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Amino Acid-Based Supramolecular Nanozyme by Coordination Self-Assembly for Cascade Catalysis and Enhanced Chemodynamic Therapy towards Biomedical Applications†

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The clinical translation of chemodynamic therapy has been highly obstructed by the insufficient intracellular H$_2$O$_2$ level in diseased tissues. Herein, we developed a supramolecular nanozyme through a facile one-step cooperative coordination self-assembly of amphipathic amino acid and glucose oxidase (GOx) in the presence Fe$^{2+}$. The results demonstrated that the supramolecular nanozyme possessed cascade enzymatic activity (i.e., GOx and peroxidase), which could amplify the killing efficacy of hydroxyl radical (·OH) by self-supplying of H$_2$O$_2$, finally achieved synergetic starvation-chemodynamic cancer therapy in vitro. Additionally, this cascade nanozyme also exhibited highly effective antibacterial activity on Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) without the need for additional H$_2$O$_2$. This work provided a promising strategy on the design and development of nanozyme for future biomedical applications.

Introduction

Chemodynamic therapy (CDT), which can convert less reactive hydrogen peroxide (H$_2$O$_2$) into most harmful hydroxyl radical (·OH) through the metal catalysts (e.g., Fe$^{2+}$, Mn$^{2+}$, Cu$^{2+}$) -mediated Fenton reaction or Fenton-like reaction,$^{1-5}$ is considered to be a promising novel modality for relevant diseases (e.g., cancer, pathogenic bacterial infection) because of its local selectivity and negligible side effect.$^{6-7}$ However, the therapeutic outcomes of CDT have been highly limited due to the insufficiencies of endogenous H$_2$O$_2$, which significantly compromises the antitumor or antimicrobial effects. Therefore, the incorporation of a H$_2$O$_2$-supplementing functionality into conventional CDT strategies have been exploited for potentiating their therapeutic efficiencies.$^{11-12}$

Glucose oxidase (GOx), has attracted particular interest as a natural H$_2$O$_2$-generating enzyme, which can catalyze glucose with O$_2$ into gluconic acid and H$_2$O$_2$. Therefore, a variety of platforms have been developed to combine GOx-based starvation therapy with CDT for cancer treatment so far. For instance, Qing$^{13}$ and co-workers developed an MnO$_2$ nanosheets-based nanoreactor for co-enhanced chemodynamic and starving therapy against tumor hypoxia, the GOx and fluorescent reporters (FRs) were co-assembled on MnO$_2$ nanosheets, which was enwrapped by hyaluronic acid for realizing tumor targeting. In another work by Ke$^{15}$ and co-workers, the nanoreactors were constructed from polyprodrug polymersomes incorporating ultrasmall iron oxide nanoparticles and GOx to activate cascade reactions for orchestrated cooperative cancer treatment. Nevertheless, these methods either suffer from safety concerns due to the premature leakage of payloads and long-term toxicity of the delivery vehicles, or are restricted by insufficient drug loading, uncontrollable drug ratio and complicated synthetic routes$^{16-18}$. Therefore, it is extremely urgent but challenging to construct a simple, safe and efficient nanoplatform integrating GOx and CDT catalysts with high loading efficiency for synergistic starvation-chemodynamic therapy.

Nanozymes have aroused increasing interests in recent years due to their superior stability, low cost, and tunable catalytic activities compared with natural enzymes$^{19-21}$. For instance, Shi et al.$^{22}$ reported the construction of an efficient dual inorganic nanozyme-based nanoplatform, which exhibits cascade enzymatic activity (i.e., GOx and peroxidase) within tumor microenvironment based on ultrasmall Au and Fe$_3$O$_4$ NPs loaded dendritic mesoporous silica NPs, whereas the long-term toxicity of inorganic material significantly hindered its clinical translation. As an alternative strategy, simple biomolecules of biological origin in the design of nanomaterials are vigorously pursued$^{23-25}$, but it still remains in the infant stage to design and engineer pluralistic nanozyme starting from small molecule combinations and their cooperative interactions.

