Mesoporous polydopamine nanoplatforms loaded with calcium ascorbate for amplified oxidation and photothermal combination cancer therapy

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Research Article

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Abstract

Background

Destruction of cellular redox homeostasis to induce cancer cell apoptosis is an emerging tumor therapeutic strategy. To achieve this goal, elevating exogenous oxidative stress or impairing the antioxidant defense system of cancer cells is an effective method. Herein, we firstly report a biocompatible and versatile nanoplatform (MpDA/Vc-Ca/PCM) based on calcium ascorbate (Vc-Ca) loaded mesoporous polydopamine (MpDA) nanoparticles, which simultaneously realized ROS generation, suppression of tumor antioxidant capability, and hyperthermia co-enhanced oncotherapy.

Methods

In this design, Vc-Ca is first loaded into MpDA, and then phase change material (PCM) is wrapped onto the surface of MpDA to form MpDA/Vc-Ca/PCM. The temperature-controlled release of Vc-Ca is characterized. The photothermal performance and GSH consumption capacity of MpDA are evaluated. The cytotoxicity mechanism of Vc-Ca is systematacially investigated. To confirm the synergistic tumor therapeutic effects, in vitro and in vivo biological tests are implemented.

Results

Vc-Ca encapsulated in MpDA by PCM is controllably released due to the melting of PCM matrix in response to photothermal heating upon a near-infrared irradiation. Vc-Ca is proved to be a pro-oxidant that can promote production of ROS (H$_2$O$_2$) in tumor site. Remarkably, MpDA can not only act as a photothermal agent, but also can break the redox balance of cancer cells through depleting the primary antioxidant glutathione (GSH), thus amplifying Vc-Ca-mediated oxidative therapy. Both in vitro and in vivo results demonstrate the significantly enhanced antitumor activity of boosted ROS combined with local hyperthermia.

Conclusion

This study highlights the potential applications of Vc-Ca in cancer treatment, and the prepared multifunctional nanoplatform provides a novel paradigm for high-efficiency oxidation-photothermal therapy.

Introduction

Cancer is a major public health problem worldwide and the second leading cause of death in the United States. According to the statistics of the World Health Organization, there are an estimated 1.9 million
new cancer cases and 0.6 million deaths in the United States in 2022[1]. Although conventional therapies (surgery, chemotherapy, and radiotherapy) are still the cornerstone for cancer treatment, the high incidence of recurrence or postoperative metastasis associated with surgical resection and the emergence of chemotherapy resistance greatly limit their clinical effectiveness[2–4]. Generally, tumor microenvironment (TME) has been considered to be the key factor affecting the therapeutic effect among various factors[5]. Compared with normal tissues, tumor cells are immersed in the oxidative stress with an excessive quantity of reactive oxygen species (ROS), mainly hydrogen peroxide (H$_2$O$_2$), which produced by the uncontrolled growth and dysfunction in physiological metabolism[6]. ROS mainly include $^1$O$_2$, •OH, H$_2$O$_2$ and O$_2$•$, which play vital roles in regulating various physiological functions of living organisms[7, 8]. The moderate ROS level can elicit cell proliferation and differentiation, once ROS concentrations beyond a certain threshold value will damage DNA, proteins, and lipids of cells, leading to cell damages or even death[9, 10]. Although cancer cells can evade oxidative damage by relying on the endogenous antioxidant system, they produce increased sensitivity for the further reinforced exogenous oxidative stress[2, 11, 12]. Thus, boosting intracellular ROS level through administration of exogenous agents to destruct the redox homeostasis in tumor cells has been developed as an effective anti-cancer strategy.

Ascorbic acid, also known as vitamin C (Vc), is a reducing antioxidant agent naturally existing in both animals and plants[13, 14]. For the past few years, the anti-cancer potential of Vc has received much attention from researchers[15]. Preclinical studies have consistently demonstrated that pharmacological pharmacologic concentrations of Vc (0.3−20 mM) can selectively kill a variety of cancer cells rather than normal cells[16, 17]. Importantly, although the mechanism of its selective toxicity is still unknown, persuasive evidence manifests that the anticancer effect of Vc depends on the autooxidation of Vc leading to increased steady-state levels of H$_2$O$_2$[18]. In addition, it is revealed that only high intracellular Vc concentration (> 3.5pm /cell) has significant tumor suppressive effect[17]. In view of these, using a versatile delivery system to load Vc, ensures sufficient drug transport and realizes programmed release in the tumor site, is an ideal strategy to strengthen its ROS therapeutic effectiveness against cancer.

In the past decades, drug-delivery systems based on engineered nanomaterials have begun to show great promise[19]. Among various nanomaterials, mussel-inspired polydopamine (pDA) has been widely explored and investigated due to its simple preparation, outstanding biocompatibility, biodegradation and easy functionalization[20, 21]. Specifically, mesoporous polydopamine nanoparticles (MpDA) with large specific surface area and ordered mesoporous structure are suitable for delivering various cargoes[20, 22]. Meanwhile, MpDA possesses superior near-infrared (NIR) absorption characteristic, which makes them ideal candidate for application in photothermal therapy (PTT)[22, 23]. Intriguingly, MpDA has also been used as a redox homeostasis regulator. Glutathione (GSH, a major ROS scavenger) is inherently excessive in the TME (up to 10 mM), and the strong reducibility of GSH consumes the cytotoxic ROS produced by therapeutic drugs, which will greatly reduce the therapeutic efficiency[24–26]. Recent study has confirmed that pDA can react with intracellular GSH, reduce the level of GSH, and damage the antioxidant defense system of cancer cells[27]. Given its inherent advantages of high drug load and GSH
consumption capacity, MpDA will be a preferred choice as a ROS drug delivery platform, and it is anticipated to implement hyperthermia and ROS-enhanced synergistic tumor therapy.

Through ingenious design, the engineered nanoplatforms can be endowed a variety of functions[28]. In the present study, we have successfully prepared a calcium ascorbate (Vc-Ca) loaded MpDA versatile nanoplatform that realized thermal stimuli-responsive drug release, ROS generation, regulation of redox homeostasis, and hyperthermia co-enhanced oncotherapy. In detail, Vc-Ca was first loaded into MpDA, and then phase change material (PCM) was wrapped onto the surface of MpDA to form MpDA/Vc-Ca/PCM (Scheme 1). Herein, Vc-Ca, as an ascorbate, has more stable physical and chemical properties than Vc, and possesses all the effects of Vc in the body. 1-tetracanol, a biocompatible PCM approved by FDA, has a phase-change temperature around 39–40°C, which has been widely used as an intelligent switch for temperature-responsive drug release[29, 30]. Under physiological conditions (approximately 37°C), the PCM existed in the solid state and the MpDA/Vc-Ca/PCM nanoplatform enabled leak-free delivery of Vc-Ca in vivo. Upon internalization by cells, the nanoplatform could be activated by an NIR irradiation, since the MpDA could trigger thermogenesis under NIR. When the temperature exceeded 39°C, rapid melting of the PCM resulted in a burst release of Vc-Ca, which selectively produced H$_2$O$_2$ in tumor lesions. Meanwhile, MpDA could react with intracellular GSH to reduce the antioxidant capacity of cancer cell, thus amplifying ROS levels. As a result, this multifunctional nanoplatform could be an efficient and safe agent for combination cancer therapy.

