Programmable starving-photodynamic synergistic cancer therapy

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ABSTRACT Synergistic therapy combines multiple therapeutic approaches in one shot, thus could significantly amplify the therapeutic effects. However, how to design the desirable combination to maximize the synergistic effect is still a big challenge in cancer management. Herein, a nanoagent composed of glucose oxidase (GOx) and upconversion nanoparticles (UCNPs) were constructed for programmable starving-photodynamic synergistic cancer therapy through cascade glucose oxidation and hydrogen peroxide photolysis. In this nanoagent, GOx modulated the tumor glucose metabolism and consumed the β -D-glucose to produce H_2O_2 . The glucose depletion induced “starvation” in cancer cells and caused cell death. Afterwards, the generated H_2O_2 was photolyzed by the invisible ultraviolet emission of UCNPs under near-infrared light excitation at 980 nm. The toxic hydroxyl radicals produced by photolysis further induced cancer cell death. Both *in vitro* and *in vivo* experiments confirmed that this starving-photodynamic synergistic therapy significantly outperforms any single therapy. This study paves an avenue to design programmable starving-photodynamic synergistic therapy for cancer management.

Keywords: upconversion nanoparticles, starvation therapy, photodynamic therapy, synergistic cancer therapy

INTRODUCTION

Synergistic therapy takes advantages of multiple therapeutic approaches at the same time, and is recognized as a revolutionary strategy for cancer therapy [1–5]. However, to achieve “1+1>2” efficiency, researchers have to design the desirable combinations that maximize the cooperative effect of both therapeutic approaches [6]. Recently, multifunctional nanoparticles that integrate

multiple therapeutic modalities have experienced rapid developments in the recent decade. Obviously, nanomaterials exhibit several prominent advantages in drug delivery and tumor targeting therapy on account of enhanced permeability and retention (EPR) effect and versatile modified properties. Rational design of nanomaterials by combining multiple therapy modalities holds super additive therapeutic effects in cancer treatment [7–10].

As an alternative to the traditional chemotherapeutics, the glucose oxidase (GOx)-based nanomedicines have aroused great research interest in cancer synergistic therapy [11–14]. In such therapeutic systems, GOx usually serves as the driving force thanks to its special biological property. GOx can catalyze the oxidation of D-glucose by O_2 into the corresponding gluconic acid and H_2O_2 [15]. This oxidation process will then lead to the exhaustion of glucose and oxygen, both of which are crucial nutrients for cells, thus greatly inhibiting the energy metabolic mode of cancer cells and causing cell death. Such therapy modality of blocking the crucial nutrients is known as the tumor starving-therapy [16,17]. In addition, the generated H_2O_2 can further induce reactive oxygen species (ROS)-dependent cell death. Gluconic acid, another product of the oxidation process, can increase the acidity in the tumor microenvironment. Due to the versatile and unique properties, GOx has attracted tremendous interest in cancer synergistic therapy. For instance, we developed GOx- and L-arginine-modified hollow mesoporous organosilica nanoparticles (L-Arg-HMON-GOx) for GOx-mediated starvation therapy and nitrogen oxide (NO) gas therapy [18]. In this system, glucose was exhausted for starving-like cancer therapy,

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while the generated gluconic acid and H_2O_2 in the process of glucose oxidation further oxidized *L*-Arg into NO for gas therapy. In addition, the GOx-modified nanomaterials have been reported for starvation/metal-ion therapy [19], starvation/chemodynamic therapy [20], and starvation/chemotherapy [21,22]. However, these synergistic therapeutic outcomes are still limited due to the uncontrollable interactions between the multiple therapeutic modalities. Therefore, it remains a challenge to design a rational GOx-modified nanomaterial system for maximizing the efficiency of chain reactions.