In nature, metal-ion binding plays key roles in regulating the supramolecular nanoarchitectonics and catalytic activity of metalloenzymes (e.g., peroxidase, catalase)$^{26-27}$. In light of this, coordination-driven self-assembly could be regarded as a versatile

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strategy to fabricate biomimetic nanozyme through the interactions of the metal ions with metal-binding amino acids\textsuperscript{28-30}. And amino acids have shown flexibility and versatility in designing self-assembled materials due to inherent advantages such as molecular simplicity, stability, low immunogenicity as well as easy availability\textsuperscript{31-33}. Moreover, GOx is also composed of multiple amino acids. Herein, we constructed supramolecular nanozyme using the strategy of amino acid coordination driven self-assembly. By using metal-binding amino acids, GOx, and ferrous ions as building blocks, well-defined, uniform supramolecular nanozyme were obtained on the basis of a combination of coordination and multiple noncovalent interactions (Fig. 1). After entering into tumor cells, the nanozyme released the GOx firstly, which could decompose glucose into gluconic acid and \(\text{H}_2\text{O}_2\), cutting off the nutrients supply to induce starvation therapy. Then, the generated \(\text{H}_2\text{O}_2\), as well as the endogenous \(\text{H}_2\text{O}_2\) in tumor cells, could activate and improve the peroxidase activity of the nanozyme for cascade catalytic generation of highly toxic \(\cdot\text{OH}\) via Fenton reaction, leading to enhanced CDT performance. What is more is that this cascade nanozyme also exhibited excellent antibacterial performance toward both \textit{E. coli} and \textit{S. aureus}, holding great promise in future biomedical applications.

Experimental

Preparation of Fmoc-L/Fe nanoparticles and Fmoc-L/Fe/GOx nanoparticles

A stock solution of Fmoc-Leu (Fmoc-L) was prepared by dissolving Fmoc-L in DMSO to give a concentration of 100 mM. Ferrous sulfate (\(\text{FeSO}_4\)) was dissolved in distilled water to form 10 mM stock solution. Afterwards, Fmoc-L stock solution (10 \(\mu\text{L}\)) and \(\text{FeSO}_4\) stock solution (100 \(\mu\text{L}\)) were added to distilled water (890 \(\mu\text{L}\)), a small amount of Tris (1 M) solution was added to adjust the final pH of the system to be neutral. The turbidity indicated the formation of Fmoc-L/Fe nanostructures. The preparation method of Fmoc-L/Fe/GOx nanoparticles is similar to that of Fmoc-L/Fe nanoparticles: 100 \(\mu\text{L}\) \(\text{FeSO}_4\) stock solution and 10 \(\mu\text{L}\) GOx (10 mg/mL) were mixed, then diluted by distilled water (880 \(\mu\text{L}\)), followed by addition of 10 \(\mu\text{L}\) Fmoc-L stock solution, then a small amount of Tris solution was added to adjust the final pH of the system to be neutral, the colloidal suspension indicating the formation of nanoparticles.

Quantitative analysis of Fmoc-L/Fe nanoparticles

The solutions of Fmoc-L/Fe nanoparticles were centrifuged at 14000 rpm for 10 min, and the residues were re-suspended in distilled water. This process was repeated for three times to remove any free \(\text{Fe}^{2+}\) not involved in the nanoparticles. These nanoparticles were then re-dispersed in distilled water (200 \(\mu\text{L}\)). After the addition of DMSO (100 \(\mu\text{L}\)) into the samples, the content of Fmoc-L was determined by measuring the UV-Vis absorption spectra and compared with the pre-established calibration curves. For the measurement of \(\text{Fe}^{2+}\), the nanoparticles were mixed with aqua regia (a mixture of nitric acid and hydrochloric acid) and measured by inductively coupled plasma-optical emission spectroscopy (ICP-OES, Perkin Elmer Avio 200).