Method Section

Materials and reagents

Dopamine hydrochloride (C$_8$H$_{11}$NO$_2$·HCl) and calcium ascorbate (Vc-Ca) were purchased from Shanghai Macklin Biochemical Co., Ltd. 1,3,5-trimethylbenzene (C$_9$H$_{12}$) and Pluronic F127 (EO$_{106}$PO$_{70}$EO$_{106}$) were obtained from Shanghai Chemical Corp. Ammonium hydroxide (NH$_3$·H$_2$O) and ethanol (C$_2$H$_6$O) were obtained from Sinopharm Chemical Reagent Co., Ltd. 1-tetradecanol (PCM) was purchased from Tianjin Xisisi Biochemical Technology Co., Ltd. The reduced GSH assay kit and H$_2$O$_2$ assay kit were obtained from Nanjing Jiancheng Bioengineering Institute. Catalase and BAPTA-AM were purchased from Sigma-Aldrich. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), LDH assay kit, 4,6-diamino-2-phenylindole (DAPI), and Mitochondrial Membrane Potential assay kit with JC-1 were bought from Beyotime Biotechnology. Phalloidine, 2,7-Dichlorodihydrofluorescein diacetate (DCFH-DA) and Calcein-AM/EthD-I double-stain kit were purchased from Suzhou Yuheng Biotechnology Co., Ltd. All reagents were used without further purification.

Preparation of MpDA nanoparticles

MpDA nanoparticles were prepared via a versatile nanoemulsion assembly approach according to a previous study[31]. Briefly, 1 g of F127 and 0.5 g of dopamine hydrochloride were dissolved in 100 mL of distilled water and ethanol mixture ($V_{water}$ : $V_{ethanol}$ = 1:1) and stirred at room temperature for 30 min to
obtain a clear solution. Then, 2 mL of TMB was slowly injected into the reaction mixture and stirred at 500 rpm for 30 min. Subsequently, 5 mL of NH$_3$·H$_2$O was dropped into the above solution to induce the self-polymerization of dopamine oligomers. After stirring for another 30 min, the final products were centrifuged and washed with water and ethanol for at least three times.

**Fabrication of MpDA/Vc-Ca/PCM**

To obtain the PCM and Vc-Ca loaded MpDA, 10 mg of MpDA powder was dissolved in 1 mL of deionized water, and 0.1 g of Vc-Ca was added to the above solution. After stirring at room temperature for 8 h, the precipitates were purified by centrifuging. Then, the products were re-dispersed in 1 mL of ethanol, 0.15 g PCM was added and further stirred at 50°C for 6 h. The resulting solution was gathered via centrifugation and carefully washed with distilled water and ethanol for several times.

**Loading capacity of Vc-Ca**

The Vc-Ca standard curve was drawn first. Typically, 10 mg of Vc-Ca was accurately weighed and prepared with deionized water into a mother solution with concentration of 100 µg/mL. Then, the mother liquor was diluted with deionized water into standard solution series of 2.125, 4.25, 8.5, 17, 34 µg/mL, and the characteristic absorption at 266 nm was measured using the UV-vis spectrometer. To determinate the loading capacity of Vc-Ca, the centrifuged supernatant was collected in the preparation process of MpDA/Vc-Ca/PCM, and the absorbance value of Vc-Ca in the medium was analyzed by UV-vis at 266 nm. The amount of Vc-Ca in the supernatant was calculated through the standard curve of Vc-Ca.

**Controlled release of Vc-Ca**

In order to study the thermo-responsive release behavior, MpDA/Vc-Ca/PCM was dissolved in deionized water at 37°C and 50°C, respectively. At predetermined time point, the supernatant was centrifuged and the releasing amounts of Vc-Ca were detected by UV-vis spectrum. The content of Vc-Ca at each point was obtained according to the standard curve of Vc-Ca.

**Photothermal performance of MpDA**

In order to evaluate the photothermal performance of MpDA, 1 mL of deionized water and MpDA solutions with various concentrations (50 µg/mL, 75 µg/mL, 100 µg/mL, 150 µg/mL and 200 µg/mL) were irradiated by the NIR laser (808 nm, 1 W/cm$^2$) for 10 min. During laser irradiation, the real-time temperature changes and thermal images were monitored using an infrared thermal imaging camera (FLIR TG165). The heating curves of MpDA (100 µg/mL) under the irradiation of an NIR laser at 1 and 1.8 W/cm$^2$ were also acquired. The photothermal stability of MpDA was investigated by periodic laser irradiation for five cycles. Briefly, MpDA solution (100 µg/mL) was firstly exposed to the NIR laser at the power density of 1 W/cm$^2$ for 10 min, followed by cooling down to room temperature prior to the next irradiation.

**GSH consumption capacity of MpDA**
In brief, different concentration of MpDA (0, 50, 75, 100, 150, and 200 µg/mL) were incubated with 20 µM of GSH for 1 h. After, the mixed solution was centrifuged, and the absorbance value of the supernatant solution was measured in line with the instructions of the reduced GSH assay kit. On the basis of the above experimental procedure, the GSH consumption performance of MpDA at different intervals was further detected by UV-vis after the reaction of MpDA (100 µg/mL) and GSH for 0, 2, 12, 24 and 48 h. In addition, when MpDA and GSH were co-incubated for 48 h, the final products were collected by centrifugation and its chemical structure was analyzed by FTIR.

**Cell culture**

MG63 cells and NIH-3T3 cells were obtained from China Center for Type Culture Collection (Wuhan, China). The two types of cells were cultured and maintained as monolayers in DMEM medium containing 10 vol% fetal bovine serum (HyClone) and 1 vol% penicillin-streptomycin (Invitrogen) at 37°C in 5% CO₂.

**Intracellular GSH consumption**

MG63 cells were seeded in 6-well plates and incubated for 24 h. Then, serum-free medium containing different concentrations of MpDA (0, 50, 75, 100, 150, and 200 µg/mL) was added. After incubation for another 12 h, cells were centrifuged and redispersed in PBS (0.5 mL, 0.1 M) solution before lysis by ultrasonic and grinding. The supernatant after treatment with precipitant was further operated according to the instruction of GSH assay kit, and the absorbance was measured at 405 nm with a microplate reader (BioTek-1807292).