To increase the flexibility and accuracy of synergistic cancer treatment, researchers usually integrate photodynamic therapy (PDT) with GOx-mediated therapy [23]. In PDT, ROS are generated by the photosensitizers (PSs) under laser irradiation for cancer treatment, thus being recognized as a minimal or noninvasive approach. For example, Chang *et al.* [24,25] developed a GOx-enhanced PDT system using light irradiation at 660 and 460 nm. Their design showed spatiotemporal controlled cancer treatment and amplified synergistic therapeutic effect. However, short-wavelength light can cause damage in normal tissues and has limited tissue-penetration depth [26–28]. Therefore, the development of long-wavelength light-responsive nanomaterials is quite demanding for GOx-involved PDT.

To address the above issues, researchers developed near-infrared (NIR) responsive materials over the last two decades, thus enabling NIR light with deep tissue penetrating and minimal photo damaging characteristics to activate biomolecules [29–33]. As a type of popular NIR materials, upconversion nanoparticles (UCNPs) absorb 980 nm light and emit invisible/ultraviolet light, which serves as the *in situ* light source for light-responsive materials in deep tissue. Here, we synthesized GOx conjugated silica-coated UCNPs (UCNPs@SiO₂@GOx) (Scheme 1a). The UCNPs can absorb NIR light at 980 nm and converted the low-energy photon to high-energy invisible ultraviolet photon that could trigger H_2O_2 photolysis to produce hydroxyl radical ($\cdot\text{OH}$) [24,34,35]. After the accumulation of UCNPs@SiO₂@GOx in tumor tissues, intratumoral glucose was oxidized into gluconic acid and produced H_2O_2 with the catalysis of GOx. Afterwards, the generated H_2O_2 could be converted into $\cdot\text{OH}$ via the photolysis. The highly toxic $\cdot\text{OH}$ could effectively increase the toxicity of the nanoagent to tumor cells (Scheme 1b). Consequently, the programmatic and spatiotemporally controlled synergistic therapy manners showed amplified synergistic effects of cancer starvation therapy and PDT with reduced side effects.

Scheme 1 (a) Synthesis of UCNPs@SiO₂@GOx; (b) schematic illustration of UCNPs@SiO₂@GOx for programmable starving-photodynamic synergistic cancer therapy.

EXPERIMENTAL SECTION

Synthesis of UCNPs

The NaYF₄:20%Yb,0.5%Tm core UCNPs were prepared by a two-step thermolysis method. In the first step, CF₃COONa (0.50 mmol), Y(CF₃COO)₃ (0.3975 mmol), Yb(CF₃COO)₃ (0.10 mmol) and Tm(CF₃COO)₃ (0.0025 mmol) precursors were mixed with oleic acid (5 mmol), oleyamine (5 mmol), and 1-octadecene (10 mmol) in a two-neck round bottom flask. The mixture was heated to 110°C to form a transparent solution followed by 10 min of degassing. Then the mixture was heated to 300°C at a rate of 15°C min⁻¹ under dry argon flow, and maintained at 300°C for 30 min to form the α-NaYF₄:20%Yb,0.5%Tm intermediate UCNPs. After the mixture was cooled to room temperature, the α-NaYF₄:20%Yb,0.5%Tm intermediate UCNPs were collected by centrifugal washing with excessive ethanol (7500 ×g, 30 min). In the second step, the α-NaYF₄:20%Yb,0.5%Tm intermediate UCNPs were redispersed into oleic acid (10 mmol) and 1-octadecene (10 mmol) together with CF₃COONa (0.5 mmol) in a new two-neck round bottom flask. After degassing at 110°C for 10 min, this flask was heated to 325°C at a rate of 15°C min⁻¹ under dry argon flow, and maintained at 325°C for 30 min to complete the phase transfer from α to β. After the mixture was cooled to room temperature, the β-NaYF₄:20%Yb,0.5%Tm UCNPs were collected by precipitation with an equal volume of ethanol and centrifugation afterwards (7500 ×g, 30 min). The UCNPs were stored in hexane (10 mL). The as-synthesized core UCNPs served as cores for the epitaxial growth of core-shell UCNPs. Typically, a hexane stock solution of core UCNPs was transferred into a two-neck round bottom flask, and the hexane was se-