Determination of GOx content involved in Fmoc-L/Fe/GOx nanoparticles

GOx was labeled firstly by fluorescein isothiocyanate (FITC), Fmoc-L/Fe/FITC-GOx nanoparticles were prepared. Then the nanoparticles were centrifuged at 14000 rpm for 10 min, the supernatant was taken to measure the absorption at 490 nm. The encapsulation efficiency (EE) was calculated according to the equation: EE (%) = (total FITC-GOx - free FITC-GOx)/total FITC-GOx ×100%. Data are expressed as EE % ± standard deviation (SD) of three independent experiments.

\(\cdot\text{OH}\) production

The solutions of 10 \(\mu\text{g}/\text{mL}\) methylene blue (MB), 50 mM \(\text{H}_2\text{O}_2\), and 2 mM Fmoc-L/Fe nanoparticles were mixed, the degradation of \(\cdot\text{OH}\)-induced MB was detected by observing the change of absorbance at 660 nm compared with control group. For terephthalic acid (TPA) oxidation, 5 mM TPA solution was prepared in 2 mM NaOH solution, then 10 mM Fmoc-L/Fe nanoparticles and 50 mM \(\text{H}_2\text{O}_2\) were added, after a few minutes, the fluorescence intensity at \(\sim 440\) nm under 312 nm wavelength excitation was measured.

Catalytic activity assessment of GOx

The GOx activity was estimated by the assessment of the product, gluconic acid, which causing pH to drop. In detail, 8 mM Fmoc-L/Fe/GOx nanoparticles ([GOx]=0.8 mg/mL) and 10 mg/mL glucose was mixed at 37 °C and the initial pH of the solution was adjusted to pH 6.0. The solution was measured with a pH meter over time.

Cell Line and culture conditions

Human breast cancer cells MCF-7 cells were maintained in RPMI-1640 medium supplemented with fetal bovine serum (10%), and penicillin-streptomycin (100 units/mL and 100 \(\mu\text{g}/\text{mL}\), respectively). The cells were grown at 37 °C in a humidified 5% CO\textsubscript{2} atmosphere.

Cellular uptake of Fmoc-L/Fe/GOx nanozymes

The MCF-7 cells were seeded onto Petri dishes (1 X 10\textsuperscript{5} cells per well) and incubated for 24 h at 37 °C. MCF-7 cells were treated with Fmoc-
L/Fe/FITC-GOx nanoparticles ([Fe] = 200 μM, [GOx] = 1 μg/mL) for 2 or 12 h. Finally, the cells were examined with a Zeiss laser scanning microscope. The FITC-GOx was excited at 488 nm and its fluorescence was monitored at 493-600 nm.

**Intracellular -OH detection**

The MCF-7 cells were seeded onto Petri dishes (1 X 10⁴ cells per well) and incubated for 24 h at 37 °C. MCF-7 cells were treated with Fmoc-L/Fe ([Fe] = 200 μM) nanoparticles + H₂O₂ (200 μM), H₂O₂. Fmoc-L/Fe nanoparticles, Fmoc-L/Fe/GOx nanoparticles ([GOx] = 1 μg/mL) + glucose (1 mg/mL), as well as Fmoc-L/Fe/GOx nanoparticles + glucose + H₂O₂ for 12 h, respectively. The medium was removed and the cells were further incubated with 2,7'-dichlorodihydrofluorescein diacetate (DCFDA, Sigma-Aldrich, 30 μM) for 30 min. Finally, the cells were examined with a Zeiss laser scanning microscope. The DCFDA was excited at 488 nm and its fluorescence was monitored at 493-600 nm. The images were digitized and analyzed using the Zen software.

**Cytotoxicity assay**

Approximately 1 x 10⁴ MCF-7 cells per well in RPMI medium were inoculated in the 96-well plates and incubated for 12 h. The cells were treated with different concentrations of Fmoc-L/Fe nanoparticles ([Fe] = 200, 100, 50, 25 and 12.5 μM) and Fmoc-L/Fe/GOx nanoparticles ([GOx] = 0.0625, 0.125, 0.25, 0.5, 1 μg/mL) nanoparticles for 12 h, under the condition existing H₂O₂ (200 μM) or not. The Fmoc-L/Fe/GOx nanoparticles group was also treated with glucose (1 mg/mL). Finally, MTT assay was used to evaluate cell viability.

