Janus \(\gamma\)-Fe\(_2\)O\(_3\)/SiO\(_2\)-based nanotheranostics for dual-modal imaging and enhanced synergistic cancer starvation/chemodynamic therapy

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**ABSTRACT**

Multimodal cancer synergistic therapy exhibited remarkable advantages over monotherapy in producing an improved therapeutic efficacy. In this work, Janus-type \(\gamma\)-Fe\(_2\)O\(_3\)/SiO\(_2\) nanoparticles (JFSNs) are conjugated with glucose oxidase (GOx) for synergistic cancer starvation/chemodynamic therapy. The \(\gamma\)-Fe\(_2\)O\(_3\) hemisphere of JFSNs can perform photoacoustic/T\(_2\) magnetic resonance dual-modal imaging of tumors. GOx on the surface of JFSNs catalyzes the decomposition of glucose and produces \(\text{H}_2\text{O}_2\) for cancer starvation therapy. Subsequently, the \(\gamma\)-Fe\(_2\)O\(_3\) hemisphere catalyzes the disproportionation of \(\text{H}_2\text{O}_2\) to generate highly reactive hydroxyl radicals in an acidic tumor microenvironment. The close distance between GOx and JFSNs ensures adequate contact between the \(\gamma\)-Fe\(_2\)O\(_3\) hemisphere and its substrate \(\text{H}_2\text{O}_2\), thus enhancing the catalytic efficiency. This synergy of glucose depletion, biotoxic \(\text{H}_2\text{O}_2\) and hydroxyl radicals significantly suppresses 4T1 mammary tumor growth with minimal adverse effects.

**Keywords:**
Synergistic therapy
Janus nanoparticles
Glucose oxidase
Fenton-like reaction
Theranostics

1. Introduction

Cancer monotherapy (i.e., chemotherapy, radiotherapy, immunotherapy) fails to eradicate the malignant tumor due to high tumor heterogeneity and complexity [1–4]. Synergistic therapy that integrates multiple treatment modalities has shown super-additive therapeutic effects and tremendous potential in cancer therapy [5–8]. Cancer starvation therapy could noninvasively exhaust tumor cells by depriving crucial nutrients of tumor cells, such as glucose and oxygen [9–11]. Among various cancer starving agents, glucose oxidase (GOx) could catalyze the decomposition of glucose, the main energy supply of tumor cells, and simultaneously produces \(\text{H}_2\text{O}_2\) and \(\text{H}^+\) for triggering subsequent treatment modalities, so GOx has attracted enormous interest in tailoring synergistic cancer therapeutic patterns [12–20]. For example, our group firstly integrated GOx-mediated starvation therapy with NO gas therapy mediated by \(L\)-arginine in the hollow mesoporous organo-silica nanoparticles (\(L\)-Arg-\(\text{HMON-GOx}\)) [21]. After accumulating in tumor tissues, glucose is converted into gluconic acid and \(\text{H}_2\text{O}_2\), which further oxidizes \(L\)-Arg into NO for gas therapy in acidic tumor microenvironment. To enhance the bio-compatibility of inorganic nanoplatforms for GOx-instructed synergistic therapy, our group recently developed a GOx-conjugated silver nanocube (AgNC-GOx) as a degradable nanosystem for synergistic starvation/metal ion therapy [16]. In this case, the \(\text{H}_2\text{O}_2\) generated from the GOx-catalyzed glucose decomposition reaction could efficiently oxidize AgNC into Ag ions in the existence of abundant \(\text{H}^+\). In addition to the oxidative activity, \(\text{H}_2\text{O}_2\) could also be converted to highly active hydroxyl radicals (\(\cdot\text{OH}\)) in the existence of iron (\(\text{Fe}^{2+}/\text{O}_{2}\)), named as Fenton and Fenton-like reactions [22–24]. For instance, Shi and co-workers [25] synthesized a dendritic mesoporous silicon nanoparticle to simultaneously load GOx and ultrasmall Fe\(_3\)O\(_4\) nanoparticles. GOx could effectively consume intratumoral glucose and then generate abundant \(\text{H}_2\text{O}_2\) for subsequent Fenton-like reaction catalyzed by Fe\(_3\)O\(_4\) nanoparticles. The combination of Fe\(_3\)O\(_4\) nanoparticles and GOx effectively inhibited the growth of subcutaneous 4T1 and U87 tumors. The synergistic therapeutic efficiency of GOx and ferromagnetic nanoparticles necessitates close contact between the nanoparticles.
and substrate H₂O₂. However, the drug leakage and instability of nanocarriers during blood circulation could enlarge the distance and reduce the catalytic efficiency of ferromagnetic nanoparticles. Therefore, it remains a challenge to guarantee an adequate contact between GOx and Fenton reaction agents to maximize the efficiency of chain reactions for synergistic cancer therapy.