quentially evaporated by heating. CF_3COONa (0.50 mmol) and $\text{Y}(\text{CF}_3\text{COO})_3$ (0.50 mmol) were introduced as UCNP shell precursors with oleic acid (10 mmol) and 1-octadecene (10 mmol). After 10 min of degassing at 110°C , the flask was heated to 325°C at a rate of $15^\circ\text{C min}^{-1}$ under dry argon flow, and maintained at 325°C for 30 min to complete the shell crystal growth. After the mixture was cooled to room temperature, the $\text{NaYF}_4\text{:}20\%\text{Yb},0.5\%\text{Tm}@NaYF_4$ core-shell UCNPs were collected by precipitation with an equal volume of ethanol and centrifugation afterwards ($7500 \times g$, 30 min). The core-shell UCNPs were stored in hexane (10 mL).

Synthesis of UCNPs@SiO₂

Twenty milliliter of water with 66 mg of cetyltrimethyl ammonium bromide (CTAB) was mixed in the 60°C water bath for 10 min. UCNPs kept in hexane (2 mL) were added into the solution, and stirred at room temperature for 48 h. The solution became clear and was added to 20 mL of water. A mixed solution of 0.15 mL NaOH (2 mol L^{-1}), 3 mL of ethanol and 20 mL water was prepared in a round bottom flask. Then the UCNPs solution was added into the flask in water bath of 70°C . After the temperature became stable, 0.15 mL tetraethyl orthosilicate (TEOS) was added into the mixed solution with magnetic stirring for 1.5 h. And then 0.05 mL 3-aminopropyl-triethoxysilane (APTES) was added into the solution with magnetic stirring for 30 min. After the reaction, the product was precipitated by addition of ethanol, separated by centrifugation at $12,000 \text{ r min}^{-1}$ for three times. The precipitate was dispersed in 10 mL ethanol and added into 10 mL ethanol containing 0.3 g NH_4NO_3 . The mixture was rapidly heated to 60°C and kept at this temperature for 2 h under vigorous magnetic stirring. After the reaction was completed, the same ethanol was centrifuged and washed for three times at $12,000 \text{ r min}^{-1}$ to obtain UCNPs coated with mesoporous silicon (UCNPs@SiO₂).

Synthesis of UCNPs@SiO₂@GOx

1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 19.2 mg), 16 mg of *N*-hydroxysuccinimide (NHS) and 5 mg of GOx were dissolved into 2 mL of phosphate buffer saline (PBS, pH 6.0) and stirred for 30 min at room temperature. Then 2 mL of UCNPs@SiO₂ aqueous solution (2.5 mg mL^{-1}) was added into the above solution. After reacting for 8 h under stirring, the UCNPs@SiO₂@GOx was separated by centrifugation and washed for three times at $12,000 \text{ r min}^{-1}$.

Characterizations

The morphology and structure of the as-synthesized nanoparticles were characterized by transmission electron microscopy (TEM, JEM-1230, JEOL). The fourier-transform infrared spectroscopy (FT-IR) spectra were measured on a FT-IR spectrometer (Spectrum TwoTM, PerkinElmer). Zeta potential was measured by a Zetasizer Nano ZS90 (Malvern Instruments, UK). The absorbance spectrum assay was tested by a UV-vis spectrophotometer (Agilent Cary60). Electron spin resonance (ESR) spectra were determined by a Bruker EMX Plus spectrometer. The emission spectra of the nanoparticles were recorded by excitation with a 980 nm laser. The *in vitro* H₂O₂ concentrations were detected with a H₂O₂ Assay Kit (Beyotime Institute of Biotechnology, China) via a UV-vis spectrophotometer.

