Article

Multifaceted Elevation of ROS Generation for Effective Cancer Suppression

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Abstract: The in situ lactate oxidase (LOx) catalysis is highly efficient in reducing oxygen to \( \text{H}_2\text{O}_2 \) due to the abundant lactate substrate in the hypoxia tumor microenvironment. Dynamic therapy, including chemodynamic therapy (CDT), photodynamic therapy (PDT), and enzyme dynamic therapy (EDT), could generate reactive oxygen species (ROS) including \( \cdot\text{OH} \) and \( \text{^1O}_2 \) through the disproportionate or cascade biocatalytic reaction of \( \text{H}_2\text{O}_2 \) in the tumor region. Here, we demonstrate a ROS-based tumor therapy by integrating LOx and the antiglycolytic drug Mito-LND into \( \text{Fe}_3\text{O}_4 / g-\text{C}_3\text{N}_4 \) nanoparticles coated with \( \text{CaCO}_3 \) (denoted as FGLMC). The LOx can catalyze endogenous lactate to produce \( \text{H}_2\text{O}_2 \), which decomposes cascades into \( \cdot\text{OH} \) and \( \text{^1O}_2 \) through Fenton reaction-induced CDT and photo-triggered PDT. Meanwhile, the released Mito-LND contributes to metabolic therapy by cutting off the source of lactate and increasing ROS generation in mitochondria for further improvement in CDT and PDT. The results showed that the FGLMC nanoplatform can multifacetedly elevate ROS generation and cause fatal damage to cancer cells, leading to effective cancer suppression. This multidirectional ROS regulation strategy has therapeutic potential for different types of tumors.

Keywords: lactate oxidase; ROS; PDT; CDT; cancer

1. Introduction

Reactive oxygen species (ROS) are active derivatives of oxygen metabolism in the microenvironment of all biological systems [1–4]. They act as a second messenger in cell signaling and are closely associated with various diseases, including cancers. It has been long postulated that cancer cells exhibit persistently high ROS levels as a consequence of genetic, metabolic, and microenvironment-associated alterations. The escalated ROS generation in cancer cells serves as an endogenous source of DNA-damaging agents that promote genetic instability and the development of drug resistance [5–8]. Although there are some negative effects of increased ROS in tumor cells, the biochemical features of ROS make it possible to explore non-surgical therapeutic strategies to kill cancer cells through ROS-mediated mechanisms.

Non-surgical therapeutic approaches based on ROS production for cancers have been developed, including chemodynamic therapy (CDT), radiotherapy, photodynamic therapy (PDT), and enzyme dynamic therapy (EDT) [9–13]. The CDT based on Fenton or Fenton-like reaction has focused on utilizing inorganic nanoparticles as nanoenzymes, which can produce the most toxic ROS (\( \cdot\text{OH} \)) through the disproportionate reaction of \( \text{H}_2\text{O}_2 \) without external instruments [14–17]. Additionally, photodynamic therapy (PDT) relies on the light activation of photosensitizers to generate cytotoxic singlet oxygen (\( \text{^1O}_2 \)) and induces cancer cell death through apoptosis or necrosis [18–21]. Furthermore, the enzyme dynamic therapy can effectively convert the endogenous ROS (\( \text{O}_2^- \) and \( \text{H}_2\text{O}_2 \)) into highly reactive \( \text{^1O}_2 \) by
the cascade biocatalytic reaction of loaded superoxide dismutase and chloroperoxidase respectively in the tumor region [22].

There are one or more enzymes (e.g., oxidase, catalase, and peroxidase) participating in the H2O2 metabolism. Among them, oxidase can oxidize substrates and reduce oxygen to H2O2. Different from other oxidases, the in situ lactate oxidase (LOx) catalysis display high efficiency due to the abundant lactate substrate in the hypoxia tumor microenvironment (TME) and the ultralow O2 reaction threshold [23–25]. The extracellular level of lactate in the TME can be 20-fold higher than that under normal physiological conditions [26,27]. Recently, the strategy of consuming lactate into H2O2 in the TME by lactate oxidase has emerged. The lactate oxidase was loaded in well-designed nanomaterials and carried to accumulate in tumor tissues [25,28,29]. Despite this approach playing important roles in lactate degradation, it failed to utilize the H2O2 to further oxidize a variety of toxic substrates and decrease the production of lactate, which can be transformed from pyruvate at the end of glycolysis. Hence, the strategy of elevating ROS generation and lactate exhaustion might have a profound antitumor effect.