**Bacterial culture and in vitro bacterial experiment**

E. coli and S. aureus single colonies were transferred to Luria-Bertani (LB) medium and shaken at 160 rpm for 16 h at 37 °C. The bacteria were then diluted in saline to 1 x 10⁸ CFU/mL. The bacterial growth inhibition was studied in LB medium: five groups of preparative bacterial suspensions were treated with Fmoc-L/Fe nanoparticles, H₂O₂, Fmoc-L/Fe nanoparticles + H₂O₂, Fmoc-L/Fe/GOx nanoparticles + glucose, and LB medium, respectively. The concentrations of Fe²⁺, H₂O₂, GOx and glucose were 1.5 mM, 100 mM, 800 μg/mL and 10 mg/mL, respectively. After incubation at a speed of 300 rpm for 4 h at 37 °C, the bacterial concentrations were investigated by detecting the optical density at 600 nm (OD₆₀₀). After the antibacterial growth inhibition evaluation, the bacteria of five groups were centrifuged and fixed with 4% formaldehyde for 30 min. Then the bacteria were treated with 30, 50, 70, 90 and 100% ethanol for 10 min in sequence. Finally, the morphology of bacteria after sputtering gold coating was observed by SEM.

**Results and Discussion**

**Self-assembly of Fmoc-L triggered by Fe²⁺**

Fmoc-L (fluorenylmethoxycarbonyl-L-Leucine) was chosen as the model amino acid owing to its inherent integration of a metal-binding essential amino acid, leucine, and a self-assembly moiety, the Fmoc group. Upon mixing a solution of Fmoc-L in dimethylsulfoxide (DMSO) and a solution of FeSO₄ in distilled water, an opalescent and turbid colloidal suspension was obtained, indicating the formation of nanoparticles. The dynamic light scattering (DLS) profile (Fig. 2a) of the resulting suspension showed that the Fmoc-L/Fe have narrow size distributions and average diameters of 161.2±68.01 nm. The scanning electron microscopy (SEM) image (Fig. 2b) and transmission electron microscopy (TEM) image (Fig. 2c) showed that Fmoc-L/Fe are spherical nanoparticles with sizes of approximately 160 nm, which consistent with DLS result. To investigate the self-assembly mechanism of the nanoparticles formation, the infrared (IR) spectrum of Fmoc-L/Fe was recorded and compared with that of Fmoc-L (Fig. 2d). The distinct bands at 1619 cm⁻¹ and 1399 cm⁻¹ in the spectrum of Fmoc-L/Fe corresponding to asymmetric and symmetric stretching vibrations appeared, indicated that the carboxyl groups of Fmoc-L have been coordinated to the Fe ions. The quantitative component analysis further revealed that the molar ratio of Fmoc-L to Fe²⁺ was close to 6:1, which conforms to the stoichiometry of ferrous coordination mode. These results, in combination our previous studies, suggested that the self-assembly process of Fmoc-L/Fe nanoparticles involved the formation of preliminary ferrous coordination complexes and further growth of the resulting complexes into advanced structures through multiple noncovalent interactions, such as hydrophobic interactions and π-π stacking of the aromatic motifs.