RESULTS AND DISCUSSION

The silica-coated NaYF₄ nanoparticles were synthesized by a two-step process according to the published protocol [36,37]. First, NaYF₄:Yb³⁺,Tm³⁺ UCNPs were synthesized by a high-temperature thermal decomposition method. The UCNPs were then coated with an amino-modified mesoporous silica layer to get the UCNPs@SiO₂. Then the GOx was covalently conjugated to UCNPs@SiO₂ via the formation of amide bonds between the carboxylic groups of GOx and amino groups on the silica layer to get UCNPs@SiO₂@GOx with the help of EDC. The morphology of the UCNPs was characterized by TEM as shown in Fig. 1a. It showed that the UCNPs were mono-dispersed and the UCNPs@SiO₂@GOx had an average diameter of around 75 nm. As shown in Fig. 1b, the absorption spectra of UCNPs@SiO₂ were then recorded. The free UCNPs@SiO₂ showed no characteristic absorption peak while the UCNPs@SiO₂@GOx showed the characteristic absorption peak of GOx at 280 nm, suggesting the successful conjugation of GOx on the surface of UCNPs@SiO₂. As shown in Fig. S1, the zeta potential of UCNPs changed from negative to positive after coating with amino-modified silica layer, due to the positively charged amino groups on the surface. After conjugation with GOx, the UCNPs@SiO₂@GOx showed a negative surface charge, which suggested the successful conjugation between the carboxylic groups of GOx and amino groups on the silica layer. Fig. S2 shows that the FT-IR curve of UCNPs@SiO₂@GOx has the characteristic peaks of C=O ($\tilde{\nu}=1650 \text{ cm}^{-1}$) and N-H ($\tilde{\nu}=3350 \text{ cm}^{-1}$) bond of GOx, further suggesting a covalent linkage between GOx and UCNPs@SiO₂. Further, the conjugation capacity of GOx on UCNPs@SiO₂@GOx was evaluated, showing the

Figure 1 (a) TEM images of the nanoparticles. Scale bar: 50 nm, 100 nm, 100 nm (from left to right). (b) UV-vis spectra of UCNPs, UCNPs@SiO₂ and UCNPs@SiO₂@GOx; (c) TG curves of UCNPs@SiO₂ and UCNPs@SiO₂@GOx; (d) the luminescence spectra of UCNPs@SiO₂ and UCNPs@SiO₂@GOx under excitation at 980 nm.

saturated GOx loading ratio of 12.3 wt% *via* thermogravimetric analysis (TGA) (Fig. 1c). Upon 980 nm NIR light irradiation, UCNPs@SiO₂@GOx emitted similar emissions in UV and visible regions compared to that of free UCNPs (Fig. 1d), which suggested that the modification with GOx did not affect the upconversion ability of UCNPs.

Afterwards, the H₂O₂-generating activity of UCNPs@SiO₂@GOx was then investigated. After the addition of UCNPs@SiO₂@GOx in the glucose solution, the H₂O₂ were immediately generated through the GOx-catalyzed glucose oxidation reaction. The concentrations of H₂O₂ were correlated to the concentrations of glucose. Furthermore, UCNPs@SiO₂@GOx had about 92% of the H₂O₂-generating activity of native GOx (Fig. 2a), indicating that the activity of GOx was well retained after conjugation. In contrast, the UCNPs@SiO₂ showed no H₂O₂-generating activity. H₂O₂ could undergo homolytic cleavage, forming two hydroxyl radicals under invisible ultraviolet light irradiation. Previously, it was reported that the generated H₂O₂ by photolysis under invisible ultraviolet light irradiation was used for antibacterial and tumor therapy [24,34,35]. Here, we also discussed the effects of H₂O₂ under light irradiation on the generation of ·OH in solution using 3,3',5,5'-tetramethylbenzidine (TMB) as the indicator. TMB can be oxidized to a blue