Janus nanoparticles (JNPs) are anisotropic particles that consist of two disparate hemispheres [26,27]. There is an ever-increasing literature describing the application of JNPs that have two or more faces in opposite directions in cancer imaging and therapy [28,29]. JNPs that simultaneously present two different theranostic materials and surfaces for combination therapy or “all-in-one” cancer theranostics are more stable in vivo compared to conventional core–shell nanoparticles, which load theranostic agents via non-covalent bonds or physical encapsulation [30–34]. For example, Hou and co-workers [31] fabricated a Janus-type Au-Fe₂C nanodumbbell via a surface nucleation and seeded growth method for multimodal imaging and photothermal therapy. The Au part could perform photoacoustic (PA)/computed tomography (CT) imaging and photothermal therapy (PTT), while the Fe₂C part could perform magnetic resonance imaging (MRI) [35]. The loaded indocyanine green (ICG) in the mesoporous silica part could be released under NIR irradiation. Simultaneously, the Au part could kill cancer cells due to the heat effect of PTT. Nevertheless, there lack reports on the application of JNPs in cancer synergistic therapy.

To address the above issues, we synthesized Janus-type γ-Fe₂O₃/SiO₂ nanoparticles (JFSNs) and conjugated GOx on their surface for synergistic cancer starvation/chemodynamic therapy (Scheme 1). Firstly, under catalysis of GOx, the intratumoral glucose decomposition reaction could be converted into gluconic acid and H₂O₂. The resultant gluconic acid could facilitate the release of Fe ions in the tumor acidic microenvironment [22]. A part of Fe³⁺ could be reduced to Fe²⁺ by intracellular GSH. Consequently, the H₂O₂ generated from the glucose decomposition reaction could be converted into OH via a Fenton-like reaction as follows:

\[
\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOx}} \text{Glucronic acid} + H_2O_2
\]

\[
\gamma-\text{Fe}_2\text{O}_3 \xrightarrow{\text{H}^+} \text{Fe}^{3+}
\]

\[
\text{Fe}^{3+} + \text{GSH} \rightarrow \text{Fe}^{2+} + \text{GSSG}
\]

\[
H_2O_2 \xrightarrow{\text{Fe}^{2+}} OH
\]

The conversion of moderately oxidative H₂O₂ to highly oxidative OH could effectively enhance the toxicity of JFSNs onto tumor cells. Combined with glucose depletion, enhanced synergistic therapeutic efficacy of chemodynamic therapy (CDT) and cancer starvation could be achieved. Compared to conventional core–shell nanoparticles that encapsulated GOx and Fenton reaction agents, the distance of GOx and γ-Fe₂O₃ in JFSNs-GOx is much closer to allow adequate catalytic effects of γ-Fe₂O₃ to H₂O₂.

2. Materials and methods

2.1. Materials

GOx was purchased from Sigma-Aldrich. 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), 3.3’5,5’-tetramethylbenzidine (TMB), and 3-aminopropyltriethoxysilane (APTES) were purchased from Macklin (China). H₂O₂ Assay Kit was purchased from Bioitome (China). Cell Counting Kit-8 (CCK8) was purchased from MCE (USA). Calcein-AM/propidium iodide (PI) were purchased from Biolegend (USA). Carbon-H₂DCFDA were obtained from ThermoFisher Scientific (USA). Penicillin-Streptomycin and Dulbecco’s modified Eagle medium (DMEM) were purchased from HyClone (USA). Fetal bovine serum was purchased from Gibco (USA). The JFSNs were synthesized by a one-step flame-assisted spray-pyrolysis (FASP) approach using an enclosed reactor according to previous methods [36].

