In-situ oxygen-generation nanoplatform with dual amplification effect for combined chemo-photodynamic therapy

Qingcheng Song  
Hebei Medical University Third Affiliated Hospital

Xiangtian Deng  
Nankai University School of Medicine

Wenbo Yang  
Wuhan Union Hospital

Yiran Zhang  
Nankai University School of Medicine

Junyong Li  
Shijiazhuang People's Hospital

Xin Xing  
Hebei Medical University Third Affiliated Hospital

Wei Chen  
Hebei Medical University Third Affiliated Hospital

Weijian Liu  
Wuhan Union Hospital

Hongzhi Hu  
Wuhan Union Hospital

Yingze Zhang (✉ zhangyz2021@163.com)  
Hebei Medical University Third Affiliated Hospital

Research

Keywords: Dual-amplification, Hypoxia, Catalase, MTH1, 8-oxo-dGTP, Photodynamic therapy, Chemotherapy

Posted Date: September 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-889561/v1

License: ✐️ This work is licensed under a Creative Commons Attribution 4.0 International License. 
Read Full License
Abstract

Background

Photodynamic therapy (PDT) is a promising method for cancer treatment because of its advantages such as easy operation, good targeting, minimal side effects, low systemic toxicity and less invasiveness. However, the hypoxic microenvironment within the tumor significantly inhibited the therapeutic effect of PDT. The development of targeted nanoplatform for regulating hypoxia microenvironment is an important method to give full play to the therapeutic effect of PDT.

Methods

In this study, we designed and prepared a novel chemo-photodynamic therapy nanoplatform, which can continuously catalyze the decomposition of H$_2$O$_2$ in tumors to generate oxygen (O$_2$) to enhance the therapeutic effect of PDT, resulting in DNA damage, while releasing MTH1 inhibitors in tumor cells to inhibit the repair process of DNA damage caused by PDT.

Results

In our work, a simple one-step reduction approach was applied to enable platinum nanoparticles (Pt NPs) growth in situ in the nanochannels of mesoporous silica nanoparticles (MSNs). After physical encapsulation of photosensitizer chlorin e6 (Ce6) and MTH1 inhibitor TH588, the drug loading nanoplatform was modified with an arginine-glycine-aspartic acid (RGD) functionalized liposome shell, resulting in the fabrication of multifunctional nanoplatform MSN-Pt@Ce6/TH588@Liposome-RGD (MPCT@Li-R) with dual amplification effect and achieve the purpose of chemo-photodynamic therapy.

Conclusions

Our study provides a new strategy for PDT to ablation tumor cells by damaging the DNA of tumor nucleus and mitochondria, meanwhile inhibiting the repair process after the damage.

Background

Cancer, as a malignant disease that seriously threatens human health, is a major cause of morbidity and mortality worldwide. Considering the high fatality rate and great harmfulness of cancer, it is particularly urgent to develop more reasonable and effective treatment approaches for cancer[1, 2]. As a non-invasive treatment strategy, photodynamic therapy (PDT) can be utilized for the treatment of many types and sites of cancer. The good therapeutic effect and the possibility of combining with other therapeutic methods make it become one of the hot spots in the field of cancer therapy[3]. The principle of PDT is that the photosensitizer that accumulates in tumor cells can be stimulated by a laser with the appropriate
wavelength and produces reactive oxygen species (ROS) in the presence of \( \text{O}_2 \)[4–7]. Therefore, PDT is the consequence of the interaction of three essential elements: light, photosensitizer and \( \text{O}_2 \). However, the hypoxic microenvironment caused by the uncontrolled proliferation of tumor cells severely limits the conversion efficiency of \( \text{O}_2 \) to ROS[8–10]. Moreover, PDT-induced microvascular collapse further impairs the \( \text{O}_2 \) supply, leading to a further deterioration of the hypoxic environment. Consequently, the vicious cycle formed by local \( \text{O}_2 \) consumption and \( \text{O}_2 \) supply disruption severely limits the efficiency of PDT, leading to incomplete ablation of tumors and metastasis[11, 12]. Therefore, it is an urgent task for cancer treatment to develop therapeutic methods to overcome tumor hypoxia and improve PDT efficiency.