product with a characteristic absorption peak at 652 nm by ·OH [38]. As shown in Fig. S3, the TMB solution showed increased absorption at 652 nm from oxidized TMB upon the light irradiation for 30 min at 450 nm with different power densities, suggesting the generation of ·OH. The blue light irradiation could be replaced by UCNPs, which emit ultraviolet/blue photon under 980 nm irradiation. As shown in Fig. S4, in the presence of UCNPs, the solution showed increased absorption at 652 nm from oxidized TMB upon the light irradiation at 980 nm in a power-dependent manner. Then, the UCNPs@SiO₂@GOx complex was demonstrated to catalyze glucose to produce H₂O₂ and emit invisible ultraviolet irradiation under excitation at 980 nm. Therefore, we chose a 980 nm light source to produce ·OH with the combination of UCNPs@SiO₂@GOx and glucose. We used UV-vis-NIR absorption and ESR spectroscopy assay to detect the ·OH generated by the photolysis of H₂O₂. As shown in Fig. 2b, the TMB solution containing UCNPs@SiO₂@GOx and glucose showed no absorption at 652 nm before light irradiation at 980 nm. Upon irradiation at 980 nm, the absorbance at 652 nm gradually increased in a time-dependent manner (Fig. 2c). This phenomenon might be attributed to the oxidation of TMB by ·OH. In this system, UCNPs@SiO₂@GOx catalyzed glucose to H₂O₂, and then H₂O₂ was photolyzed to

Figure 2 (a) Relative activity of GOx, UCNPs@SiO₂, and UCNPs@SiO₂@GOx; (b) absorption spectra of TMB in the presence of glucose and UCNPs@SiO₂@GOx with different times of light irradiation at 980 nm (1 W cm^{-2}) ([TMB] = 10 mmol L^{-1} , [glucose] = 20 mmol L^{-1} , [UCNPs@SiO₂@GOx] = 2 mg mL^{-1}); (c) absorption intensity of the oxidized TMB at 652 nm as a function of irradiation time for the UCNPs@SiO₂@GOx. A and A_0 were the absorption intensities of the TMB system solution without light irradiation at 980 nm. The error bars represent the standard deviations of three separate measurements. (d) ESR spectra of different groups treated with glucose and 5,5-dimethyl-1-pyrroline N-oxide (DMPO) under light irradiation at 980 nm.

$\cdot\text{OH}$ that oxidized colorless TMB to chromogenic TMB. In addition, the characteristic $\cdot\text{OH}$ -induced 1:2:2:1 quadruple peaks were observed in the ESR spectra in the glucose solution after treatment with UCNPs@SiO₂@GOx under 980 nm light irradiation (Fig. 2d), further confirming the generation of $\cdot\text{OH}$. In contrast, when the glucose solution was treated by the PBS (control), GOx or UCNPs@SiO₂, there was no $\cdot\text{OH}$ characteristic ESR peak, which further suggested that GOx first catalyzed the oxidation of glucose with the production of H₂O₂ and the generated H₂O₂ was photolyzed by the invisible ultra-violet emission of the UCNPs under NIR excitation at 980 nm. This cascade system exhibited spatiotemporally controlled cancer therapy.

After that, the effects of UCNPs@SiO₂@GOx on 4T1 cancer cells were studied. The intracellular H₂O₂ production ability was tested *via* a reactive oxygen probe (DCFH-DA) [39]. As shown in Fig. S5, the 4T1 cells showed no green DCF fluorescence in the control and UCNPs@SiO₂ groups. But a bright DCF fluorescence was

found in 4T1 cells after treatment with GOx and UCNPs@SiO₂@GOx, which was ascribed to the generation of H₂O₂ by GOx. Next, the cytotoxicity of UCNPs@SiO₂@GOx at different concentrations was investigated. For comparison purpose, the cytotoxicities of UCNPs@SiO₂, GOx, and PBS were also measured. As depicted in Fig. 3a, UCNPs@SiO₂ at low concentrations ($\leq 40 \mu\text{g mL}^{-1}$) had no obvious cytotoxicity. However, after conjugation with GOx, the cytotoxicity of UCNPs@SiO₂@GOx was greatly increased and caused about 65% of cell death at the concentration of $40 \mu\text{g mL}^{-1}$ in the dark condition. This is probably due to the high level of H₂O₂ and glucose exhaustion caused by GOx. Subsequently, the synergistic effect of PDT on starving therapy on 4T1 cancer cells was investigated. As shown in Fig. 3b, the 4T1 cell viability in the UCNPs@SiO₂ after exposure to light illumination for 20 min (980 nm, 0.6 W cm^{-2}) had a weak decrease compared with that in dark condition. However, the cell viability in the group of UCNPs@SiO₂@GOx significantly decreased