Herein, we designed and developed a pH-responsive nanoplatform to multifacetedly elevate ROS generation by integrating LOx and the antiglycolytic drug Mito-LND into Fe3O4/g-C3N4 nanoparticles coated with CaCO3 (denoted as FGLMC). The mechanisms of which are as follows (Scheme 1): (1) LOx consumes lactic acid in tumor cells, while oxidizing lactic acid to H2O2, which was further converted to ·OH by Fe3O4 NPs through Fenton-like reaction; (2) g-C3N4 combined with O2 under 660 nm activation can generate 1O2 to initiate PDT; (3) The antiglycolytic drug Mito-LND, which was obtained by connecting lonidamine (LND) and triphenylphosphine cation (TPP+) through a long alkyl chain, can accumulate and generate ROS in the mitochondria of tumor cells to cause mitochondrial dysfunction for further dynamic therapy enhancement. This nanoparticle platform based on the multifaceted elevation of ROS generation could achieve the purpose of efficient cancer treatment and has the potential to realize multiple functions of biomedical ROS regulation as a safe and efficient treatment strategy for ROS-related tumors.

![Scheme 1](image_url)

Scheme 1. Scheme illustration of the composition of FGLMC and its therapeutic effect on breast cancer.
2. Results and Discussion

2.1. Synthesis and Characterization of FGLMC

The synthetic procedure for the nanoplatform FGLMC (Fe$_3$O$_4$-LOx-MLND@g-C$_3$N$_4$@CaCO$_3$) involved two main steps. Firstly, the Lonidamine derivative (MLND) with mitochondrial targeting was obtained from Lonidamine and (2-aminoethyl)triphenylphosphonium bromide through amide condensation (Scheme S1). The structure of FGLMC was confirmed by $^1$H-NMR, $^{13}$C-NMR, and electrospray ionization mass spectrometry (ESI-MS) (Figure S1–S3). There were 22 aromatic hydrogen signals in the range $\delta$ 7.23–8.20 ppm, which was consistent with the five benzene ring skeletons of MLND. The NH signal at 8.93 ppm allowed us to confirm the successful construction of the amide bond. The single peak at 5.69 ppm was the result of the coupling of the methylene group with its adjacent N atom. The positions at 4.13 and 4.02 ppm corresponded to two adjacent methylene groups, respectively. Secondly, the Fe$_3$O$_4$@g-C$_3$N$_4$-LOx-MLND (FGLM) nanosystem was constructed by loading MLND, Fe$_3$O$_4$ nanoparticles, and LOx-NH$_2$ on g-C$_3$N$_4$, which possess a sheet structure. Specifically, MLND and Fe$_3$O$_4$ nanoparticles, g-C$_3$N$_4$, and LOx-NH$_2$ were sonicated together, placed in the dark, and then lyophilized to remove the solvent. Then, the CaCl$_2$ powder was dissolved in an aqueous solution of FGLM and saturated with carbon dioxide, volatilized by ammonium bicarbonate in a sealed environment to obtain the final nanoplatform FGLMC encapsulated by CaCO$_3$.

Transmission electron microscopy (TEM) (Figure 1A) and dynamic light scattering (DLS) (Figure 1B) determination indicated that Fe$_3$O$_4$ NPs presented regular squares and the diameter in an aqueous solution was about 5 nm. The g-C$_3$N$_4$ nanosheets had a sheet-like and porous structure with a diameter of 65 nm. In addition, it can be observed that the g-C$_3$N$_4$ nanosheets were loaded with uniform-sized Fe$_3$O$_4$ NPs and the diameter was 95 nm and the hydrated particle size was 115.4 nm. The surface of FGLMC was covered with a calcium carbonate film, and its hydrated particle size was 130 nm. In addition, the TEM-Element Mapping image of FGLMC indicated the presence of Ca elements, which further proved the successful loading of CaCO$_3$ (Figure 1C). The ultraviolet-visible absorption spectroscopy revealed the successful encapsulation of MLND in FGLMC (Figure 1D).

2.1.1. LOx Loading

The successful loading of LOx was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie Brilliant Blue Staining. It showed that the FGLMC group and the LOx group were on the same line, indicating that LOx was widely retained in the FGLMC nano-drug delivery system (Figure 1E).