At present, various strategies have been developed to alleviate hypoxia in tumors. One approach is to construct NPs with an \( \text{O}_2 \) donor as the functional center for the targeted delivery of \( \text{O}_2 \) for PDT therapy. For example, Tang et al. innovatively utilized red blood cell (RBC) as \( \text{O}_2 \) carrier to achieve the RBC-facilitated PDT therapy, which effectively solved the problem of tumor hypoxia[13]. However, due to the need for repeated light irradiation during PDT treatment, additional requirements have been placed on the design of nanocarriers with high oxygen capacity. In addition, uncontrolled irradiation dose and excessive \( \text{O}_2 \) carrier can generate abundant ROS, causing unnecessary damage to peripheral tissue surrounding the tumor[14, 15]. Another popular approach is to utilize the catalytic properties of nanoenzymes to build nanoplatform that can generate \( \text{O}_2 \) in situ within the tumor[16]. Thanks to the abundant expression of hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) in tumor cells, a variety of nanoenzymes/catalysts such as manganese dioxide (\( \text{MnO}_2 \)), copper oxide (CuO) or catalase have been constructed that can catalyze the decomposition of \( \text{H}_2\text{O}_2 \) into \( \text{O}_2 \) to alleviate the hypoxia problem inside the tumor[17–20]. However, this method also has problems such as narrow pH response range and unstable catalyst and so on.[21, 22].

In recent years, Pt NPs have been regarded as the ideal nanoenzymes for catalyzing \( \text{H}_2\text{O}_2 \) decomposition due to their antioxidant properties, excellent catalytic efficiency, durable catalytic and good biocompatibility[23, 24]. More importantly, Pt NPs has excellent stability and will not be decomposed in the tumor slight acidic and \( \text{H}_2\text{O}_2 \) micro-environment during the catalytic process like other catalysts (\( \text{MnO}_2 \))[24]. However, Pt NPs are usually small in volume (< 10nm), which makes them easy to be cleared by the urinary system, resulting in a short residence time in the body[25]. MSN has many attractive properties, such as high specific surface area, adjustable pore size, high porosity, excellent biocompatibility and easy surface modification, which make it an ideal nanovehicle for loading other drugs or reagents for the purpose of chemotherapy (CHT), photothermal therapy (PTT) or PDT combination therapy[26, 27]. It is encouraging that the inner surface of MSN nanochannels modified by abundant amino groups (\(-\text{NH}_2\)) can easily coordinate with Pt ions by substituting chloride ligand, and then achieve the purpose of in situ growth of Pt NPs after NaBH\(_4\) reduction reaction[28, 29]. In addition, due to the presence of plentiful electronegative -SH on the outer surface of silica and positively charged \(-\text{NH}_2\) in the inner wall of mesopore, the negatively charged PtCl\(_6^{2-}\) were driven into the mesopore under electrostatic action.
ROS are the consequence of PDT treatment and simultaneously become the cause of DNA mispairing[30]. High tension ROS can directly lead to DNA damage, or cause the oxidation of bases in the deoxynucleoside triphosphate (dNTP) pool, leading to DNA mispairing, mutations, and cell necrosis[31]. It has been reported that guanine can be oxidized by ROS to generate 8-oxoguanine, which can be further converted to 8-oxo-2'-deoxyguanosine triphosphate (8-oxo-dGTP), leading to nuclear or mitochondrial DNA mispairing[32–34]. The MTH1 protein can sanitize the oxidized dNTP pool by converting 8-oxo-dGTP to 8-oxo-dGMP, thus avoiding the integration of these oxidized nucleotides into DNA. MTH1 is unnecessary for normal cells, but it is closely related to the migration and metastasis behavior of tumor cells, so it is of great significance for the survival of tumor cells and the reduction of tumor-related damage[35]. Therefore, specific chemotherapy (CHT) targeting MTH1 is a very promising anticancer approach. Moreover, the therapeutic effect of CHT can be amplified by ROS-induced intracellular base oxidation. Predictably, amplified PDT can collaborate with MTH1 inhibitors to generate large amounts of 8-oxo-dGTP, resulting in DNA structure destruction and cell death.