Figure 3 (a) Cell viabilities of 4T1 cancer cells incubated with PBS (control), UCNPs@SiO₂ and UCNPs@SiO₂@GOx with different concentrations for 6 h in dark conditions; (b) cell viabilities of 4T1 cancer cells pretreatment with PBS (control), UCNPs@SiO₂ and UCNPs@SiO₂@GOx for 6 h and treated with light irradiation at 980 nm (0.6 W cm^{-2} , 20 min). The error bars represent the standard deviations of three separate measurements.

after light irradiation under the same condition compared with that in dark condition, indicating the efficient synergistic effects of PDT on starving therapy.

Moreover, the effective synergistic therapeutic performance was also intuitively confirmed by the live/dead (Calcein-AM/propidium iodide (PI)) cell co-staining assay (Fig. 4). The live/dead staining results exhibited obvious cell death after treatment with UCNPs@SiO₂@GOx and light irradiation compared with the control group (PBS) or in the absence of light irradiation. It is worth mentioning that the free GOx at low concentrations ($\leq 30 \text{ ng mL}^{-1}$) had obvious cytotoxicity to 4T1 cancer cells (Fig. S6). But it did not produce any phototoxicity on the cells after irradiation at 980 nm (Fig. S6), which further suggested that the synergistic effects of combination PDT and starving therapy only existed in the UCNPs@SiO₂@GOx system. In addition, some reports have proven that the free GOx failed to exhibit effective antitumor activity *in vivo* [40].

Therefore, it is needed to develop the multifunctional nanoparticles to optimize the GOx-involved synergistic therapy and guarantee an effective therapy efficacy in animal study.

The *in vivo* anticancer effects of UCNPs@SiO₂@GOx under light irradiation at 980 nm were evaluated on 4T1 cancer-bearing mouse model (see the detail experimental procedures in the Supplementary information). All the animal experiments were conformed to the guidelines set by the Animal Ethical and Welfare Committee of Shenzhen University (AEWC-SZU). As shown in Fig. 5a, rapidly progressive growth of tumors was observed after treatment with free GOx and light irradiation compared with the control group treatment with PBS under light irradiation, which suggested that the GOx was negative in inhibiting tumor growth compared with PBS and the light

Figure 4 Fluorescent images of 4T1 cancer cells with Calcein-AM/PI staining after different treatment. Cells were pre-treatment with PBS (control) or UCNPs@SiO₂@GOx (0.05 mg mL^{-1}) for 6 h, and then treated with light irradiation at 980 nm (0.6 W cm^{-2} , 20 min). Calcein-AM: $\lambda_{\text{ex}} = 488 \text{ nm}$, $\lambda_{\text{em}} = 515 \text{ nm}$; PI: $\lambda_{\text{em}} = 600\text{--}630 \text{ nm}$, $\lambda_{\text{ex}} = 536 \text{ nm}$. Scale bar: $200 \mu\text{mol L}^{-1}$.

irradiation at 980 nm could not directly provide synergistic therapy effect without the UCNPs. UCNPs@SiO₂ were found to have a weak inhibition of tumor growth under light irradiation. Moreover, UCNPs@SiO₂@GOx exhibited a certain tumor growth inhibition in the absence of light, which was ascribed to its starvation therapeutic effects. Furthermore, the mice after treatment with UCNPs@SiO₂@GOx and light irradiation showed the most effective tumor inhibition compared with all other control groups. The results indicated that the UCNPs@SiO₂@GOx-mediated starving- and photodynamic therapy had efficiently synergistic therapy efficacy, much better than mono treatment.