2.1.2. Mitochondrial Targeting

We further explored the mitochondrial targeting capabilities of different components by using the JC-1 mitochondrial membrane potential probe. After the MCF-7 cells were stained and performed by flow cytometry, we found that the FGLM and FGLMC groups showed superior membrane potential to the control group (Figure 1F).

2.1.3. Ca$^{2+}$ Releasing

The fluorescence intensity and semi-quantitative results of the calcium ion indicator probe Rhod-2AM co-incubated with MCF-7 cells were used as the standard for Ca$^{2+}$ release in the CaCO$_3$ surface layer of FGLMC. First of all, the Ca$^{2+}$ release of FGLM and different concentrations of FGLMC after co-incubation with MCF-7 cells were evaluated. The results showed that, compared with the control group (PBS treatment), there was no fluorescence signal in the cells after FGLM (10 $\mu$g mL$^{-1}$) was incubated with MCF-7 cells for 2 h, indicating that there was no Ca$^{2+}$ release in FGLM. In contrast, the fluorescence of Rhod-2AM grew stronger as the concentration of FGLMC increased. Furthermore, the fluorescence was the strongest when FGLMC had a concentration of 20 $\mu$g mL$^{-1}$, which emphasized the successful coating of CaCO$_3$ in FGLMC (Figure 2A). Additionally, the linear relationship between the fluorescence intensity and the concentration of FGLMC was
also consistent with the semi-quantitative analysis (Figure 2B), which previously implied that the CaCO$_3$ coating in FGLMC can cause tumor cell damage through Ca$^{2+}$ overload. Next, the sensitivity of the CaCO$_3$ coating to different pH environments was studied. It demonstrated that the fluorescence intensity of Rhod-2AM in pH 5.0 was higher than pH 7.4 (Figure 2C), and the semi-quantitative data also supported the above results (Figure 2D). Based on the above analysis, we concluded that FGLMC can respond and release Ca$^{2+}$ to play a vital role in the acidic environment of tumors. The relationship between the release of Ca$^{2+}$ in FGLMC and the incubation time was further detected. It was found that the fluorescence intensity of Rhod-2AM ameliorated with the extension of the incubation time of FGLMC and MCF-7 cells and reached the maximum Ca$^{2+}$ release after incubating for 3 h (Figure S4), which was also consistent with the results of semi-quantitative analysis (Figure 2E). It revealed that the incubation time of FGLMC and tumor cells increased appropriately can enhance the release of Ca$^{2+}$ to effectively damage tumor cells.

**Figure 1.** Material characterization. (A) TEM images and (B) Hydrodynamic diameters of Fe$_3$O$_4$, g-C$_3$N$_4$, Fe$_3$O$_4$@g-C$_3$N$_4$, and FGLMC. (C) TEM-Element mapping of FGLMC (scale bar: 20 nm). (D) UV-vis absorption spectra of LND, MLND and FGLMC. (E) SDS-PAGE protein analysis of LOx. (F) Mitochondrial membrane potential detection.
emphasized the successful coating of CaCO₃ in FGLMC (Figure 2A). Additionally, the linear relationship between the fluorescence intensity and the concentration of FGLMC was also consistent with the semi-quantitative analysis (Figure 2B), which previously implied that the CaCO₃ coating in FGLMC can cause tumor cell damage through Ca²⁺ overload. Next, the sensitivity of the CaCO₃ coating to different pH environments was studied. It demonstrated that the fluorescence intensity of Rhod-2AM in pH 5.0 was higher than pH 7.4 (Figure 2C), and the semi-quantitative data also supported the above results (Figure 2D). Based on the above analysis, we concluded that FGLMC can respond and release Ca²⁺ to play a vital role in the acidic environment of tumors. The relationship between the release of Ca²⁺ in FGLMC and the incubation time was further detected. It was found that the fluorescence intensity of Rhod-2AM ameliorated with the extension of the incubation time of FGLMC and MCF-7 cells and reached the maximum Ca²⁺ release after incubating for 3 h (Figure S4), which was also consistent with the results of semi-quantitative analysis (Figure 2E). It revealed that the incubation time of FGLMC and tumor cells increased appropriately can enhance the release of Ca²⁺ to effectively damage tumor cells.