2.2. Synthesis of JFSNs-GOx

The 5 mg JFSNs were introduced into 5 mL ultrapure water. The resultant suspension was ultrasonicated for 5 min. 12.5 μL of (3-aminopropyl) triethoxysilane was added to the solution and then ultrasonicated for another 10 min. After reaction under stirring overnight, the JFSNs were collected by centrifugation or magnetic force and then washed by ultrapure water several times, and finally dispersed in 5 mL ultrapure water for further use. The 2.4 mg 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and 4 mg N-hydroxysuccinimide (NHS) were dissolved into 600 μL PBS (pH 6.0) and above solution was used to dissolve 2 mg GOx. Mix reaction components well and react for 15 min at room temperature. Then the activated GOx was added to the amine-JFSNs solution. After reacting for 2 h under stirring, the JFSNs-GOx were separated by centrifugation or magnetic force.

2.3. Characterization of JFSNs-GOx

The morphology and structure of JFSNs-GOx were characterized by a low-resolution transmission electron microscope (TEM, JEM-1230, JEOL) and high-resolution transmission electron microscopy (HRTEM, JEM-3200FS, JEOL). Zeta potential were determined by a Zetasizer Nano ZS90 (Malvern Instruments). UV–vis absorbance spectra were recorded by UV–vis spectrophotometer (Agilent Cary60). FTIR spectra were collected on a FTIR spectrometer (Agilent Cary60). FTIR spectra were recorded on a FTIR spectrometer (Spectrum Two™, PerkinElmer). A Rigaku D/Max 2550VB/PC X-ray diffractometer (XRD) with a Cu Kα radiation source and energy-dispersive X-ray spectroscopy (EDS, JEM-3200FS) were used to obtain chemical composition and structural information about JFSNs. ESR spectra were measured by Bruker EMX Plus spectrometer. 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) was applied to assess the hydroxyl radical generation in the nanomedical catalysts. Thermogravimetric analysis (TGA) was measured by a thermogravimetric analyzer (Q50, TA Instrument). The content ofScheme 1. (Color online) Schematic of JFSNs-GOx mediated dual-modal imaging and enhanced synergistic cancer starvation/chemodynamic therapy.
Fe in JFSNs is detected by ICP. The H₂O₂ concentration in vitro were measured by a H₂O₂ Assay Kit (Biyotime).

2.4. Catalytical activity of glucose oxidase

The production of H₂O₂ and the change in the pH of the solution are used to evaluate the activity of glucose oxidase. Time-dependent changes of generated H₂O₂ and pH values during the reaction between JFSNs-GOx ([JFSNs] = 0.1 mg/mL) and glucose (10 mmol/L). The concentrations of H₂O₂ and pH values arising from the reaction between JFSNs-GOx and glucose ([JFSNs] = 0.1 mg/mL, [glucose] = 0.16, 0.33, 3.3, 10, 33 mmol/L). The H₂O₂ concentration in vitro were measured by a H₂O₂ Assay Kit (Biyotime.). pH value was measured by a pH meter (Orion Star A111, Thermo Scientific).

2.5. Catalytical activity of JFSNs

The 300 μL JFSNs (0.1 mg/mL) were in-cubated with different concentrations of H₂O₂ containing TMB (500 μmol/L). After 30 min incubation, UV–vis spectra of solutions were scanned by a microplate reader.

2.6. Catalytical activity of JFSNs-GOx

The 300 μL JFSNs-GOx and JFSNs/GOx mixture ([JFSNs] = 0.1 mg/mL, [GOx] = 0.019 mg/mL) were incubated with glucose at various concentrations of TMB (500 μmol/L) overnight. After incubation, UV–vis-NIR spectra of solutions were scanned by a microplate reader.