**Cytotoxicity mechanism of Vc-Ca**

MG63 cells were seeded in 96-well plates and incubated for 24 h. Then, cells were treated with different concentrations of Vc-Ca (0, 0.05, 0.1, 0.5, 1.0, 2.5 and 5.0 mM) dispersed in serum-containing medium for another 24 h. Subsequently, the culture medium was discarded, and 200 µL of serum-free media containing 10% MTT reagent were added to each well. After 4 h of incubation, the original culture medium was replaced with 150 µL of dimethyl sulfoxide followed by further incubation for 10 min. Finally, the absorbance was measured at 492 nm using a microplate reader.

The cell viability of MG63 cells treated with Vc-Ca and catalase were also detected. Briefly, MG63 cells were seeded in 96-well plates. After 24 h of incubation, different concentrations of Vc-Ca (0, 0.05, 0.1, 0.5, 1.0, 2.5 and 5.0 mM) were added, at the same time, 20 µL of catalase (250 µg/mL) was supplied to each well. After cultured for 24 h, the cells were operated in accordance with the above procedures.

In order to explore the effect of Ca²⁺ in Vc-Ca on cell activity, we used BAPTA-AM chelated Ca²⁺ ion. The procedure was the same as above, only replacing the catalase with BAPTA-AM (80 µM). In addition, to further investigate the cell damage mechanism of Vc-Ca, a series of comparative experiments were set up. In brief, MG63 cells were seeded in 96-well plates and incubated for 24 h. Then, the cells were divided into six groups: blank group (control group), catalase group (250 µg/mL), BAPTA-AM group (80 µM), Vc-
Ca group (5.0 mM), Vc-Ca + catalase group, and Vc-Ca + BAPTA-AM group. After 24 h of incubation, the cells were treated following the above steps.

**Extracellular H$_2$O$_2$ level**

MG63 cells were seeded in 6-well plates and incubated for 24 h. Then, serum-containing medium with different concentrations of Vc-Ca (0, 0.05, 0.1, 0.5, 1.0, 2.5 and 5.0 mM) was added into each well. After further culture for 30 min, 60 min, 90 min and 120 min, respectively, the cells were washed twice with PBS. The extracellular H$_2$O$_2$ level was determined using the H$_2$O$_2$ assay kit in line with the manufacturer's instructions.

**Cell viability detection**

MG63 cells were seeded in 96-well plates and incubated for 24 h. Then, different concentrations of MpDA (0, 50, 75, 100, 150 and 200 µg/mL) and MpDA/Vc-Ca/PCM (containing MpDA: 0, 50, 75, 100, 150 and 200 µg/mL) was added into the plate. After 12 h of incubation, the laser irradiation groups were treated with an 808 nm laser at a power density of 1 W/cm$^2$ for 10 min, and then the cells were continued to be cultured for 5 h. Afterward, the absorbance of each group was measured by a standard MTT assay. Additionally, to evaluate cell compatibility, NIH-3T3 cells were seeded in 96-well plates and incubated for 24 h. Then, different concentrations of MpDA/Vc-Ca/PCM were added into the plate and incubated for another 17 h. Next, the handling method for the NIH-3T3 cells was also using the MTT method.

**Cell cytotoxicity detection**

MG63 cells were seeded in 96-well plates and incubated for 24 h. Subsequently, the cells were treated with different groups such as blank (control group), MpDA (100 µg/mL), MpDA/Vc-Ca/PCM (MpDA: 100 µg/mL), Vc-Ca (5.0 mM), MpDA + NIR (100 µg/mL) and MpDA/Vc-Ca/PCM + NIR (containing MpDA: 100 µg/mL). In subsequent experiments, all groups followed this concentration unless otherwise specified. After 12 h of incubation, the laser irradiation groups were exposed to an 808 nm laser at a power density of 1 W/cm$^2$ for 10 min, and the cells were incubated for another 5 h. After that, the culture medium collected from each well was centrifuged at 400 g for 5 min, and 120 µL of the supernatant in each group was incubated with 60 µL of LDH reagent for 30 min at 25°C. Finally, the absorbance was determined on a microplate reader at 492 nm.

**Cell morphology observation**

MG63 cells were seeded in 24-well plates and incubated for 24 h. Subsequently, the cells were treated with different groups such as blank (control group), MpDA, MpDA/Vc-Ca/PCM, Vc-Ca, MpDA + NIR and MpDA/ Vc-Ca/PCM + NIR for 12 h. Specifically, the laser irradiation groups were exposed to an 808 nm laser at a power density of 1 W/cm$^2$ for 10 min, and the cells were incubated for another 5 h. Secondly, the cells were washed with PBS, fixed with 3% glutaraldehyde for 15 min, and then permeabilized with 0.5% Triton X-100 for 10 min. Then, the cells were dyed with DAPI for 10 min and fixed by 2.5%
glutaraldehyde. The cell cytoskeleton and nuclei were stained with phalloidin and DAPI for 20 min, respectively, before observing the cell images using a fluorescence microscope (NiKon-H550S, Japan).

**Live/dead staining**

Calcein-AM and ethidium homodimer-1 was used to stain the live and dead cells. MG63 cells were seeded in 24-well plates and incubated for 24 h. Subsequently, the cells were treated with different groups such as blank (control group), MpDA, MpDA/Vc-Ca/PCM, Vc-Ca, MpDA + NIR and MpDA/Vc-Ca/PCM + NIR for 12 h. The laser irradiation groups were exposed to an 808 nm laser at a power density of 1 W/cm$^2$ for 10 min, and the cells were incubated for another 5 h. Next, the dye mixture of calcein-AM and ethidium homodimer-1 was added into each well and incubated for 15 min in dark condition. After thoroughly rinsed with PBS, cells were observed under the fluorescence microscope.

**ROS generation assay**

The ROS generation was examined by using a DCFH-DA method. MG63 cells were seeded in 24-well plates and incubated for 24 h. Subsequently, the cells were divided into six groups: blank group (control group), MpDA group, MpDA/Vc-Ca/PCM group, Vc-Ca group, MpDA + NIR group and MpDA/Vc-Ca/PCM + NIR group. After 12 h of incubation, the laser irradiation groups were treated with an 808 nm laser at a power density of 1 W/cm$^2$ for 10 min, and the cells were incubated for another 5 h. Then, 400 µL of the DCFH-DA solution (1 µM in serum-free medium) was added into the cells and followed by additional incubation for 30 min at 37°C. After thoroughly rinsed with PBS, the intracellular ROS levels were observed by fluorescence microscope.