**Figure 2.** Calcium release of (A) FGLM and FGLMC with different concentrations and (B) semi-quantitative analysis (Scale bars represent 20 μm, *p < 0.05, **p < 0.005, ***p < 0.0005). (C) Calcium release of FGLMC under different pH conditions and (D) semi-quantitative analysis (Scale bars represent 20 μm, *p < 0.05). (E) Fluorescence semi-quantitative analysis of Ca²⁺ release with different incubation times (Scale bars represent 20 μm, ***p < 0.0005). Each value represents means ± SD (n = 3).

### 2.2. ROS Generation

Because of the outstanding Fenton-like activity of FGLMC in solution, 2′,7′-dichlorofluorescin diacetate (DCFH-DA) was employed as the ROS fluorescent probe to determine intracellular ROS generation. The inverted fluorescence image indicated that MCF-7 cells treated with PBS and LOx showed negligible fluorescence, implying relatively low ROS levels. Alternatively, there was an improved fluorescence signal when FGLMC was added, while without laser irradiation. Notably, strong green fluorescence was observed for the treatment of FGLMC and 660 nm laser irradiation, which demonstrated that FGLMC could generate much more ROS within MCF-7 cells (Figure 3A). Semi-quantitative analysis of the fluorescence intensity was also consistent with the above results (Figure 3B). Since LOx cannot penetrate the cell membrane, its function was severely limited by adding LOx alone. On the other hand, g-C₃N₄ could not absorb laser light when FGLMC was without laser irradiation, which caused the nanoplatform to not exert its PDT function. Therefore, only when FGLMC was irradiated with the 660 nm laser could the PDT effect of g-C₃N₄ be exerted to the greatest extent, and most ROS could be produced. In addition, flow cytometry was further applied to assess the ROS generating ability of different components. Compared with the control group (only PBS treatment), FGLMC exhibited more ROS generation than other groups, indicating that only co-loading of Fe₃O₄ and LOx can generate the maximum amount of ROS (Figure 3C).
All in all, the results confirmed that FGLMC has a strong ability to consume lactic acid and can oxidize lactic acid to H$_2$O$_2$.

### 2.2. ROS Generation

Because of the outstanding Fenton reaction with Fe$_3$O$_4$ and produce hydroxyl free radicals to synergistically enhance tumor lethality. Therefore, the H$_2$O$_2$ detection kit can be used to reveal the H$_2$O$_2$ production and lactic acid consumption capacity of FGLMC NPs. The generation of H$_2$O$_2$ was enhanced with the up-regulated concentration of the LOx in FGLMC. Moreover, the H$_2$O$_2$ concentration produced by 100 μg mL$^{-1}$ of FGLMC was the same as that produced by 49.6 μg mL$^{-1}$ of LOx. The loading rate of LOx in FGLMC was calculated as 49.6% (Figure 3D,E). All in all, the results confirmed that FGLMC has a strong ability to consume lactic acid and can oxidize lactic acid to H$_2$O$_2$.

### 2.3. H$_2$O$_2$ Detection

FGLMC NPs can consume lactic acid in tumor cells and oxidize it to H$_2$O$_2$ for further Fenton reaction with Fe$_3$O$_4$ and produce hydroxyl free radicals to synergistically enhance cell death. Therefore, the H$_2$O$_2$ detection kit can be used to reveal the H$_2$O$_2$ production and lactic acid consumption capacity of FGLMC NPs. The generation of H$_2$O$_2$ was enhanced with the up-regulated concentration of the LOx in FGLMC. Moreover, the H$_2$O$_2$ concentration produced by 100 μg mL$^{-1}$ of FGLMC was the same as that produced by 49.6 μg mL$^{-1}$ of LOx. The loading rate of LOx in FGLMC was calculated as 49.6% (Figure 3D,E). All in all, the results confirmed that FGLMC has a strong ability to consume lactic acid and can oxidize lactic acid to H$_2$O$_2$.

### 2.4. In Vitro Cytotoxicity

The methyl thiazolyl tetrazolium (MTT) assay was preliminary carried out to investigate the cytotoxicity of Fe$_3$O$_4$ and g-C$_3$N$_4$. The results showed that Fe$_3$O$_4$ and g-C$_3$N$_4$ were basically non-toxic to MCF-7 cells in the concentration range of 0–300 μg mL$^{-1}$, indicating the safety of the two carrier materials (Figure S5).