Based on current background knowledge, we tactfully and innovatively designed a dual amplification therapeutic efficacy nanotherapy platform (MSN-Pt@Ce6/TH588@Li-R) in which MSN was taken as the template, MSN-Pt formed by in-situ growth of Pt NPs in MSN nanochannels was used as the core, after physical encapsulation of Ce6 and TH588, the RGD functionalized liposome shell was modified on the periphery to effectively prevent the premature release of Ce6 and TH588 in the blood circulation[36, 37]. NPs can reach the tumor site through the active targeting by RGD and the enhanced permeability and retention (EPR) effect. After being internalized through membrane fusion, the liposome layer was destroyed and Ce6 and TH588 were subsequently released into the cytoplasm. Pt NPs exposed to the H₂O₂ microenvironment of tumor cells catalyzed the decomposition of H₂O₂ to generate O₂. Under the irradiation of 660nm wavelength laser, the continuously generated O₂ interacts with Ce6 to produce sufficient ROS, which effectively leads to the occurrence of base mispairing. Simultaneously, the release of TH588 further blocked the repair pathway of oxidized nucleotides. In conclusion, the catalase activity of Pt NPs amplifies the therapeutic effect of PDT, while the presence of TH588 further amplifies the effect of PDT-induced DNA damage, and finally triggers the end point event: p53-mediated apoptosis in the reciprocal progression of the two above processes.

**Materials And Methods**

**Materials**

Chlorin e6 (Ce6), RGD peptide, 3-aminopropyltriethoxysilane (APTS), triethanolamine (TEA), cetyltrimethylammonium chloride (CTAC, 25 wt%), 1,2 distearoyl-sn-glycero-3–phosphoethanolamine-N-[amino(polyethylene-glycol)] with a PEG length of 2000 (DSPE-PEG2K-NH₂), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), cholesterol were purchased from Xi’an ruixi Biological Technology Co., Ltd. Hydrogen hexachloroplatinate (IV) hexahydrate (H₂PtCl₆·6H₂O, 99%), sodium borohydride (NaBH₄) were purchased from Beijing Hongke Chemical Products Co. Tetraethoxysilane (TEOS) and sodium hydroxide (NaOH) were purchased from Aladdin (Shanghai China). TH588 was purchased from Selleckchem.
(Houston, TX, USA). All other chemical reagents were analytical grade and do not require further purification.

**Preparation of MSN-NH<sub>2</sub>**

15g CTAC and 0.6g TEA were dissolved in 150ml water and incubated in a trimethyl silicone bath at 80°C, followed by intensive magnetic stirring at 200 rpm for 1 h until completely dissolved. Subsequently, 10ml of TEOS was added to the reaction system drop wise at the rate of two seconds per drop, the reaction was carried out at 80°C for 2h. After cooling, the precipitation was collected by centrifugation at 12000rpm for 10min, followed by alternately washed with water and ethanol for 3 times. The removal process of the template was further performed by dissolving the reaction product in an ethanol/hydrochloric acid solution and carried out at 70°C overnight. The MSN were centrifuged at 12000rpm for 10min, then washed alternately with ddH<sub>2</sub>O and ethanol for 3 times, and dissolved in water for later use. Finally, 50μL APTS and 100μl glacial acetic acid were added to the MSN aqueous solution and stirred at room temperature for 24h. After centrifugation at 12000rpm for 15min, precipitation was collected and vacuum freeze-dried to obtain MSN-NH<sub>2</sub>.

**In situ growth of Pt NPs in nanochannels**

150mg MSN-NH<sub>2</sub> was dispersed in 20ml ddH<sub>2</sub>O, and 20mL H<sub>2</sub>PtCl<sub>6</sub>·6H<sub>2</sub>O aqueous solution was added into the NPs suspension and stirred at room temperature for 1h. NaBH<sub>4</sub> was then added to the reaction system and stirred for another 1h. NPs were centrifuged at 12000rpm for 10min, washed with ddH<sub>2</sub>O for 3 times and dried in vacuum to obtain MSN-Pt.