**ΔΨm assessment**

MG63 cells were seeded in 24-well plates and incubated for 24 h. Then, the cells were divided into two groups: MpDA/Vc-Ca/PCM group and MpDA/Vc-Ca/PCM + NIR group. After 12 h of incubation, the laser irradiation groups were treated with an 808 nm laser at a power density of 1 W/cm$^2$ for 10 min. and the cells were incubated for another 5 h. Afterward, the cells were stained with JC-1 dyestuff for 20 min, and the excess dye was washed with PBS. Ultimately, the cell images were captured using a fluorescence microscope.

**In Vivo thermal imaging**

The photothermal effects of composite nanoparticles in mice were investigated by infrared thermal image. Nude mice bearing 143B tumors were randomly assigned to five groups: PBS + NIR group, Vc-Ca + NIR group, MpDA + NIR group, MpDA/Vc-Ca/PCM + NIR group, and MpDA/Vc-Ca/PCM group. The mice of laser irradiation groups were exposed to an 808 nm laser at a power density of 1 W/cm$^2$ for 10 min. During this period, an infrared thermal camera was used to record the temperatures every 30 s in tumor sites. At the same time, the infrared thermal images of mice were taken at 0 min, 1 min, 3 min, 5 min and 10 min.
In Vivo antitumor therapy

All animal experiments were approved by the Animal Ethics Committee of Wuhan Servicebio Technology Co., Ltd (SYXK(W)2015 – 0693). Twenty nude mice (BALB/c, 5 weeks old) were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (China; animal quality certificate NO.110011211108440457). 143B cells (5×10^7) suspended in 125 µL of serum free cell medium were subcutaneously injected into the back of each mouse to establish the tumor model. When the tumor volume reached ~ 100 mm^3, mice were intratumorally injected with 50 µL of PBS, Vc-Ca (2 mg/mL), MpDA (8 mg/mL) and MpDA/Vc-Ca/PCM fluid (containing MpDA: 8 mg/mL), respectively. The mice were randomized into five groups (4 mice/group): PBS + NIR group, Vc-Ca + NIR group, MpDA + NIR group, MpDA/Vc-Ca/PCM + NIR group, and MpDA/Vc-Ca/PCM group. For the laser irradiation treatment groups, after 0, 3, 6 days injection, the mice were irradiated by the 808 nm laser at the power density of 1 W/cm^2 for 10 min. The non-laser group (MpDA/Vc-Ca/PCM) also received injection on day 0, 3, 6 without laser irradiation. Body weight and tumor volume were both recorded every two days from day 0 (after the initial drug injection) until the 14st day. All the mice were euthanized on day 14, and the tumor tissues were dissected to weigh and collected for photographing. In addition, the tissue sections of tumors were subjected for histological analysis with H&E, TUNEL and GPX4 staining.

Statistical analysis

Each experiment in this study was repeated three times. For samples that were used for each experiment, an n = 5 or 6 was used. All data were expressed as mean ± standard deviation (SD). Each experiment was repeated three times.

Results

Synthesis and characterization of MpDA/Vc-Ca/PCM

The MpDA nanoparticles were synthesized via a versatile nanoemulsion assembly method in the ethanol/water system by using Pluronic F127 as a template, dopamine as a raw material, and TMB as a mediator[31]. Both scanning electron microscope (SEM) and transmission electron microscope (TEM) observations demonstrated that MpDA had well-demarcated spherical morphology and ultralarge mesoporous structure with uniform size distribution (Fig. 1a, 1b and 1e). Upon loading with the Vc-Ca and PCM, it could be seen from SEM (Fig. 1c and 1d) and TEM (Fig. 1f) images that the mesopores on the surface of MpDA/Vc-Ca/PCM nanoparticles disappeared, and the average diameter of nanoparticles had an obvious increase (Fig. S1, Supporting Information), suggesting the success of loading. This result was also confirmed by the element mapping of MpDA/Vc-Ca/PCM. As shown in Fig. 1g, MpDA/Vc-Ca/PCM exhibited homogenous distribution of the C, N, and Ca elements throughout the nanoparticles. Further, Fourier transform infrared (FTIR) spectroscopy was used to characterize the chemical constitution of composite nanoparticles, and relevant spectra were represented in Fig. 1h. The spectra of MpDA showed the absorption bands at 3350 and 3248 cm^{-1}, which were assigned to the characteristic peaks of the
primary amine bonds in dopamine; and the peaks at 1597 and 1508 cm$^{-1}$ corresponded to N-H stretching and scissoring vibrations, respectively[32]. In the FTIR spectra of MpDA/Vc-Ca/PCM, the absorption band at 1622 cm$^{-1}$ was the superposition peak of MpDA, PCM and Vc-Ca; while the peaks at 2916 and 2850 cm$^{-1}$ belonged to the characteristic peak of PCM. In addition, the peaks at 3427 and 3380 cm$^{-1}$ deriving from the characteristic absorption of Vc-Ca were also detected in spectrum of MpDA/Vc-Ca/PCM. These results confirmed the successful loading of both PCM and Vc-Ca. We also detected the UV-vis spectra of Vc-Ca, MpDA, MpDA/Vc-Ca and MpDA/Vc-Ca/PCM (Fig. 1i). The absorption peak of MpDA/Vc-Ca at 266 nm corresponded to the characteristic absorption peak (AscH$^-$) of Vc-Ca, indicating that Vc-Ca has been successfully filled into the mesoporous structure of MpDA. Specially, the peak of AscH$^-$ was not detected in the UV-vis spectra of MpDA/Vc-Ca/PCM, which was mainly attributed to the encapsulation of PCM. In this study, the Vc-Ca loading efficiency of MpDA was calculated to reach as high as 42.33% according to the standard curve of Vc-Ca (Fig. S2, Supporting Information). We investigated the release profiles of Vc-Ca from MpDA/Vc-Ca/PCM nanoparticles under different temperature. As can be seen from Fig. 1j, only about 10% of Vc-Ca was released at 37 °C. When the temperature was 50 °C, the release amount of Vc-Ca from MpDA/Vc-Ca/PCM was significantly increased; and the cumulative release percentage of Vc-Ca reached 61.4% after 20 min. These results suggested that MpDA/Vc-Ca/PCM had controlled drug release properties under thermal stimulation.