2.7. In vitro synergistic cancer starvation/chemodynamic therapy

Cells were cultured with high-glucose DMEM containing 10% FBS and 1% Penicillin-Streptomycin at 37 °C in humidified atmosphere with 5% CO₂. For cytotoxicity assays, 4T1 cells were inoculated into 96-well plate with a density of 7 × 10³ per well overnight, and then the medium was replaced by fresh medium containing various concentrations of JFSNs-GOx for 12 h. After 12 h incubation, 10 mmol/L glucose, JFSNs-GOx (1 μg/mL) and L-ascorbic acid at different concentrations were added to the fresh medium of 96-well plate. The cell cytotoxicity was assessed by standard MTT assay and Calcein-AM/propium iodide (PI) staining. For cytotoxicity assays with L-ascorbic acid addition, 4T1 cells were inoculated into 96-well plate with a density of 7 × 10³ per well. After 12 h incubation, 10 mmol/L glucose, JFSNs-GOx (1 μg/mL) and L-ascorbic acid at different concentrations were added to the fresh medium of 96-well plate. The cell cytotoxicity was assessed by standard MTT assay. For ROS generation, the carboxy-H₂DCFDA (E₀/Eₘ = 495/529 nm) was used as a ROS indicator to monitor ROS by a fluorescence microscope (Nikon Eclipse Ts2, Japan). 4T1 cells were inoculated into 12-well plate with a density of 7 × 10⁴ per well overnight, and then the medium was replaced by fresh medium containing various concentrations of glucose (5 and 10 mmol/L) and different concentrations of samples before incubating the cells for 4 h. For cellular uptake assay, 4T1 cells incubated with JFSNs (10 μg/mL) for 24 h were collected and then fixed with cell fixative reagents overnight at 4 °C. Then the samples were treated by the standard procedures for biotransmission electron microscopy (Bio-TEM, FEI Tecnai Spirit T12, USA).

2.8. Animal model

Female BALB/C mice or nude mice aged 3–4 weeks were purchased from Guangdong Medicinal Laboratory Animal Center (Guangzhou, China) and all animal experiments were performed under the regulations of the Animal Ethical and Welfare Committee of Shenzhen University (AEWC-SZU). 2 × 10⁶ 4T1 cells were subcutaneously inoculated into the right hind leg of the mice to develop subcutaneous tumor-bearing mouse model. The mice were administered with drugs once the tumors reached ~50 mm³.

2.9. Photoacoustic (PA) imaging

For in vitro PAI, 200 μL of JFSNs-GOx (20, 50, 100, 500 and 1000 μg/mL) were added into PCR tubes to record PA imaging. For in vivo experiments, 50 μL of JFSNs-GOx (1 mg/mL) was intratumorally injected into 4T1 tumor-bearing mice. The Vevo 2100 LAZR system (VisualSonics, USA) with 808 nm excitation filters was used to perform PA imaging. For intratumoral blood oxygen saturation (SO₂) evaluation, the PA images were acquired using ‘Oxyhemo’ mode. After intratumoral injection of JFSNs-GOx (1 mg/mL, 50 μL), the PA images were captured in 0, 0.5 and 2 h to monitor the intratumoral O₂ concentration. The PA signal intensity of the region of interest (ROI) was then measured using the Vevo LAZR imaging system software package.

For in vitro MRI, the JFSNs-GOx of different Fe concentrations were solubilized in PBS and shaken at 37 °C for 6 h. Afterwards, the T₂ relaxation time of the supernatant was recorded on a 3.0T MR scanner (uMR 770, United-Imaging, Shanghai).

For in vitro MRI, when the tumors reached ~120 mm³, 50 μL of JFSNs-GOx (1 mg/mL) was intratumorally injected into 4T1 tumor-bearing mice. In vivo T₂-weighted MR imaging was performed 0, 1, and 24 h after intratumoral injection of JFSNs-GOx on a 3.0T MR scanner.

2.10. In vivo synergistic cancer starvation/chemodynamic therapy

Mice bearing 4T1 tumors (about 50 mm³) were divided into five groups (n = 5 per group): (1) Control; (2) JFSNs (1 mg/mL, 50 μL); (3) GOx (0.19 mg/mL, 50 μL); (4) JFSNs + GOx (1 mg/mL JFSNs mixed with 0.19 mg/mL GOx, 50 μL); (5) JFSNs-GOx (1 mg/mL, 50 μL). The administration time was 1, 3, 5 d, and the administration method was intratumoral injection. During 15 d, the tumor volume was measured every other day and calculated by the following equation: volume = width² × length/2. At day 15, the tumors and major organs were collected and sectioned into slices for Hematoxylin and eosin (H&E) staining.