**Synthesis of MSN-Pt@Ce6/TH588**

For successful loading of Ce6, MSN-Pt (75mg) was dispersed in phosphate buffered saline (PBS), 5mg Ce6 (dissolved in 750μl DMSO solution) was added and stirred at room temperature for 24h in the dark. The mixture was centrifuged at 12000rpm for 20min, the supernatant was collected, and the precipitation was washed with PBS for 3 times. The loading process of TH588 is similar to that of Ce6, MSN-Pt/Ce6 75mg was dispersed in PBS, 7.5mg TH588 (20mg/mL,375μl DMSO solution) was added and stirred overnight at room temperature. The precipitation and supernatant were collected after centrifugation at 12000rpm for 20min. The absorbance of Ce6 and TH588 in the supernatant was detected by fluorescence spectrophotometer to determine the drug loading content and encapsulation efficiencies, respectively.

**Fabrication of DSPE-PEG-RGD**

In order to synthesize the RGD-modified phospholipid (DSPE-PEG-RGD), DSPE-PEG-NHS and RGD (molar ratio 3:1) were co-incubated in DMF solution for 24 h under nitrogen flow at 25°C. The excess RGD and DSPE-PEG-NHS were removed by dialysis (MWCO:3500) for 48 hours to achieve the purpose of
separation and purification of the products. Finally, the products were freeze-dried and stored at -80°C for later use.

**Synthesis of MSN-Pt@Ce6/TH588@Li-R, MSN-Pt@Ce6/TH588@Li**

Liposome shell was prepared by thin film hydration method[38]. Briefly, cholesterol, DPPC, DSPE-PEG-RGD, DSPE-PEG, and DOTAP (molar ratio: 40:50:3:3:6) were placed in a round flask containing 30 mL chloroform. The organic solvent was removed in a rotary evaporator under a vacuum environment of 40°C and a thin lipid film was obtained at the bottom of the flask. The flask was then dried in a vacuum desiccator for 24 h until the organic solvent completely evaporated. Subsequently, 15ml of deionized water was added to the flask and the liposome sample was obtained by ultrasonic oscillation at 30°C for 15min. The prepared MSN-Pt/CE6/TH588 and liposome sample were dispersed in 25ml PBS and continuously stirred in the dark for 12h. The mixture was then centrifuged at 12000 rpm for 10 min to collect the precipitate (MSN-Pt/CE6/TH588@Li-R). Non-RGD-targeted liposome encapsulated MPCT was synthesized in a similar method as described previously without the addition of DSPE-PEG-RGD. All nanoparticles were re-suspended in saline or PBS for later use.

**Characterization**

The particle size and morphology of NPs at each stage of synthesis process were characterized by transmission electron microscopy (TEM, JEOL, JEM F200) and scanning electron microscopy (SEM, ZEISS, Gemini 300). Size distribution, zeta potential and polydispersity index (PDI) of NPs were monitored by Malvern zeta sizer Nano-ZS90. The UV-vis spectra of the samples were obtained by using a UV-vis spectrophotometer (Perkin-Elmer, Lambda Bio40).

**Drug release in vitro**

To explore the drug release kinetics and pH responsive release properties of MPCT@Li-R, two kinds of NPs (MPCT, MPCT@Li-R) with equal amounts were immersed in different aqueous solutions (pH=7.4, 5.0) at room temperature, respectively. The supernatant of the samples was extracted at different time points, and the release amount of TH588 and Ce6 was determined by UV-vis spectrophotometry. All the release tests were repeated three times in parallel and the average of the results were taken.