**Stimuli-responsive properties and peroxidase-like activity of Fmoc-L/Fe nanoparticles**

Now that the formation of Fmoc-L/Fe nanoparticles is based on synergy of coordination and other weak interactions, the complexes should be sensitive to environmental stimulus, such as, overexpressed GSH in cancer cells, which could competitively bind with Fe²⁺. The release of Fe²⁺ from Fmoc-L/Fe nanoparticles was examined using phenanthroline, which can react with Fe²⁺ forming orange red complexes with the maximum absorption at 510 nm. As Fig. 2e showed, Fmoc-L/Fe nanoparticles stayed nearly stable in the absence of GSH, whereas the release efficiency of Fe²⁺ in the presence of 5 mM GSH significantly increased up to 75% during 48 h. These results indicated that Fmoc-L/Fe could be triggered by GSH to release rapidly Fe²⁺, which would act as core reagent to initiate Fenton reaction for generating •OH. Next, we evaluated the production of •OH of Fmoc-L/Fe nanoparticles using terephthalic acid (TPA) as a probe, which can be transformed into fluorescent 2-hydroxyterephthalic acid with a characteristic peak at 426 nm in the presence of •OH. As shown in Figure 2f, the fluorescence intensity for Fmoc-L/Fe nanoparticles with H₂O₂ treated-group increased significantly compared with that for Fmoc-L/Fe nanoparticles or H₂O₂ alone group. In addition, methylene blue (MB) can be degraded quickly when H₂O₂ was added to Fmoc-L/Fe nanoparticles (Fig. 51), whereas the degradation rate of MB by only H₂O₂ or Fmoc-L/Fe nanoparticles were relatively low. These results suggested the peroxidase-like activity of Fmoc-L/Fe nanoparticles to generate •OH.

**Preparation and characterization of Fmoc-L/Fe/GOx cascade system**

Encouraged by coordination self-assembly of Fmoc-L with Fe²⁺ and Fe²⁺-mediated robust peroxidase-like ability, we next studied the feasibility for construction of cascade nanozyme system with consideration of GOx. Multicomponent, cooperative coordination of Fmoc-L and GOx in the presence of Fe²⁺ in a one-step self-assembled process induced a colloidal suspension with average sizes of 187.1±69.54 nm for Fmoc-L/Fe/GOx (Fig. 3a). The SEM (Fig. 3b) and TEM image (Fig. 52) further confirmed its spherical shape and size distribution, indicated that the GOx incorporation has negligible influence on the morphology of the Fmoc-L/Fe. The encapsulation efficiency of GOx in Fmoc-L/Fe/GOx was determined to exceed 99% by fluorescence measurements using fluorescein.
isothiocyanate (FITC)-labeled GOx. This high encapsulation efficiency was probably the result of cooperative coordination of GOx with Fmoc-L to Fe²⁺. The DLS results also revealed that the zeta potential value of Fmoc-L/Fe/GOX nanoparticles was -27.12±4.50 mV, which was more negative than Fmoc-L/Fe nanoparticles, i.e., -6.54±2.52 mV. After validating successful preparation, cascade catalytic reaction of Fmoc-L/Fe/GOX was then detected. First, the catalytic capabilities of GOx were evaluated based on gluconic acid generation. The pH values were tested to represent gluconic acid generation. As expected, a significant pH decline from 6.0 to 3.57 occurred after treatment with Fmoc-L/Fe/GOX while the pH of the solution stayed stable in absence of GOx (Fig. 3c), indicating the primary GOx activity of Fmoc-L/Fe/GOX. Subsequently, the peroxide-like activity of Fmoc-L/Fe/GOX was studied. As Fig. 3d showed, the Fmoc-L/Fe/GOX catalyzed the hydroxylation of TPA and caused the enhanced fluorescence intensity in the presence of glucose, and required no additional H₂O₂. The absorption of MB also exhibited significant decrease after addition of Fmoc-L/Fe/GOX nanoparticles and glucose (Fig. S3). By contrast, the fluorescence intensity of TPA and the absorption of MB in solution treated with Fmoc-L/Fe nanoparticles and glucose showed no change. The effect of nanoparticles or free GOx alone on MB degradation is also very negligible. These observations are obviously resulting from the H₂O₂ generation in the process of glucose being catalyzed by GOx and the following ·OH generation catalyzed by Fe²⁺ using H₂O₂ as substrate through Fenton reaction. Thus, a pluralistic nanozyme platform with cascade enzymatic activities was achieved by incorporating natural GOx into peroxidase-mimicking Fmoc-L/Fe.

