytotoxicity of  $\text{Fe}_3\text{O}_4\text{-PLGA}$  and  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  on PC12 cells was evaluated by using CCK-8



colorimetric assay. PC12 cells were respectively incubated with  $\text{Fe}_3\text{O}_4$ -PLGA and  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA for 24 h, and cell viability was evaluated by CCK-8 colorimetric assay. As shown in Fig. 4a, no obvious cytotoxicity was observed after 24 h incubation in the concentration range of  $0.1$ – $20\ \mu\text{g mL}^{-1}$ , and the cell viability was over 90% even at high concentration ( $20\ \mu\text{g mL}^{-1}$ ), which validated that  $\text{Fe}_3\text{O}_4$ -PLGA and  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA nanocomposites presented low cytotoxicity. No significant decreases of cell viabilities in both cases may be ascribed to excellent biocompatibility of nanocomposites based on PLGA and  $\text{Fe}_3\text{O}_4$ . It also provided a basis for the dose design of nanocomposites for subsequent oxidative stress experiments.

### 3.5 $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA NCs-induced oxidative stress in HeLa cells

The inhibitory effect of oxidative stress from  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA on HeLa cells were evaluated at the concentration of  $1$ – $20\ \mu\text{g mL}^{-1}$  based on the results of cytotoxicity analysis on PC12 cells. HeLa cells were used as a model of tumor cells to verify the ability of  $\text{Fe}_3\text{O}_4$ -PLGA and  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA to induce oxidative stress. Under the condition with high concentration of glucose at pH 7.4,  $\text{Fe}_3\text{O}_4$ -PLGA showed no obvious cytotoxicity at the concentration of  $1$ – $20\ \mu\text{g mL}^{-1}$  for HeLa cells (Fig. 4b). Compared with  $\text{Fe}_3\text{O}_4$ -PLGA, the higher inhibition rate from  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA meant more efficient oxidative stress and

apoptosis inducement caused by a large amount of  $\cdot\text{OH}$  generated from glucose by cascade catalysis of  $\text{GO}_x$  and  $\text{Fe}_3\text{O}_4$  in glucose-rich TME. The cytotoxicity of  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA at pH 6.0 and 7.4 was respectively evaluated within the concentration range of  $1$ – $20\ \mu\text{g mL}^{-1}$ . In Fig. 4c, it was found that the  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA showed obvious inhibition on HeLa cells in a dose-dependent manner both at pH 6.0 and 7.4, but higher inhibition rate at pH 6.0.

To visually observe distributions of the viable and dead cells, HeLa cancer cells were stained with calcein-AM and PI solution after separate incubation with  $\text{Fe}_3\text{O}_4$ -PLGA and  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA at varied concentrations ( $2$ ,  $6$ , and  $10\ \mu\text{g mL}^{-1}$ ) under neutral conditions for 6 h. Likewise, HeLa cells were respectively incubated with  $\text{Fe}_3\text{O}_4$ -PLGA and  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA nanocomposite at varied concentrations ( $1$ ,  $4$ , and  $10\ \mu\text{g mL}^{-1}$ ) in both acidic (pH = 6.0) and neutral (pH = 7.4) culture mediums for 6 h. The viable and dead cells were respectively stained with green and red fluorescence before fluorescence and CLSM observation. The fluorescence and CLSM images showed that no significant damage on HeLa cells for  $\text{Fe}_3\text{O}_4$ -PLGA at pH 7.4 (Fig. S6 and S7<sup>†</sup>), and influence of cell apoptosis on the varied concentration of  $\text{Fe}_3\text{O}_4$ -PLGA and  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA ( $1$ ,  $4$ ,  $10\ \mu\text{g mL}^{-1}$ ) were assayed under neutral and acidic conditions, respectively. More dead cells were observed when incubated with  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA at varied concentration ( $1$ ,  $4$ ,  $10\ \mu\text{g mL}^{-1}$ ) under acid condition (Fig. 4d and S8<sup>†</sup>). Obviously, under acidic