**Cytotoxicity assay**

The cytotoxicity of MPCT@Li-R NPs was evaluated by Cell Counting Kit-8 (CCK-8) assay. Specifically, HOS cells were seeded into 96-well plates at a density of 5000 cells per well and co-incubated with fresh medium at 37°C in 5% CO₂ for 24h. Next, the cells were treated with a series of increasing concentrations of MPCT@Li-R (0, 6.25, 12.5, 25, 50, 100, 200 μg/ml) for 24h and 48h. Then, 10μl of CCK8 solution was added to each well, and incubate for 2h. The absorbance values at the 450nm test wave were determined using an enzyme immunoassay analyzer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The experiment was performed in quintuplicate.
The PDT-CHT combination treatment efficacy of MPCT@Li-R was monitored by CCK8 assay. Generally, HOS cells were seeded into a 96-well plate (5000 cells per well) and cultured in humidified 5% CO₂ at 37°C for 24 h until completely attached. Subsequently, the cells were washed once with PBS, fresh medium containing a range of concentrations of free Ce6, MPC@Li-R or MPCT@Li-R was then added. After co-incubation with HOS cells for 6 h, the cells were irradiated with 660 nm laser (400 mW cm⁻²) for 10 minutes and incubated for another 24 h. Finally, the HOS cell viabilities were determined by CCK8 method in vitro.

**Calcein AM/PI staining**

Calcein AM/PI staining was used to further investigate the antitumor effect of MPCT@Li-R in vitro. HOS cells were inoculated in 24-well plates (5x10⁴ cells per well) and cultured at 37°C with 5% CO₂ for 24 hours until completely adherent. TH588 (250 μL, 20 μg/ml), Ce6 (250 μL, 20 μg/ml), MPC@Li-R (250 μL, equivalent MSN concentration: 20 μg/ml), MPCT@Li-R (250 μL, equivalent MSN concentration: 20 μg/ml) were added to displace the medium. PBS was added as negative control group. After incubation for 4 h, the treatment group was irradiated with a 660 nm laser with an energy density of 2.5 W cm⁻² for 5 min. Finally, Calcein AM/PI detection solution was added and incubated in dark for 30 minutes at 37°C. After incubation, the staining effect was observed under an inverted fluorescence microscope.

**Hemolysis Assay**

To test the blood biocompatibility of MPCT@Li-R, hemolysis assay was performed. Venous blood was extracted from BALB/c mouse and centrifuged at 8000 rpm for 5 min. The serum was discarded and 2 ml PBS was added to resuspend the red blood cells (RBC). Then, 200 μl RBC suspension was added to 800 μl PBS, in which the concentration of MPCT@Li-R ranged from 12.5-400 μg/ml. Moreover, 200 μl RBC suspension were added to 800 μl PBS and deionized water as negative and positive controls, respectively. The mixture was shaken at 37°C for 2 hours and centrifuged at 12000 rpm for 3 minutes. Finally, 8 treatment groups were photographed, 100 μl supernatant was taken and placed in 96-well plate. The absorbance was measured by a microplate reader (Bio-Rad, Model 550, USA).

**Cellular uptake experiments**

To measure the efficiency of the cellular nanoparticle uptake, Human osteosarcoma HOS cells were cultured in a 24-well plate at 37°C and 5% CO₂ for 24 h. After the complete application, the cells were washed once with PBS. Then, fresh medium containing different formulations were added to incubate for another 12 hours. The cells were then washed with PBS and stained with Hoechst 33342 for 15 min, followed by cell imaging with an inverted fluorescence microscope.

**In vitro catalysis and ROS generation experiment**

MPCT and MPCT@Li-R NPs were dissolved in 4 ml 3% H₂O₂ solution or deionized water, respectively, and co-incubated for 30 minutes followed by detection using HI-2400 dissolved oxygen meter to demonstrate
O₂ generation. Subsequently, we further explored the catalytic performance of MPCT@Li-R. The MPCT@Li-R was resuspended in H₂O₂ solutions of different concentrations (0, 5, 10, 20mM). The oxygen electrode probe was inserted into the solution and the change of O₂ concentration in the solution was measured in real time within 15 min. To test the catalytic stability, H₂O₂ (1mM) solution was repeatedly added to the reaction system every 1h and continued to incubate with MPCT@Li-R, followed by measuring the concentration of H₂O₂.