For biomedical applications, the high stability of nanoparticles is one of necessary requirements. The stabilities of Fmoc-L/Fe and Fmoc-L/Fe/GOX nanoparticles were investigated firstly through creating 10-fold (v/v) dilutions in pure water and phosphate buffer (PBS) (pH 7.4). From the Fig. S5, both of Fmoc-L/Fe nanoparticles and Fmoc-L/Fe/GOX nanoparticles are highly stable as their size and size distributions have no discernable change. In addition, the stabilities of these nanoparticles were also evaluated in DMEM containing 10% FBS at 37 °C to mimic the physiological environment. The DLS results exhibited that the size of Fmoc-L/Fe nanoparticles and Fmoc-L/Fe/GOX nanoparticles remained unaltered (Fig. S5). The high stability of these nanoparticles under different conditions indicated their great promise in biomedical applications.

![Fig. 2](image_url) Fig. 2 (a) DLS profile with digital picture, (b) SEM image and (c) TEM image of as-prepared Fmoc-L/Fe nanoparticles. (d) FTIR spectra of Fmoc-L/Fe and Fmoc-L. (e) GSH-responsive release profile of Fe²⁺ from Fmoc-L/Fe nanoparticles. (f) The change of fluorescence emission spectra of TPA after treatment with Fmoc-L/Fe nanoparticles with or without H₂O₂.

![Fig. 3](image_url) Fig. 3 (a) DLS profile with digital picture. (b) SEM image of Fmoc-L/Fe/GOX nanoparticles. (c) pH value changes of Fmoc-L/Fe/GOX and Fmoc-L/Fe solutions in the presence of glucose. (d) The change of fluorescence emission spectra of TPA after treatment with Fmoc-L/Fe/GOX or Fmoc-L/Fe in the presence of glucose.
Intracellular ROS generation and cytotoxicity of Fmoc-L/Fe/GOx

Since excellent cascade enzymatic activity and high stability of Fmoc-L/Fe/GOx has been verified, in vitro catalytic cytotoxicity was further evaluated. The cellular uptake was firstly examined after incubating MCF-7 cells with the Fmoc-L/Fe/FITC-GOx nanoparticles. The confocal laser scanning microscopy (CLSM) images (Fig. 4a) show that the GOx fluorescence is mainly aggregated on the membranes at 2 h and entered gradually into cytoplasm with the incubation time increase, presumably by endocytosis. Next, the feasibility of cascade catalytic reactions occurring in living cells was studied based on •OH detection using dichlorofluorescein diacetate (DCF-DA) as indicators. As shown in the CLSM images (Fig. 4b), only weak green fluorescence in cells was observed after treating with Fmoc-L/Fe nanoparticles or H$_2$O$_2$ alone. After incubation with Fmoc-L/Fe/GOx nanoparticles, the cells exhibited brighter green fluorescence, and the intensity was comparable with that for Fmoc-L/Fe nanoparticles with additional H$_2$O$_2$, suggesting that glucose decomposition by GOx caused the elevated H$_2$O$_2$ level, as well as the subsequent enhanced •OH generation catalysing by Fmoc-L/Fe in cells. So, when the cells were incubated with Fmoc-L/Fe/GOx nanoparticles plus H$_2$O$_2$, the fluorescence intensity became strongest. These observations were further reflected clearly by the quantified fluorescence intensity summarized in Fig. 4c. In one word, these results confirmed that the cascade catalytic activity of Fmoc-L/Fe/GOx nanoparticles could be realized in cancer cell to decompose glucose and generate higher amounts of •OH, which would achieve the combination of chemodynamic therapy and starvation therapy.