**Photothermal performance and GSH consumption capacity of MpDA**

The photothermal performance of MpDA was tested. As shown in Fig. 2a, after irradiation by an 808 nm laser at a power density of 1 W/cm$^2$ for 10 min, solution containing MpDA nanoparticles showed rapid warming. The temperature of MpDA solution (100 µg/mL) increased to 52.5 °C from 24.1 °C within 10 min, while the temperature of water only increased to 29.2 °C from 24.0 °C under the same irradiation condition (Fig. S3, Supporting Information). Notably, the photothermal effect of MpDA displayed both concentration-dependent and power-dependent behaviors (Fig. 2b). In addition, no obvious changes in the ability to raise solution temperature was observed after five lasers on/off cycles (Fig. 2c), suggesting the excellent photothermal stability of MpDA.

To explore the ability of MpDA to regulate the redox balance, the consumption performance of MpDA on GSH was systematically verified. Generally, GSH can react with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to produce a yellow compound whose absorption peak at 405 nm, which can be used as an indicator to monitor the levels of GSH. As shown in Fig. 2d, the consumption of GSH was quantitated after adding different concentrations of MpDA into a fixed amount GSH. It could be seen that the concentration of GSH decreased significantly with the increase of MpDA concentration, and 100 µg/mL of MpDA could deplete approximately 7 mM of GSH. In addition, we detected the UV-Vis spectra of MpDA after its reaction with GSH at different times. The results in Fig. 2e showed that the relative absorbance intensity of MpDA decreased gradually with the passage of time in the presence of GSH. These results indicated that MpDA could react with GSH and consume it effectively, which was a potential antioxidant scavenger.
to impair the antioxidant defense system of cancer cells. Based on the evidence of CSH depletion by MpDA, we further analyzed the consumption capacity of MpDA on intracellular GSH. As shown in Fig. 2f, after MG63 cells incubated with different concentrations of MpDA, intracellular GSH levels decreased obviously compared with the control group. At the same time, the relative amount of intracellular GSH dropped gradually as the increase of MpDA concentration, revealing that MpDA were able to consume GSH in tumor cells.

**Anti-tumor mechanism of Vc-Ca**

Here, the cytotoxicity mechanism of Vc-Ca on MG63 cells *in vitro* was explored. First, we investigated the effects of different concentrations of Vc-Ca on the viability of MG63 cells using the MTT assay. As shown in Fig. 3a, the killing effect of Vc-Ca on tumor cells was dose-dependent, and the cell survival rate was only 53.6% when the concentration of Vc-Ca was 5 mM. It has been proved that the anti-tumor effects of Vc is mainly due to the production of H$_2$O$_2$ by Vc autooxidation[13]. To verify this inference, MG63 cells were co-incubated with Vc-Ca of various concentrations for different periods, and the extracellular H$_2$O$_2$-producing level was furtherly detected. The results in Fig. 3b showed that the amount of H$_2$O$_2$ produced in extracellular fluid was positively correlated with the concentration of Vc-Ca. It was worth noting that H$_2$O$_2$ level reached the maximum value when cells were treated by Vc-Ca for 30 min, whereas the H$_2$O$_2$ production gradually decreased with the extension of treatment time. Further, when antioxidant (catalase) was used to withstand the possible generated H$_2$O$_2$, the cytotoxicity of Vc-Ca was significantly reduced, and the cell survival rate was still higher than 90% even at a relatively high concentration of 5 mM (Fig. 3c).

Since calcium overload induced by calcium ions can also induce programmed cell death[33]. Subsequently, we further verified whether calcium ions in Vc-Ca played a role in inducing cell death. BAPTA-AM is a transmembrane calcium chelating agent. As shown in Fig. 3d, when MG63 cells were treated with different concentrations of Vc-Ca in the presence of BAPTA-AM, cell viability still decreased with the increase of Vc-Ca concentration, which showed no significant difference from that without BAPTA-AM. This result indicated that calcium ions might not contribute to the process of Vc-Ca-induced cell death, while extracellular H$_2$O$_2$ produced by AscH$^-$ played a constructive role. To further confirm the above conclusion, a series of comparative experiments were set up. As presented in Fig. 3e, when MG63 cells were treated with catalase or BAPTA-AM alone, there was no obvious difference in cell viability compared with the blank control group. The cell viability of MG63 cells incubated with Vc-Ca decreased significantly, while the cell survival rate was only slightly increased after the addition of BAPTA-AM. Definitely, after the cells were treated with Vc-Ca and catalase simultaneously, the metabolic activity was much higher than that of the Vc-Ca group, which was the same as the above experimental results.

*In Vitro synergistic anticancer effect*
Prior to study the anticancer performance of MpDA/Vc-Ca/PCM, we first evaluated the biocompatibility of MpDA/Vc-Ca/PCM by selecting the mouse embryonic fibroblast cell line NIH-3T3 as a model cell type. As shown in Fig. 4a, even at the concentration of 200 µg/mL, NIH-3T3 cells treated with MpDA/Vc-Ca/PCM still exhibited high cell viability, and the survival rate was higher than 90%. This result indicated that MpDA/Vc-Ca/PCM had good cytocompatibility and no cytotoxicity effect on normal cells. To evaluate the anticancer capacity of MpDA/Vc-Ca/PCM composite nanoplatform in vitro, the cell viability of MG63 cells was further performed. As illustrated in Fig. 4b, the cell survival rate of MG63 cells treated with MpDA nanoparticles of different concentrations alone exceeded 100%, suggesting the excellent biocompatibility of MpDA. In this regard, MpDA has been widely reported as an ideal therapeutic platform for drug delivery. When irradiated with 808 nm laser, the cell viability of MG63 cells incubated with MpDA (MpDA + NIR) was significantly lower than that of the group without irradiation at the same concentration, indicating that the temperature rise caused by photothermal therapy could effectively kill cancer cells. Remarkably, compared with single photothermal therapy (MpDA + NIR), MpDA/Vc-Ca/PCM showed enhanced cell killing effect under laser irradiation, with a sharp decrease in cell viability under the same conditions. This was mainly because the photothermal effect of MpDA increased the temperature of the whole system, leading to the melting of PCM and thus triggering the controlled release of Vc-Ca. The synergistic effect of photothermal and Vc-Ca oxidation could effectively boost the antitumor efficacy of the composite nanoplatform. In addition, it was worth mentioning that MG63 cells still maintained good survival rate even at a higher concentration of MpDA/Vc-Ca/PCM without light treatment. This was attributed to the fact that PCM was solid in the absence of light, which prevented the release of Vc-Ca.

In general, the cell membrane damage caused by either apoptosis or necrosis would induce lactate dehydrogenase (LDH) releasing from the cytoplasm into the culture medium [34]. Here, the LDH activity in the corresponding culture medium of MG63 cells treated in different ways was detected. As shown in Fig. 4c, the LDH activity of MpDA and MpDA/Vc-Ca/PCM groups were not significantly different from that of blank control group. However, the LDH activity matching with Vc-Ca group had an obvious elevation, manifesting that Vc-Ca had toxicity against MG63 cells. Compared with other groups, LDH activity of laser treatment group was significantly increased. Especially, the MpDA/Vc-Ca/PCM + NIR group showed highest LDH release, indicating that PTT and Vc-Ca had synergistic enhancement of cytotoxicity.