In the absence of light irradiation (laser off), g-C$_3$N$_4$, FGLM, and FGLMC showed lower cytotoxicity at low doses. However, the all-active (laser on) three materials showed a certain degree of toxicity at high doses, especially the FGLMC group, which was attributed to the ROS-dependent promotion of cell death (Figure 4A,B). Under the laser-on conditions, there was a significant difference between the half-maximal inhibitory concentration (IC$_{50}$) of g-C$_3$N$_4$ and FGLMC ($p < 0.001$) (Figure 4C). Such behavior could be explained by the fact that CDT and PDT synergistically enhanced ROS generation better than PDT individually. Furthermore, to fully prove the superorities of FGLMC under 660 nm laser, CLSM images of calcein-AM and propidium iodide (PI) co-staining of dead/live cells after receiving different formulations were carried out. Compared to the control group and FGLMC without the laser group, FGLM and FGLMC with laser irradiation exhibited significant cell lethality. Furthermore, the MCF-7 cells incubated with FGLMC displayed the highest red fluorescence and negligible green fluorescence when exposed to the 660 nm laser irradiation, confirming the therapeutic synergistic effect of CaCO$_3$ on killing cancer cells, which was consistent with the cell viability results (Figure 4D). Furthermore, cell apoptosis induced by different formulations was examined by flow cytometer after AV/PI co-staining. The
FGLM and FGLMC group without a laser showed better early and late apoptosis rates than other treatments (Figure 4E). However, the apoptotic rate was significantly increased when the laser was on. It was worth mentioning that FGLMC induced the highest early (18.74%) and late (76.67%) apoptosis percentages (Figure 4F,G). These results jointly demonstrated that CDT, PDT, and Ca\textsuperscript{2+} overloading collaborated with MLND and exerted the best efficacy.

2.5. FGLMC Suppress Cancer Cell Migration and Invasion

As cell migration and invasion are critical for tumor growth and metastasis, Transwell assays and Scratch assays were performed. Microscopic images revealed that compared with the control group, the migration distance of MCF-7 cells was fundamentally unchanged after FGLMC treatment for 12 h. Nevertheless, the distance was relatively smaller after incubating for 24 h, and the cell spacing was significantly reduced after treatment for 48 h, which indicated that FGLMC can effectively inhibit cell proliferation and migration (Figure 5A). To compare the inhibitory ability of different drugs, LND, MLND, and FGLMC were cultured with MCF-7 cells for 24 h to observe cell spacing. Interestingly,
changed after FGLMC treatment for 12 h. Nevertheless, the distance was relatively smaller after incubating for 24 h, and the cell spacing was significantly reduced after treatment for 48 h, which indicated that FGLMC can effectively inhibit cell proliferation and migration (Figure 5A). To compare the inhibitory ability of different drugs, LND, MLND, and FGLMC were cultured with MCF-7 cells for 24 h to observe cell spacing. Interestingly, FGLMC showed a cell migration inhibitory ability comparable to MLND, which was significantly different from the control group and the LND group (Figure 5B).

Western blotting was applied to further verify the effects of different formulations. Compared with the control and LND group, peroxiredoxin 3 (Prx3) in the cells treated with MLND was significantly oxidized, the degree of mitochondrial depolarization was strong, and the expression of Prx3 was reduced (Figure 5C). This can be attributed to the enhanced mitochondrial targeting ability after triphenylphosphine modification. In addition, due to the damage to mitochondria caused by Ca\(^{2+}\) overload, FGLMC exhibited the greatest inhibition of Prx3 expression. Moreover, a different concentration of FGLMC was added followed by 660 nm laser irradiation, the expression of Prx3 was suppressed, which revealed that PDT could also enhance mitochondria damaging capability (Figure S6). PINK1 is a mitochondrial-dependent protein kinase, which is located in the inner mitochondrial

![Figure 5. Migration and invasion evaluation. (A) Wound healing assay and (B) anti-invasion effects of different formulations on MCF-7 cells (The distance between the red dotted lines is the cell spacing after different treatments). (C) Western blot analysis for the expression of Prx3 and PINK1 on MCF-7 cells treated with different formulations. GAPDH was used as the loading control. Quantification of Prx3 (D) and PINK1 (E) expression intensity. Data are presented as mean ± SD (** p < 0.01, *** p < 0.005).](image-url)
membrane of the cell, and its expression is generally very low. The expression of PINK1 in the MLND and FGLMC group was higher than that in the control and LND groups, which indicated that the treatment with the drug MLND would cause the mitochondria in the cells to depolarize and cause mitochondrial damage (Figure 5C). The quantitative analysis data of Prx3 and PINK1 indicate that mitochondrial damage is an important factor in cancer cell apoptosis (Figure 5D,E).