To visually determine the therapeutic outcome of different treatments, the representative photos of tumor-

Figure 5 (a) Tumor growth curves after different treatments with PBS (control), GOx, UCNPs@SiO₂ and UCNPs@SiO₂@GOx in dark or light conditions (dark indicates a dark condition; light indicates light irradiation at 980 nm of 0.6 W cm⁻² for 30 min. Data are shown as mean $\pm$ standard deviation ($n = 5$; *** $P < 0.001$). (b) Survival curves of the mice after the treatments obtained from (a). (c) Representative H&E staining images of major organs (heart, liver, spleen, lung and kidney) after different treatments. Scale bar: 100 μ m.

bearing mice were photographed after the therapy process (Fig. S7). These photos directly demonstrated the most efficient tumor inhibition after treatment with UCNPs@SiO₂@GOx and light irradiation. As a result, the mice after treatment with UCNPs@SiO₂@GOx and light irradiation exhibited a much better animal survival in 35 days than the other groups (Fig. 5b), which further confirmed the enhanced *in vivo* therapeutic efficacy of the synergistic starving- and photodynamic therapy. In addition, no significant body weight change was observed in all groups during the 15 days of the treatment process (Fig. S8). No obvious hemolysis was induced by UCNPs@SiO₂@GOx at a high concentration of 100 μ g mL⁻¹ (Fig. S9), indicating that the injection of UCNPs@SiO₂@GOx had no significant toxicity during

the treatment. The glutamic-pyruvic transaminase (ALT), urea nitrogen (BUN), glutamic oxalacetic transaminase (AST) and creatinine (CREA) levels from healthy nude mice at 15 days post-injection of UCNPs@SiO₂@GOx were found to be kept stable levels compared with the control group. No significant change in major organ histomorphology was observed after different treatments (Fig. 5c and Fig. S10). These results indicated that the UCNPs@SiO₂@GOx had good biocompatibility and did not cause severe toxicity *in vivo* during the therapy.

CONCLUSIONS

In summary, a type of NIR-responsive GOx-conjugated UCNPs, UCNPs@SiO₂@GOx, was constructed to improve the anticancer efficiency through cascade glucose

oxidation and hydrogen peroxide photolysis. The UCNPs@SiO₂@GOx agents absorb the 980 nm light and convert the low-energy light into high-energy invisible ultraviolet radiation for PDT. After administration, the UCNPs@SiO₂@GOx efficiently catalyzed the oxidation of glucose with production of H₂O₂, producing the anticancer activities of starving therapy and H₂O₂ therapy. Meanwhile, the H₂O₂ could be photolyzed to generate two ·OH radicals by UCNPs@SiO₂@GOx with the irradiation at 980 nm, which greatly improved the anticancer ability and inhibited the tumor growth with less side effects. This study would provide a new insight into the design and construction of multifunctional nanoparticles for cancer synergistic therapy.

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Conflict of interest The authors declare that they have no conflict of interest.

Supplementary information Supporting data are available in the online version of the paper.

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程序化饥饿与光动力协同癌症治疗研究

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摘要 协同治疗是指将多种治疗方法联合在一起使用, 从而显著增强治疗效果. 然而, 如何设计出理想的组合以最大限度地发挥协同效应仍是肿瘤治疗的一大挑战. 在此, 我们构建了一种由葡萄糖氧化酶修饰的上转换纳米制剂, 用于程序化的肿瘤饥饿-光动力协同治疗研究. 葡萄糖氧化酶催化氧化肿瘤内的葡萄糖并产生过氧化氢, 该过程消耗葡萄糖和氧气, 使得肿瘤细胞缺乏营养物质处于“饥饿”状态, 导致细胞死亡. 并且在980 nm的近红外光激发下, 上转换纳米颗粒激发产生紫外可见光, 将双氧水裂解成毒性更强的羟基自由基, 进一步杀死肿瘤细胞. 体外和体内实验均证实这种饥饿-光动力协同治疗明显优于任何单一治疗. 本研究为设计程序可控的饥饿-光动力协同治疗提供了理论支撑.