The outcome of combination treatment was further evaluated using fluorescence imaging. Figure 4d showed the number and morphology of MG63 cells incubated with different materials were obviously different. MG63 cells without any treatment (control group) spread adequately and F-actin was well-organized. After co-culture with MpDA, MG63 cells showed better elongation and stronger cytoskeleton development than control group, mainly due to the obvious promotion of MpDA on cell adhesion and spreading. In contrast, cells in the Vc-Ca treatment group displayed decreased cell density and condensed F-actin, indicating that Vc-Ca was not conducive to the tumor cells. For the MpDA with laser exposure, cells also showed a poor cell spread phenotype, which was mainly associated with the photothermal heating of MpDA. In particular, when MG63 cells were cultured with the MpDA/Vc-Ca/PCM nanoparticles under laser irradiation, a more pronounced collapse of cell structures was observed, confirming the reinforced cytotoxicity of photothermal and ROS therapy to cause severely damage to the cytoskeleton.
As expected, in the presence of MpDA/Vc-Ca/PCM nanoparticles but with no laser exposure, the cells still diffused well due to the limited release amount of Vc-Ca from MpDA/Vc-Ca/PCM without irradiation. Additionally, this observation was also confirmed using live/dead staining based on calcein-AM and ethidium homodimer-1 dyes, respectively. As shown in Fig. S4 (Supporting Information), compared with the blank control group, there was a lot of dead cells (indicated by red fluorescence) in the Vc-Ca group, which was mainly attributed to the cytotoxic effect of Vc-Ca. However, almost no cell death could be observed in MpDA and MpDA/Vc-Ca/PCM groups, indicating that MpDA and MpDA/Vc-Ca/PCM did not have any killing ability on MG63 cells in the absence of NIR irradiation. By comparison, MpDA/Vc-Ca/PCM and MpDA-treated cells exhibited a large number of red fluorescence after NIR irradiation. In particular, all cells in the MpDA/Vc-Ca/PCM + NIR group showed a state of death, additionally demonstrating that the combination of photothermal and oxidative therapy provided a synergistic effect to more effectively kill cancer cells than the monotherapy model.

**ROS generation and \( \Delta \psi_m \) detection**

In order to explore the feasibility of MpDA/Vc-Ca/PCM in enhancing ROS generation in cancer cells, MG63 cells with different treatments were stained with a ROS fluorescent probe, dichlorofluorescein diacetate (DCFH-DA). As shown in Fig. 5a, almost no green fluorescence signal was observed in control, MpDA and MpDA + NIR group. However, MG63 cells incubated with Vc-Ca appeared green fluorescence, indicating efficient ROS generation triggered by Vc-Ca. In contrast, the green fluorescence intensity was enhanced in the cells treated with MpDA/Vc-Ca/PCM + NIR, suggesting that MpDA protected the exogenous ROS produced by Vc-Ca by consuming intracellular GSH. As expected, only a weak green fluorescence signal was detected in MpDA/Vc-Ca/PCM group without irradiation; and this small amount of ROS might be generated by Vc-Ca adsorbed on the surface of MpDA/Vc-Ca/PCM nanoparticles.

To detect mitochondrial damage induced by ROS, we further analyzed the changes of mitochondrial membrane potential (\( \Delta \psi_m \)) using JC-1 as a probe. JC-1 is a cationic mitochondrial selective dye that produces red fluorescence (J-aggregate) at high membrane potential and green fluorescence (J-monomer) at low membrane potential. Therefore, the switch of JC-1 from red fluorescence to green fluorescence indicates the decrease in \( \Delta \psi_m \). As shown in Fig. 5b, the mitochondria of MG63 cells in MpDA/Vc-Ca/PCM group simultaneously showed green and red fluorescence when there was no irradiation, indicating the preservation of \( \Delta \psi_m \). For comparison, the MpDA/Vc-Ca/PCM group was irradiated with 808 nm laser at the power of 1 W/cm\(^2\), the red fluorescence significantly decreased while the green fluorescence relatively increased, suggesting the loss of \( \Delta \psi_m \).

**In Vivo thermal imaging**

In order to investigate the photothermal effect of MpDA/Vc-Ca/PCM *in vivo*, we used an infrared thermal imager to monitor the temperature changes of tumor-bearing mice under different treatments. As shown in Fig. 6a and 6b, after 808 nm (1 W/cm\(^2\)) laser irradiation for 10 min, the temperature of PBS + NIR group and Vc-Ca + NIR group only increased to 39.2°C and 37.7°C after intratumoral injection, respectively,
indicating that NIR light irradiation alone cannot generate enough heat \textit{in vivo} in the absence of photothermal conversion materials. In contrast, the tumor site temperature of MpDA + NIR group and MpDA/Vc-Ca/PCM + NIR group increased rapidly to 60.2°C and 59.1°C with irradiation time, respectively. This result indicated that the photothermal effect of MpDA/Vc-Ca/PCM was not affected by the load of Vc-Ca and PCM, which was suitable for photothermal therapy \textit{in vivo}. As expected, tumor temperature of MpDA/Vc-Ca/PCM group was basically stable at 33.5–35.5°C due to the lack of NIR irradiation.

\section*{In Vivo tumor therapeutic efficacy}

The antitumor effect of MpDA/Vc-Ca/PCM nanoplatform \textit{in vivo} was investigated on 143B tumor-bearing BALB/c mice. When the tumor grew to approximately 100 cm$^3$, the mice were randomly divided into five groups. The therapeutic materials were injected into the tumor on day 0, day 3, and day 6, and irradiated with 808 nm NIR laser for 10 min, respectively (Fig. 7a). From the first treatment, changes in tumor volume were recorded every two days during the 14-day course of treatment. As shown in Fig. 7c, tumor volume in the PBS + NIR group and the MpDA/Vc-Ca/PCM group without laser irradiation continued to increase rapidly with increasing days. In comparison, tumor growth was significantly inhibited in the Vc-Ca + NIR, MpDA + NIR and MpDA/Vc-Ca/PCM + NIR groups. Remarkably, the MpDA/Vc-Ca/PCM + NIR group showed a stronger inhibitory effect on tumor growth compared with Vc-Ca + NIR and MpDA + NIR groups, confirming that localized combination therapy of photothermal and ROS inhibit tumor multiplication more effectively than monotherapy. In addition, from the photos and weights of the tumors in each treatment group in Fig.s 7b and 7d, it could also be seen intuitively that the volume and weight of tumors in the MpDA/Vc-Ca/PCM + NIR group were the smallest. Comparatively, the MpDA/Vc-Ca/PCM group without laser exposure showed no suppression of tumor growth compared with the Vc-Ca + NIR group, mainly because the PCM was solid when no light was applied, which effectively prevented the release of Vc-Ca. The weight of the mice was weighed during the whole experiment, and relevant data were shown in Fig. 7e. No distinct fluctuation of body weight was detected among the groups, indicating insignificant side effects in each treatment sample.