2.11. Hemolysis assay

0.5 mL mice blood sample was collected and added to 1 mL PBS solution. The Red blood cells (RBCs) were separated from serum by centrifugation (4500 r/min, 3.5 min) and wash the RBCs three times with PBS and then disperse the RBCs to 4 mL PBS. 0.3 mL RBCs suspension was added to four times volume of PBS solution with various concentrations of JFSNs-GOx. The solutions were vortexed and stand at room temperature. After 3 h, samples were centrifuged (4500 r/min, 3.5 min). The absorbance of supernatants at 541 nm were measured by a microplate reader. RBCs treated with ultrapure water and PBS were set as negative and positive controls.

2.12. Statistical analysis

Data represent the mean ± SD statistical differences were calculated with unpaired two-tailed Student’s t-test or one-way analysis of variance (ANOVA) followed by Tukey’s multiple comparison post-hoc test using GraphPad Prism 8.2 (GraphPad software).
3. Results and discussion

3.1. Characterization of JFSNs-GOx

Firstly, the asymmetric JFSNs were synthesized using a one-step flame-assisted spray-pyrolysis (FASP) approach in a sealed reactor according to previous report [36]. The TEM images (Fig. 1a) clearly indicated the formation of Janus-type spherical $\gamma$-Fe$_2$O$_3$/SiO$_2$ nanoparticles with a diameter of around 30 nm. The high-resolution TEM (HRTEM) images (Fig. 1b, c) showed a lattice spacing between two (3 1 1) adjacent planes in the dark side of 0.252 nm, which was in good agreement with the interplanar distance of $\gamma$-Fe$_2$O$_3$ [37]. The light side exhibited amorphous structure of SiO$_2$. Element mapping (Fig. S1 online) clearly showed that the dark side of JFSNs mainly contained Fe and O, while the light side consisted of Si and O. Results of EDS (Fig. S2 online) also showed that JFSNs had the peaks of Si, O and Fe. XRD pattern of JFSNs (Fig. S3 online) contained the typical peaks of both SiO$_2$ and $\gamma$-Fe$_2$O$_3$. These results further consolidated the composition and crystal phases of JFSNs. To perform cancer starvation therapy, the carboxyl group of GOx reacted with the amino group of JFSNs-NH$_2$ to form the amide bond. The as-prepared JFSNs-GOx could be well dispersed in aqueous solution. When a magnet was put close to the vial, the nanoparticles were immediately moved onto the vial wall under this magnetic field, indicating that JFSNs remained good magnetic response after conjugation (Fig. 1d). The absorption spectrum of JFSNs-GOx showed an increase compared to that of JFSNs probably owing to the improved dispersity and aqueous solubility after introduction of GOx (Fig. 1e). The zeta potential of JFSNs recovered to negative after conjugation of GOx, indicating the successful conjugation of GOx to the $\gamma$-Fe$_2$O$_3$ groups-modified surface of JFSNs (Fig. 1f). The released Fe ions then catalyze the disproportionation of H$_2$O$_2$ to generate highly toxic $\cdot$OH in an acidic environment. In order to verify the generation of $\cdot$OH, the mixture of JFSNs-GOx and glucose was added with the nitrogen spin trap DMPO and the obtained long-lived radical-DMPO adducts were measured by electron spin resonance (ESR) spectroscopy. The mixture of JFSNs-GOx and glucose generated considerable amount of $\cdot$OH, as indicated by the characteristic 1:2:2:1 $\cdot$OH signals (Fig. 2c). The JFSNs and glucose, by contrast, failed to produce $\cdot$OH due to the lack of H$_2$O$_2$ that generated in the glucose decomposition reaction catalyzed by GOx. During the process, there was no noticeable change for the UV–Vis-NIR absorption of JFSNs, indicating good stability of JFSNs toward H$_2$O$_2$ (Fig. S8 online).