The in vitro therapeutic efficacy of Fmoc-L/Fe/GOx nanoparticles on MCF-7 cells was then examined through 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyldtetrazolium bromide (MTT) assays. As Figure 4d shown, Fmoc-L/Fe nanoparticles exhibited limited cytotoxicity with cell viability of about 90%, upon addition of H$_2$O$_2$, the cell viability decrease to below 50% due to H$_2$O$_2$-mediated chemodynamic therapy, because H$_2$O$_2$ itself is not cytotoxic at this concentration (Fig. S4). Since GOx can consume glucose and cut off supply of energy and nutrients of cancer cell to perform starvation therapy, free GOx showed apparent cytotoxicity to MCF-7 cells. Notably, Fmoc-L/Fe/GOx nanoparticles in the presence H$_2$O$_2$ exhibited the strongest cytotoxicity with cell viability of about 10% as compared to that of monotherapy groups. More importantly, even in the absence of H$_2$O$_2$, the Fmoc-L/Fe/GOx nanoparticles-treated group still exhibited excellent killing efficacy, indicating the synergistic therapy of starvation and potentiated chemodynamic therapy.

Antimicrobial activity evaluation

Bacterial infection is a frequently encountered disease that severely threatens the health of human beings. Inspired by the excellent anticancer activity of Fmoc-L/Fe/GOx nanozyme, we then evaluated its antibacterial activity in inhibiting the growth of Gram-negative E. Coli and Gram-positive S. aureus. The excellent antibacterial activity
of Fmoc-L/Fe was proved by the fact that the viabilities of *E. coli* and *S. aureus* were lower than 20% upon Fmoc-L/Fe nanoparticles treatment in the presence of H$_2$O$_2$ (Fig. 5a, b). As a control, the viabilities of *E. coli* and *S. aureus* were above 95% for Fmoc-L/Fe nanoparticles and 60% for H$_2$O$_2$-treated groups. Notably, the viabilities of *E. coli* and *S. aureus* were reduced to 20% in the Fmoc-L/Fe nanoparticles-treated group in the absence of H$_2$O$_2$, indicating the high catalytic efficiency of Fmoc-L/Fe/GOx in the cascade reaction of glucose to highly toxic •OH. Visible colonies of bacterial growth were further evaluated for antibacterial activity measurement (Fig. 5c, d). Almost no colonies were observed in Fmoc-L/Fe nanoparticles with H$_2$O$_2$ and Fmoc-L/Fe/GOx nanoparticles treated-groups, and the antibacterial activity results were anastomotic with those of the viability measurements. The morphology of bacteria was then characterized via SEM (Fig. 5e, f). The *E. coli* and *S. aureus* in Fmoc-L/Fe nanoparticles-treated group presented a typical rod and spherical morphology with a smooth surface and intact cell walls, same with that of blank group, demonstrated almost no toxicity toward *E. coli* and *S. aureus*. The H$_2$O$_2$ treated-group resulted in a few disruptions on the cell wall of the bacteria, indicating the slight cytotoxicity of H$_2$O$_2$. But the groups treated with Fmoc-L/Fe/GOx nanoparticles or co-incubated with Fmoc-L/Fe nanoparticles and H$_2$O$_2$ induced more serious damage to the bacterial cells, as reflected by compressed morphology and rough surface. Thus, the prepared Fmoc-L/Fe/GOx nanoparticles possessed strong antibacterial activity with biocompatible glucose as the substrate, without the introduction of toxic H$_2$O$_2$.

**Conclusion**

In conclusion, we fabricated a supramolecular nanozyme based on the multicomponent coordination self-assembly strategy using amino acid, GOx and Fe$^{2+}$ as building blocks for enhanced chemodynamic therapy towards relevant diseases. The resulting nanozyme exhibited spherical shape, narrow size distribution, and impressive encapsulation efficiencies of hydrophilic GOx. The in vitro studies showed that this nanozyme could be internalized well by
cancer cell and possessed strong cytotoxicity, which has also been proved from the combination of starvation and chemodynamic therapeutic effects. Importantly, Fmoc-L/Fe/GOx exhibited an excellent antibacterial efficiency without the additional H$_2$O$_2$, which indicated the occurrence of cascade reaction from H$_2$O$_2$ generation by glucose oxidation to the production of highly active •OH via Fenton reaction. Overall, this work offered a useful strategy via employing bio-derived molecule for preparing efficient nanozyme for future biomedicines.

Conflicts of interest

There are no conflicts of interest to declare.

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Reference