To further evaluate the antitumor activity, histological hematoxylin/eosin (H&E) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) analyses were applied to tumor tissues from different group (Fig. 7f). From the H&E staining images, a large number of tumor cells were found in the PBS + NIR and the MpDA/Vc-Ca/PCM without laser irradiation groups, and the nuclei of tumor cells were dark purple and fusiform. In marked contrast, the tumor cells in the other groups showed different degrees of reduction and damage, particularly for the MpDA/Vc-Ca/PCM + NIR group, which mainly indicated as broken cell membrane structure and nuclear lysis. Similarly, the TUNEL assay also showed that the Vc-Ca + NIR, MpDA + NIR and MpDA/Vc-Ca/PCM + NIR groups had remarkable antitumor effects, especially the MpDA/Vc-Ca/PCM + NIR exhibited a mass of apoptotic tumor cells as evidenced by the largest area of green fluorescence. Moreover, an immunohistochemical analysis was further performed to evaluate the expression of GPX4 (a typical indicator of oxidative stress). The GPX4 presented normal expression levels in the PBS + NIR, MpDA + NIR and MpDA/Vc-Ca/PCM groups, while this indicator had significantly upregulated in Vc-Ca + NIR and MpDA/Vc-Ca/PCM + NIR groups.
Discussion

Due to the limitations of conventional cancer-treatment methods used in clinical practice (e.g., surgery, chemotherapy, and radiotherapy), it is still necessary to continuously develop new treatment strategies to effectively fight against cancer[35]. In this study, we successfully prepared a biocompatible and versatile nanoplatform based on MpDA nanoparticles and a phase-change material for delivering Vc-Ca, simultaneously enabling combination therapy of hyperthermia, ROS generation, and suppression of tumor antioxidant capability.

The large specific surface area and ordered mesoporous structure of MpDA provides a possibility for efficient loading of drugs. The present research demonstrated the optimal Vc-Ca loading efficiency of MpDA was 42.33%, indicating that sufficient Vc-Ca could be transported into tumor cells for effective ROS therapy. PCM based on fatty acids and their derivatives have a sensitive response to temperature changes[36]. Thus, drug release can be precisely controlled by the change of molecular mobility of PCM during solid-liquid phase transition. In this study, the PCM acted as a gating material for Vc-Ca, which existed in a solid state at low temperature to prevent Vc-Ca leakage; however, when the temperature exceeded 39 °C, the Vc-Ca trapped in the solid matrix of the PCM was released rapidly along with the melt of PCM. This was in good agreement with previous studies[29, 33]. Based on the results of photothermal performance detection of MpDA, it was suggested that a thermal-responsive drug delivery system has been successfully constructed.

In recent years, MpDA has been widely reported as a drug carrier and photothermal agent, but its ability to regulate redox homeostasis has received little attention. The elevated antioxidant defense system in cancer cells can lead to resistance to treatments involving ROS, which inevitably compromises the efficacy of ROS therapy[37–39]. In this study, the consumption performance of MpDA on GSH was methodically investigated. Figure 2d exhibited dramatically less GSH in MpDA solution; moreover, a gradually decreased absorbance intensity of MpDA was observed over time in the presence of GSH (Fig. 2e). These results indicated that MpDA could interact with GSH and reduce GSH levels. In biological systems, GSH is a major scavenger of ROS. With the increase of MpDA concentration, the relative content of intracellular GSH decreased gradually (Fig. 2f), which further revealed that MpDA could consume the GSH in tumor cells. From these results, it was concluded that the MpDA nanoplatform could significantly enhance ROS production by consuming GSH, which will provide the possibility of efficient ROS treatment.

Early studies have confirmed that Vc is a potential chemotherapy drug, showing selective toxicity to a variety of cancer cells[16, 18]. Herin, we conducted an in-depth discussion on the anti-tumor mechanism of Vc-Ca. The MTT result (Fig. 3b) revealed that the extracellular $\text{H}_2\text{O}_2$-producing was positively correlated with the concentration of Vc-Ca. However, when the antioxidant was used to strive against the possible produced $\text{H}_2\text{O}_2$, the cytotoxicity of Vc-Ca observably improved (Fig. 3c) compared to that of no catalase addition (Fig. 3a). These results confirmed that increased $\text{H}_2\text{O}_2$ levels in cells were the main cause of cytotoxicity of Vc-Ca, which was consistent with previous studies[30].
As mentioned before, inhibition of ROS feedback scavenging by modulating redox homeostasis during ROS treatment would amplify oxidative stress\[40, 41\]. With the proof of GSH, consumption by MpDA in hand, we investigated the feasibility of ROS promotion in cancer cells by MpDA with a ROS fluorescent probe (DCFH). The fluorescence images in Fig. 5a showed that upon incubating the cells with Vc-Ca green luminescence appeared, suggesting the efficient ROS generation triggered by Vc-Ca. However, irradiation treatment led to a brighter green fluorescence incubated with MpDA/Vc-Ca/PCM, thanks to the ROS protection through GSH depletion by MpDA. All the above results manifested that MpDA/Vc-Ca/PCM was an ideal platform for tumor therapy, which could amplify intracellular ROS levels by regulating the redox balance, thus achieving enhanced ROS therapy and photothermal combination therapy. The synergistic therapeutic effect could be sufficiently demonstrated, as evidenced by decreased cell activity (Fig. 4b), increased cell toxicity (Fig. 4c) and depressed cytoskeleton development (Fig. 4d). Mitochondria are known to be the best-characterized sources and primary target of intracellular ROS\[42, 43\]. Increased intracellular ROS levels can adversely affect mitochondrial membrane integrity and associated transmembrane potential\[44\]. Notably, compared with the group without light exposure, mitochondria of MG63 cells in the MpDA/Vc-Ca/PCM group under light irradiation presented significant loss of $\Delta \psi_m$ (Fig. 5b). The reduction of $\Delta \psi_m$ has been proved to be a landmark event in the early stage of cell apoptosis\[45\]. It could be concluded that MpDA/Vc-Ca/PCM nanoplatform with synergistic ROS therapy and hyperthermia was able to lead to mitochondrial dysfunction, thereby inducing apoptosis of cancer cells.