To detect ROS generation in cancer cells, five groups were set up (control, TH588, Ce6 + laser, MPC@Li-R + laser, MPCT@Li-R + laser). The HOS cells (1×10⁵ per well) were inoculated into 6-well plates and cultured for 24 hours until they adhered completely. Then, different formulations (TH588, Ce6, MPC@Li-R, MPCT@Li-R) were added into the corresponding wells according to the treatment of the above 5 groups. After the cells were incubated at 37°C for another 6 hours, the culture medium was replaced with fresh MEM again. The cells were irradiated with a 660nm, 500mW cm⁻² laser for 5 min, and then co-incubated with the ROS probe: 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) for 30 min, followed by ROS fluorescence imaging.

**Animal and tumor models**

Female BALB/c nude mice (weight 15-19g, age 4-6 weeks) were provided by the Hebei Ex & Invivo Biotechnology Co. Ltd (Shijiazhuang, China). All animal experiments were carried out according to protocols approved by the Experimental Animal Ethics Committee of Hebei Ex & Invivo Biotechnology Co., Ltd. Each mouse was injected with 200μL PBS containing 6×10⁶ HOS cells under the axilla. Vernier caliper was utilized to measure the volume of the tumor according to the formula: 1/2 × (width²) × length. HOS tumor-bearing mice can be used for further experiments after the tumor volume has reached about 80-100mm³.

**In vivo imaging**

MPCT@Li-R NPs (equivalent Ce6 amount :2mg/kg) was injected into tumor-bearing mice through the tail vein. Fluorescence imaging of mice was performed with the IVIS Lumina III imaging system (Perkin Elmer, Caliper Life Sciences, MA) at different time points under gas anesthesia. At the end of 24h fluorescence imaging, the mice were sacrificed, and their tumors and major organs were harvested for in vitro imaging analysis.

**In vivo cancer treatment**

Tumor-bearing mice were randomly divided into 5 treatment groups (n=3 each group): group1, PBS; group2, TH588; group3, Ce6 + laser; group4, MPC@Li-R + laser; group5, MPCT@Li-R + laser. The mice in the 4, 5 groups received laser irradiation (660nm, 1 W cm⁻²) for 5 minutes. Body weight and tumor volume were monitored every other day for 14 consecutive days during treatment. Then, the mice were sacrificed and the tumor tissues were removed and weighed.
To further evaluate the efficacy of different treatment modalities, terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling (TUNEL) staining was used to evaluate the apoptotic response of tumor tissues. Hematoxylin and eosin (H&E) and Ki67 staining were used to evaluate the efficacy of chemo-photodynamic combination therapy on tumor tissues. The main organs (heart, liver, spleen, lungs, kidneys) of mice were extracted, embedded in paraffin and sectioned for H&E staining to study the toxicity of NPs in vivo.

**Statistical analysis**

The values are expressed as mean ± standard deviation (SD). GraphPad Prism (version 7.0.0.159) was used to conduct the two-tailed Student's t tests for multiple comparisons. P < 0.05 was considered statistically significant.

**Results And Discussion**

**Preparation of MPCT@Li-R NPs**

The synthesis process of MPCT@Li-R is illustrated in Scheme 1. MSN-based drug vehicles were first synthesized and then modified with -NH$_2$ to effectively chelate Pt ions while improving water stability, then Pt ions were reduced by NaBH$_4$, so as to achieve the goal of in-situ growth of Pt NPs in the nanochannels. After the adsorption of photosensitizer Ce6 and MTH1 inhibitor TH588 through electrostatic action, the RGD-functionalized liposome shell was introduced to the periphery of the NPs in order to further realize the controllable release and precise targeting of Ce6 and TH588.