Then the oxygen consumption profile of the chain reactions was examined (Fig. S9 online). In contrast to the drop of O$_2$ concentration from 7.4 to 1.9 ppm (parts per million) in the GOx-mediated glucose decomposition process, the JFSNs-mediated Fenton-like reaction exhibited a slight increase of O$_2$ concentration from 7.4 to 8.0 ppm, because the Fenton reaction in an H$_2$O$_2$-rich environment could continuously generate O$_2$ [38]. Nevertheless, the amount of O$_2$ generated from Fenton-like reaction could not slow down the decrease of O$_2$ concentration in the JFSNs-GOx-mediated chain reactions. This experiment indicated that although JFSNs-mediated Fenton-like reaction could moderately enhance...
the O₂ concentration in the presence of H₂O₂ corresponding to the previous report, it was far less than the O₂ consumption of the GOx-mediated catalysis [23]. A probe of -OH, 3,3′,5,5′-tetramethyl benzidine (TMB), was applied to investigate the catalytic kinetics of JFSNs. TMB shows blue color and absorption increase in the presence of -OH. The TMB solution turned bright blue once addition of H₂O₂ and JFSNs, and its absorption peak localized at ~652 nm (Fig. 2d). This phenomenon might be attributed to the chain reactions among H₂O₂, JFSNs and TMB [39]. First, H₂O₂ was converted into -OH catalyzed by the JFSNs. Second, -OH oxidized colorless TMB to chromogenic TMB cation-free radicals. The Michaelis-Menten steady-state kinetics of JFSNs-GOx were then investigated. The absorbance of TMB increased with the concentration of added H₂O₂ (pH 7.4) and the corresponding initial velocities (v₀, ΔA/Δt, relative units of absorbance produced per minute) were recorded (Fig. 2e). The chromogenic changes after 20 min upon the addition of H₂O₂ at corresponding concentrations were also depicted (Fig. 2e-(i)). To calculate the Michaelis-Menten constant (Kₘ) and maximum velocity (Vₘₐₓ), a linear double-reciprocal plot of the Michaelis-Menten curve was obtained via plotting v₀ against the H₂O₂ concentrations (Fig. 2e-(ii)). According to the Eq. (5),

\[
\frac{1}{v_0} = \frac{K_m}{v_0} \frac{1}{[S]} + \frac{1}{V_{\text{max}}},
\]

where v₀ is the initial velocity of the reaction, Vₘₐₓ is the maximal rate of reaction, [S] is the substrate concentration, and Kₘ is the Michaelis-Menten constant, which is equivalent to the substrate concentration at which the rate of conversion is half of Vₘₐₓ and denotes the affinity of the enzyme. The Kₘ and Vₘₐₓ values were calculated to be 1423 mmol/L and 985.6 mmol/(L min). This relatively high Kₘ value indicated that a high concentration of H₂O₂ was necessary for JFSNs to reach maximal catalytic activity. A typical TMB absorption profile that plotted against glucose concentrations was demonstrated (Fig. 2f). As the concentration exceeded 100 μmol/L, the blue color variation of TMB could also be observed by naked eyes, and the blue color of JFSNs-GOx group (Fig. 2f-(i)) was deeper than that of the mixture group (Fig. 2f-(ii)). At the glucose concentration of 4 mmol/L, the absorption of TMB of JFSNs-GOx group exceeded 0.3, while that of JFSNs and GOx mixture group was <0.2 (Fig. 2f), indicating better catalytic efficiency of JFSNs-GOx group. Considering that the serum glucose concentrations in healthy individuals are 3–8 mmol/L, the JFSN-GOx was expected to generate plenty of -OH in vivo for efficient cancer treatment.