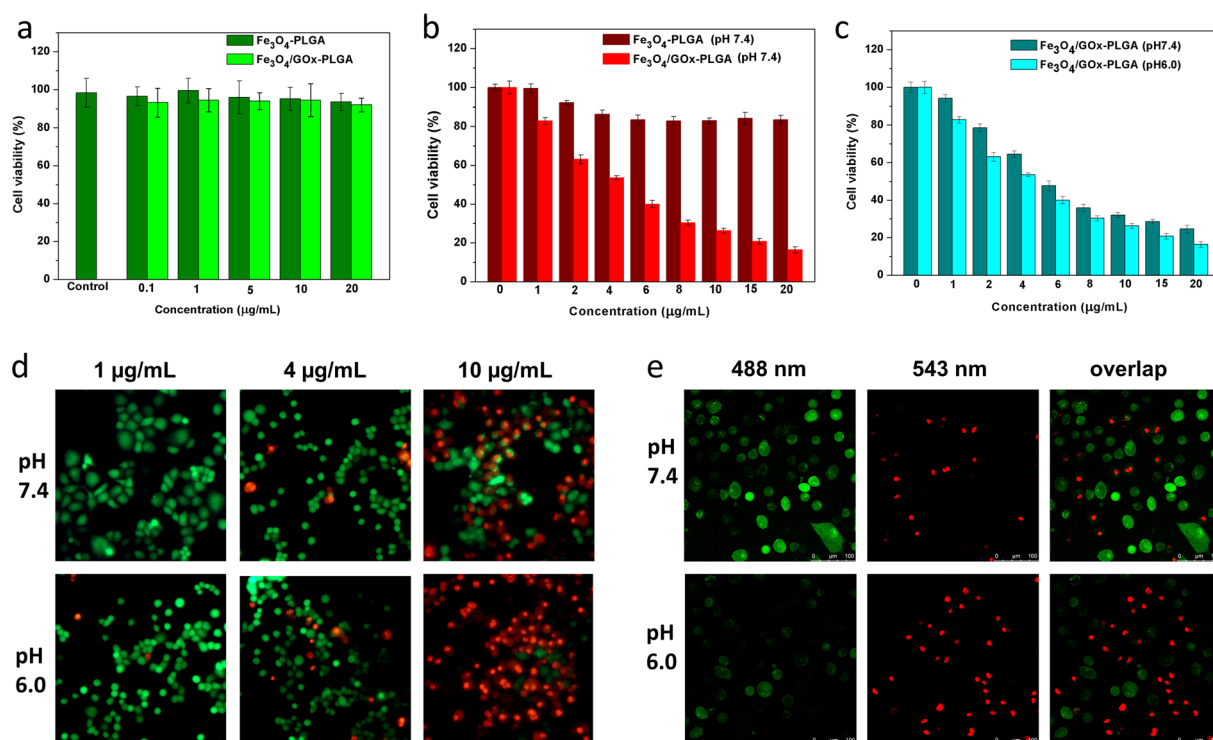


Fig. 4 (a) PC12 cell viability of  $\text{Fe}_3\text{O}_4$ -PLGA and  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA with different concentrations from  $0.1$  to  $20\ \mu\text{g mL}^{-1}$  for 24 h, respectively. (b) Concentration effects of  $\text{Fe}_3\text{O}_4$ -PLGA and  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA under neutral (pH = 7.4) and acidic (pH = 6.0) conditions on the viability of HeLa cells. (c) Concentration effects of  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA under neutral (pH = 7.4) and acidic (pH = 6.0) conditions on the viability of HeLa cells. (d) Fluorescence images of viable and dead cell distributions after co-incubation with  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA under neutral (pH = 7.4) and acidic (pH = 6.0) conditions at varied concentrations for 6 h. (e) CLSM images of HeLa cells after co-incubation with  $\text{Fe}_3\text{O}_4/\text{GO}_x$ -PLGA under neutral (pH = 7.4) and acidic (pH = 6.0) conditions at  $10\ \mu\text{g mL}^{-1}$  for 6 h.



condition, almost all HeLa cancer cells were dead at  $10\ \mu\text{g mL}^{-1}$  of  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  (Fig. 4e). The improved ability of killing cancer cells for  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  at pH 6.0 might be related to the  $\text{GO}_x$ -initiating and  $\text{Fe}_3\text{O}_4$ -mediated generation of  $\cdot\text{OH}$  and cell starvation resulting from consumption of intracellular glucose. The above results confirmed cooperative enhancement interactions between starvation therapy and cascade cancer therapy by self-sufficient  $\text{H}_2\text{O}_2$  *in situ* and the continuous generation of  $\cdot\text{OH}$  inducing oxidative stress and apoptosis.

## 4 Conclusions

In summary, a new strategy responding to TME was developed by intelligently integrate  $\text{GO}_x$  and  $\text{Fe}_3\text{O}_4$  NPs into PLGA NPs with emulsification-solvent evaporation method, realizing a tumor-selected combination of chemodynamic and starving therapy. The  $\text{GO}_x$  effectively converted glucose into  $\text{H}_2\text{O}_2$ , accompanied by decline of localized  $\text{O}_2$  content and pH. The pH decline in turn promoted the degradation of PLGA and sequent  $\text{Fe}_3\text{O}_4$  release, to give rise to Fenton reaction between Fe ions and  $\text{H}_2\text{O}_2$  for generation of  $\cdot\text{OH}$ . The synthesized  $\text{Fe}_3\text{O}_4\text{-PLGA}$  nanocomposite was proved to show remarkable Fenton catalytic activity and better affinity to substrate  $\text{H}_2\text{O}_2$  in tumor micro-environment, indicating excellent capability of producing  $\cdot\text{OH}$  to kill cancer cells. After loading  $\text{GO}_x$ , intracellular oxidative stress suggested that  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  nanocomposite could enhance the ability to kill cancer cells through continuous conversion of intratumoral glucose into  $\text{H}_2\text{O}_2$  for Fenton reaction and cell starvation due to glucose consumption. Thus,  $\text{Fe}_3\text{O}_4/\text{GO}_x\text{-PLGA}$  nanocomposite as an excellent cascade reaction-based nanoplateform will provide a new perspective for cancer therapy.

## Conflicts of interest

There are no conflicts to declare.

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