**Characterization of MPCT@Li-R NPs**

The synthesis process of the core-shell MPCT@Li-R was monitored by TEM. As shown in Figure. 1a, b, MSN was spherical, with uniform particle size (about 95nm by TEM measurement) and possessed homogeneous mesoporous structure, in which the black part is the skeleton of MSN and the bright area is the mesoporous channel. After the reduction of NaBH$_4$, it can be observed that the bright black dot structure with an average diameter of 3.4 nm is evenly distributed in the mesoporous structure of MSN, indicating the successful grafting of Pt NPs. The morphology of MPCT@Li-R was monitored by TEM, in which the thickness of the liposome shell was about 7nm, and the particle size increased to nearly 105nm compared with MSN-Pt, indicating the successful aggregation of lipid bilayer on the surface of the NPs (Figure. 1c, d). SEM was used to further observe the uniform spherical structure of MPCT@Li-R and the corresponding element distribution (Figure. S1). The polydispersity index (PDI), as an evaluation of the inhomogeneity of size distribution, showed that the PDI of the final product in deionized water was 0.265, which proved its good dispersion stability. As shown in the Figure. S2, both the intermediate product and the final product MPCT@Li-R exhibited good colloidal stability in aqueous solution. In addition, the particle sizes of MSN and MPCT@Li-R were measured by dynamic light scattering (DLS), and the results were similar to those measured by TEM (Figure. 1e, f). Studies have shown that the cell uptake efficiency of MSN is negatively correlated with particle size, and smaller particle size means
higher uptake[39]. Fang et al. reported that MSN with a particle size of around 100nm exhibited the best intracellular uptake rate and endosomal escape efficiency, which perfectly matched our results. In addition, energy dispersive spectrometer (EDS, OXFORD, Xplore 30) was used to analyze the composition of different elements in MPCT@Li-R, in which Si, O, Pt, C and other elements can be clearly observed, further indicating the successful preparation of the composite material (Figure. 1g-i).

For effectively induce the growth of Pt NPs in mesoporous of MSN, the MSN was modified with -NH₂, resulting in the formation of positively charged nanochannels which further promotes the electrostatic bonding of negatively charged PtCl₂⁻ with -NH₂. Zeta potential is shown in Figure. 2a: in the aqueous solution, the surface of MSN was covered by hydroxyl groups (-OH) due to the presence of SiO₂ molecule and exhibited negative potential. After APTS grafting, the potential of MSN was reversed and exhibited positive potential, indicating its successful amination. Due to the positive charge of Pt NPs, the potential of MSN-Pt was further increased after the successful reduction of Pt ions in the nanochannels. Then, photosensitizer Ce6 and MTH1 inhibitor TH588 were successively loaded into the synthesized MSN-Pt through electrostatic adsorption to form MPCT NPs, which were further coated with RGD functionalized liposome shell. According to the UV-vis absorption spectra analysis, as shown in Figure. 2b, there are two obvious characteristic absorption peaks at 400nm and 660nm for Ce6. Meanwhile, MPC and MPCT@Li-R also exhibited similar characteristic absorption peaks, demonstrating the successful loading of Ce6.

**Drug loading and TH588/Ce6 pH responsive release in vitro**

Firstly, ultraviolet absorption curves of Ce6 and TH588 with different concentrations were measured by ultraviolet spectrophotometer (Figure. S3), and the concentration-fluorescence intensity standard curve was drawn according to the results, as shown in the Figure. 2c, d. The drug loading capacity and encapsulation rate of TH588 were 8.67% and 94.93%, and that of Ce6 were 9.08% and 99.85%, respectively.

The pH-responsive release of TH588 and Ce6 is an inevitable requirement for PDT-CHT combination therapy after the targeted arrival of NPs to tumor cells. Different pH values (pH 7.4, 5.0) were selected to simulate the pH values of normal physiological environment and tumor acidic environment. As shown in Figure. 2e, explosive drug release of MPCT NPs was observed in PBS solutions at all pH values, the release proportion of TH588 and Ce6 from MPCT was nearly 90% and 85% at 10h, respectively. In contrast, MPCT@Li-R has a completely different TH588 and Ce6 release profile. As shown in Figure. 2f, MPCT@Li-R is pH-dependent on the release of TH588 and Ce6. For TH588, only 30% was released at pH 7.4 within 30 h, in contrast to more than 75% at pH 5.0. The release behavior of Ce6 was similar to that of TH588, with only 17% release at pH 7.4 and more than 60% release at pH 5.0 within 36 hours. In general, the gatekeeper liposomes can be cleaved in acidic environments and unlock mesoporous channels, eventually leading to the release of TH588 and Ce6. This property provides a strong guarantee for the continuous drug release of NPs in acidic medium after entering tumor cells.