### 3.3. In vitro synergistic cancer starvation/chemodynamic therapy

The above results demonstrated the efficient generation of -OH in vitro by JFSNs-GOx upon additions of glucose. Next, the JFSNs-GOx-mediated synergistic starvation/chemodynamic therapy on tumor cells were investigated. 4T1 mammary tumor cell line was chosen as the model owing to its high glucose concentration [40]. The efficient intracellular incorporation of JFSNs-GOx was demonstrated by the bio-TEM images (Fig. 3a). JFSNs at low concentrations (≤1 μg/mL) had no obvious cytotoxicity, probably due to the low content of H₂O₂ in tumor cells (Fig. S10a online). After conjugation with GOx, the cytotoxicity of JFSNs was significantly enhanced (Fig. S10b online) and induced nearly 80% of cell death at the concentration of 0.5 μg/mL. To evaluate the synergistic effect of JFSNs and GOx, 4T1 tumor cells were incubated with JFSNs-GOx and glucose at various concentrations (1, 2, 5 and 10 mmol/L) for 24 h. The results of MTT assay showed that JFSNs were noncytotoxic at this concentration, but JFSN/GOx mixture caused around 20% of cell death in the presence of 10 mmol/L glucose, indicating the elevated therapeutic efficacy upon addition of GOx (Fig. 3b). Nevertheless, the higher cytotoxicity of JFSNs-GOx were achieved compared to the mixture group, which further demonstrated the enhanced synergistic efficiency after conjugation of JFSNs and GOx. To visually observe the generation hypoxic radicals in the tumor cells, 6-carboxy-2,7′-dichlorodihydrofluorescein diacetate (carboxy-H₂DCFDA), a green fluorescence probe for...
reactive oxygen species (ROS), was applied to stain the cells with different treatment. The JFSNs-GOx group generated much stronger green fluorescence than other groups, indicating a massive ROS production (Fig. 3c). The synergistic therapeutic outcome could also be visually observed by the live/dead cell staining assay (Fig. 3d). To verify the contribution of Fenton-like reaction to the cytotoxicity, a typical anti-oxidant agent L-ascorbic acid (200, 100, 50, 20, 10 and 0 mg/mL) were added to the cell medium to eliminate the \( \cdot \mathrm{OH} \) generated by JFSNs-catalyzed disproportionation of \( \mathrm{H}_2\mathrm{O}_2 \). The addition of L-ascorbic acid successfully elevated the cell viability of the JFSNs-GOx group from about 23% to 42%, indicating that the cytotoxicity of JFSNs-GOx mainly originated from the oxidative injury by \( \cdot \mathrm{OH} \) (Fig. 3e).

3.4. In vivo MR/PA dual-modal imaging

The integration of therapeutic and diagnostic functions in a single nanoplatform may provide more comprehensive and accurate information to guide the therapeutic process [41,42]. Therefore, the MR and PA imaging capability of magnetic JFSNs were assessed both in vitro and in vivo. The darker \( T_2 \)-weighted MR images of JFSNs-GOx (\([\text{JFSNs]} = 1, 0.8, 0.5, 0.3 \) and 0.1 mmol/L) were then captured (inset of Fig. 4a). The slope, as given by the \( r_2 \) value, was evaluated to be 56.82 L/(mol s). JFSNs-GOx at a high concentration (\( \geq 50 \mu\text{g/mL} \)) could also achieve PA imaging, and the PA values exhibited good linear correlation with the concentration of JFSNs (Fig. 4b and c). After injection of JFSNs-GOx, the PA signal at tumor sites increased two-fold and lasted for over 24 h (Fig. 4d and e). Likewise, the \( T_2 \)-MR signals of JFSNs distributed all over the tumor tissues and lasted for over 24 h (Fig. 4f and g). These results not only indicated the dual-modal imaging function of JFSNs, but also consolidated the good permeation of JFSNs-GOx in the tumor tissues after injection.