Guided by the results of in vitro anticancer effect, the tumor therapeutic efficacy of MpDA/Vc-Ca/PCM nanoplatform in vivo was further evaluated in a 143B tumor-bearing BALB/c mice model. These experimental results in Fig. 7 confirmed that MpDA/Vc-Ca/PCM had a synergistic effect of amplified oxidation and hyperthermia under laser irradiation to effectively inhibit tumor growth in vivo. GPX4 is a typical indicator of oxidative stress\[46\]. Compared with other groups, the GPX4 expression level in the Vc-Ca + NIR group and MpDA/Vc-Ca/PCM + NIR group was significantly up-regulated (Fig. 7f), suggesting that oxidation damage occurred in tumor tissues due to the presence of Vc-Ca, evidencing the potential of Vc-Ca for oxidation therapy in vivo, consistent with the previous reports\[13\]. Taken together, such an ingenious nanoplatform with the dual therapy of hyperthermia and oxidation would be a powerful weapon to induce tumor ablation, showing great potential in cancer synergetic therapy.

**Conclusions**

We have developed a MpDA/Vc-Ca/PCM intelligent nanoplatform for combination cancer treatment. Specifically, Vc-Ca was encapsulated with a PCM in the mesoporous of MpDA for photothermally triggered release by melting the PCM with NIR irradiation. More importantly, the nanoplatform could consume intracellular GSH and amplify Vc-Ca-mediated ROS therapy by regulating the redox balance. In vitro and in vivo experiments have confirmed that the boosted ROS combined with local hyperthermia could act synergistically to effectively kill cancer cells. Collectively, the MpDA/Vc-Ca/PCM nanosystem
developed in this study was a promising multi-functional platform for cancer treatment with reduced adverse impacts and side effects.

**Abbreviations**

MpDA: Mesoporous polydopamine; PCM: Phase-change material; Vc: Vitamin C; Vc-Ca: Calcium ascorbate; ROS: Reactive oxygen species; TME: Tumor microenvironment; pDA: Polydopamine; NIR: Near-infrared; PTT: Photothermal therapy; GSH: Glutathione; SEM: Scanning electron microscope; TEM: Transmission electron microscope; FTIR: Fourier transform infrared spectroscopy; LDH: Lactate dehydrogenase; DCFH-DA: Dichlorofluorescein diacetate; H&E: Histological hematoxylin/eosin; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labeling.

**Declarations**

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/XXX.

**Additional file 1.** Figures S1–S4; particle size distributions of MpDA and MpDA/Vc-Ca/PCM; standard curves of Vc-Ca; infrared thermal images of MpDA/Vc-Ca/PCM; live/dead stain images of MG63 cells.

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**Authors’ contributions**

MZ designed the experiments, analyzed data, and wrote the manuscript. SR and XY did cell culture experiments. JZ characterized the materials. XS and WS supervised the research. ZZ reviewed the manuscript and provided funding support. All authors read and approved the final manuscript.

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**Availability of data and materials**

Not applicable.

**Ethics approval and consent to participate**
All animal experiments were approved by the Animal Ethics Committee of Wuhan Servicebio Technology Co., Ltd (SYXK(W)2015-0693). Twenty nude mice were purchased from the Beijing Vital River Laboratory Animal Technology Co., Ltd. (China; animal quality certificate NO.110011211108440457).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References


Scheme 1

Scheme 1 is available in the Supplementary Files section.

Figures
Figure 1

a,b) SEM images of MpDA at different magnifications. c,d) SEM images of MpDA/Vc-Ca/PCM at different magnifications. e,f) TEM images of MpDA and MpDA/Vc-Ca/PCM. g) TEM elemental mappings of C, N, and Ca of MpDA/Vc-Ca/PCM. h) FT-IR spectra of different samples. i) UV-vis absorption spectra of different samples. j) The cumulative release curves of Vc-Ca from MpDA/Vc-Ca/PCM at 37 °C and 50 °C, respectively.
Figure 2

Measurements of photothermal and GSH depletion properties of MpDA. a) Temperature changes of MpDA at various concentrations under irradiation of 808 nm laser irradiation (1 W/cm², 10 min). b) Temperature changes of MpDA (100 μg/mL) under 808 nm laser irradiation with different power densities. c) Photothermal cycle stability test of MpDA. d) GSH concentration after treating with different concentrations of MpDA. e) UV-vis absorption spectra of MpDA after reacting with GSH for different time. f) The relative levels of intracellular GSH in MG63 cells treated with different concentrations of MpDA.
Figure 3

Study on the cytotoxic mechanism of Vc-Ca. a) Relative viabilities of MG63 cells after incubation with different concentrations of Vc-Ca. b) H2O2 formation in extracellular fluid of MG63 cells treated with different concentrations of Vc-Ca at different time. c) Relative viabilities of MG63 cells after incubation with Vc-Ca and catalase. d) Relative viabilities of MG63 cells after incubation with Vc-Ca and BAPTA-AM. e) Relative viabilities of MG63 cells after different treatments.
Figure 4

a) Relative viabilities of NIH-3T3 cells after incubation with different concentrations of MpDA/Vc-Ca/PCM in the absence of light irradiation. b) Relative viabilities of MG63 cells after incubation with different concentrations of MpDA or MpDA/Vc-Ca/PCM in the absence or present of light irradiation. c) Detection of LDH activity in culture medium of MG63 cells after different treatments. d) F-actin morphology of MG63 cells after different treatments.
Figure 5

a) Detection of intracellular ROS in MG63 cells after different treatments. b) Fluorescence microscopy images of JC-1-stained MG63 cells after treated with MpDA/Vc-Ca/PCM in the absence or present of light irradiation. The red and green fluorescence colors indicate the preservation and loss of mitochondrial membrane potential, respectively.
Figure 6

a) Infrared thermal images of 143B tumor-bearing mice intratumorally injected with PBS, Vc-Ca, MpDA, and MpDA/Vc-Ca/PCM with NIR laser irradiation (1 W/cm², 10 min) or not. b) Curve of tumor temperature rose with irradiation time.
Figure 7

In vivo tumor therapeutic effect of MpDA/Vc-Ca/PCM. a) Schematic representation of the in vivo therapeutic experiment. b) Photographs of representative tumors dissected from each group at 14th day. c) Tumor growth curves of 143B tumor-bearing mice during the administration of different treatments. d) Tumor weight after various treatments at 14th day. e) Body weight changes of 143B tumor-bearing mice
during the administration of different treatments. f) Histochemical analyses (H&E, TUNEL, GPX4) of
tumor tissues harvested from mice after different treatments at 14th day.

Supplementary Files

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