2.6. Antitumor Effect In Vivo

Studies were also carried out to examine the in vivo therapeutic efficacy of FGLMC. Mice bearing MCF-7 tumors were, respectively, injected with (1) PBS (25 µL); (2) LND (25 µL, 10 mg kg⁻¹); (3) FGLMC (25 µL, 10 mg kg⁻¹); (4) FGLMC + 660 nm laser (25 µL, 10 mg kg⁻¹, 100 mW cm⁻² for 5 min) (Figure 6A). After treatment, the tumor size was measured every other day in each group to identify the tumor growth inhibition effect. Compared with the rapid tumor growth curve of the PBS group, groups treated with LND and FGLMC displayed relatively slower growth rates. Furthermore, the FGLMC + 660 nm laser group showed significant tumor growth suppression (Figures 6B and S7). The weights and images of tumors were well consistent with the measurement of tumor volume in vivo (Figure 6C,D). The body weights of mice in all groups increased, indicating that all formulations had negligible toxicity (Figure 6E). Similarly, images of hematoxylin and eosin (H&E) stain slices implied no obvious histological changes in major organs of the control and FGLMC + 660 nm laser group (Figure 6F). As expected, another mice treatment with FGLMC with 660 nm laser irradiation completely ablated the tumor during the 30 days, as well as produced the highest survival rate with notable elongation of life span compared to other groups, after which these mice were sacrificed (Figure 6G). These results were attributed to the synergistic effect of PDT and CDT to enhance the generation of ROS and the mitochondrial damage caused by chemotherapeutic and Ca²⁺ overload.

Figure 6. In vivo therapeutic effects of LND, FGLMC without or with laser. (A) Schematic illustration of the administration route for in vivo therapy. (B) Relative tumor volume, (C) tumor weight, (D) tumor image of MCF–7 tumors from different groups. (E) Bodyweight of MCF-7 tumor-bearing mice administrated with different formulations. (F) H&E–staining images of major organs (scale bar represents 100 µm). (G) The survival rate of MCF–7 tumor-bearing mice received different treatments. Error bars indicate SD (n = 4). (* p < 0.05, ** p < 0.01, *** p < 0.005).
3. Conclusions

In summary, we successfully developed a versatile nanoparticle platform FGLMC based on multifaceted elevation of ROS generation by integrating LOx and the antiglycolytic drug Mito-LND into Fe$_3$O$_4$/g-$C_3$N$_4$ nanoparticles coated with CaCO$_3$. The combination of dynamic therapy (CDT/PDT/EDT) synergistically enhanced ROS generation. Additionally, the antiglycolytic drug Mito-LND caused mitochondrial dysfunction and generated ROS for further dynamic therapy enhancement. Remarkably, this nanoplatform exhibited excellent ROS-generation ability and confirmed potent in vivo anticancer efficacy, as evidenced by the inhibition efficiency on MCF-7 tumor growth in a nude mice model with good biocompatibility. Briefly, the multifunctional collaborative nanoplatform provided a promising strategy for breast cancer therapy.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/nano12183150/s1, Scheme S1: Synthesis of MLND; Figure S1: $^1$H NMR of MLND; Figure S2: ESI-MS spectrum of MLND; Figure S3: Calcium release of FGLMC with different incubation times (Scale bars represent 20 µm); Figure S4: Cell viability of MCF-7 cells incubated with Fe$_3$O$_4$ and g-$C_3$N$_4$; Figure S5: (A) Western blot analysis and (B) quantification for the expression of Prx3 on MCF-7 cells treated with different concentrations; Figure S6: Digital photographs of MCF-7 tumor-bearing mice before and after different treatments. Reference [30] are cited in the Supplementary Materials.

Author Contributions: P.W. and H.T. conceived the idea, guided the project, and wrote the manuscript. H.W. and M.C. performed the experiments, analyzed the results and wrote the manuscript. Y.X. and T.L. helped in data analysis. H.W., M.C., Y.X., T.L., Y.G., P.W. and H.T. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data generated or analyzed during this study are included in this published article and its additional information files.

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