3.5. In vivo synergistic cancer starvation/chemodynamic therapy

Encouraged by the above results, the synergistic therapeutic efficacy of JFSN and GOx was then evaluated on the 4T1-tumor bearing mice model. As Eq. (1) showed, the GOx-catalyzed glucose decomposition reaction consumes massive oxygen. To monitor the content of oxygen in tumor tissues, PA imaging of oxyhemoglobin (\( \text{HbO}_2 \)) and hemoglobin (\( \text{Hb} \)) in tumor sites was performed. The blood oxygen saturation (\( \text{sO}_2 \)) of tumor tissue quickly dropped from about 55% to 32% within 2 h post-injection of JFSNs-GOx (\([\text{JFSNs]} = 1 \text{ mg/mL} \)), while mice in the PBS group maintained their original \( \text{sO}_2 \) levels (about 50%) (Fig. S11 online). After consolidating the oxygen consumption in tumor tissues, the 4T1 tumor volumes were measured every two days after different treatments. The results indicated that JFSNs-GOx presented the comparatively most satisfactory suppression effects on tumors (Fig. 5a). To be specific, the suppression rates of different treatment groups against the PBS group were calculated by the tumor weights (Fig. 5b). Interestingly, the suppression rate of JFSNs-GOx mixture group were similar to that of GOx group (52.4% and 47.0%), while the suppression rate of JFSNs-GOx were as high as 72.6%. The results indicated that the covalent conjugation of GOx to the surface of JFSNs had shorten the distance between JFSNs and GOx for efficient synergistic therapy. To visually compare the therapeutic outcome of different treatments, the mice and tumors were photographed after the measurement process (Figs. 5c and S12 online). The photograph directly demonstrated the efficient inhibition of tumor growth by JFSNs-GOx. The significant damage of tumor cells could be directly observed in the H&E staining images of 4T1 tumor tissue sections (Fig. 5d). No obvious hemolysis had been caused by JFSNs-GOx (200 mg/mL) in the whole blood of healthy mice (Fig. S13 online). During the 15 days of the treatment process, the body weight of mice in all groups showed negligible
Fig. 4. (Color online) In vitro and in vivo MR/PA dual-modal imaging. (a) $1/T_2$ versus Fe concentration curve of JFSNs in agarose gel. Inset: the corresponding $T_2$-weighted MRI image. (b) In vitro PA images and (c) the corresponding PA amplitudes of JFSNs-GOx. (d) In vivo PA images and (e) the corresponding PA amplitudes of JFSNs-GOx. (f) $T_2$-weighted MRI and (g) the corresponding tumor grey values of the 4T1 tumor 0 h, 5 min, 1 and 24 h after intratumoral injection of JFSNs-GOx. $n = 3$, mean ± SD.

Fig. 5. (Color online) In vivo synergistic cancer therapy. (a) The tumor growth curve of 4T1 tumor-bearing mice receiving different treatments. (b) The weight and (c) photograph of isolated 4T1 tumors 15 d after different treatments. (d) Representative H&E staining images of tumor tissues 15 d after different treatments. Scale bar: 50 μm. (e) ROS staining images of tumor tissues 15 d after different treatments. Scale bar: 500 μm. $n = 5$, mean ± SD. *$P < 0.05$, ***$P < 0.001$. 
4. Conclusion

To summarize, GOx-conjugated Janus-type $\gamma$-Fe$_2$O$_3$/SiO$_2$ nanoparticles (JFSNs-GOx) have been fabricated for T$_2$-MR/PA dual-modal imaging and cancer synergistic therapy. The JFSNs-GOx have an average diameter of around 30 nm, and the $\gamma$-Fe$_2$O$_3$ hemisphere maintains high magnetism and catalytic activity towards H$_2$O$_2$. Due to the covalent conjugation of GOx on the surface of JFSNs, the distance between GOx and $\gamma$-Fe$_2$O$_3$ hemisphere is close enough for an adequate contact between $\gamma$-Fe$_2$O$_3$ hemisphere and H$_2$O$_2$ to generate sufficient hydroxyl radicals for cancer chemodynamic therapy. The enhanced synergistic efficiency of GOx-mediated starvation therapy and JFSNs-catalyzed chemodynamic therapy has been verified in a 4T1 mammary tumor-bearing mouse model. After administration of JFSNs-GOx, the tumor suppression rate reaches to 72.6%, while the mixture of JFSNs and GOx only inhibits 52.4% of the tumor growth. Therefore, it is believed that Janus nanoparticles can present a feasible approach to maximize the cascade reactions-based cancer synergistic therapy.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgments

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Author contributions

Peng Huang, Longhua Tang and Yifan Zhang conceived the study and participated in the experiment design. Yifan Zhang, Yilin Wan, Yunyan Liao and Tao Jiang participated in the fabrication and characterization of JFSNs-GOx. Ting He and Wei Bi participated in the experiment design. Yifan Zhang, Yilin Wan and Yunyan Liao completed the subsequent experiments, drew all figures and wrote this manuscript. Peng Huang, Longhua Tang, Yanjie Hu and Peng Gong helped to write and revise this manuscript.

Appendix A. Supplementary materials

Supplementary materials to this article can be found online at https://doi.org/10.1016/j.scib.2019.12.024.

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