**Evaluation of O₂ and ROS generation in vitro**
As shown in Figure. 2g, obvious transparent bubbles were attached to the tube wall after MPCT and MPCT@Li-R were co-incubated with H$_2$O$_2$ solution for 30min. The catalytic performance of Pt NPs was found to be excellent by dissolved oxygen meter, and Ce6, TH588, even liposome shell had a negligible influence on its performance. In addition, the catalase activity of the final product was further investigated. As shown in Figure. 2h, in the absence of H$_2$O$_2$, the content of O$_2$ in the solution hardly changes. With the increase of H$_2$O$_2$ concentration, the content of dissolved O$_2$ also increased gradually, indicating that MPCT@Li-R had excellent catalase-like activity. Time-dependent detection of H$_2$O$_2$ concentration showed that more than half of the H$_2$O$_2$ was decomposed within 30 minutes (Figure. S4). After repeated addition of H$_2$O$_2$ for several times, its catalytic activity was still excellent, indicating its good catalytic stability (Figure. 2i).

ROS can ablate tumors by destroying nucleic acids and proteins in tumor cells[40]. In order to investigate whether MPCT@Li-R can produce ROS under the irradiation of 660nm laser, Ce6, TH588, MPC@Li-R and MPCT@Li-R were co-incubated with HOS cells and DCFH-DA probe was used to detect ROS generation. As shown in the Figure. 3a, negligible fluorescence was observed in the control group, while relatively obvious green fluorescence was observed in the other 5 treatment groups, indicating that CHT, PDT or NPs + laser can effectively produce ROS in tumor cells. Compared with Ce6 + laser, MPC@Li-R + laser exhibited a stronger green fluorescence, which may be attributed to the catalase properties of Pt NPs or the targeting ability of RGD peptide. In all treatment groups, MPCT@Li-R + laser (660nm, 500mW cm$^{-2}$, 5min) exhibited the highest green fluorescence intensity, which demonstrated the unique advantage of CHT-PDT combination therapy with dual amplifying effect in ablation of tumor cells.

Since MTH1 inhibitor TH588 can inhibit the purication of 8-oxo-dGTP, resulting in DNA damage. Therefore, immunofluorescence staining was utilized to observe the content of 8-oxo-dGTP in different treatment groups. There is no doubt that the dissipation of MTH1 protein can lead to the accumulation of 8-oxo-dGTP in DNA, as demonstrated by pink fluorescence. In addition, the single PDT or MSN-Pt based in situ oxygen-generation promoting PDT process, and the reciprocal effect of TH588 and O$_2$-facilitated PDT could significantly increase the intracellular content of 8-oxo-dGTP, resulting in oxidative damage to DNA (Figure. 3b).

**Cancer cellular uptake of NPs**

To evaluate the uptake capacity of HOS cells to MPCT@Li-R, different formulations (free Ce6, MPCT@Li, MPCT@Li-R) were co-incubated with HOS cells for 12h and fluorescein imaging was performed using a fluorescence microscope. As shown in Figure. 3c, after being co-incubated with free Ce6, although the blue fluorescence of the nucleus was clearly visible, only a small amount of red fluorescence was observed in the cytoplasm, which may be due to the poor solubility of Ce6 and its inability to enter HOS cells effectively. In contrast, there was a strong red fluorescence signal in the cytoplasm of MPCT@Li-R group, indicating that RGD peptides enhanced the endocytosis of NPs by interacting with integrin receptors.
Antitumor efficacy of NPs in vitro

Excellent biocompatibility is the prerequisite for composite to play the therapeutic role. Hemoglobin can be released from the broken red blood cells, generating the red supernatant and resulting in enhanced absorbance at 570nm. In the hemolysis experiment, composites with a series of gradient concentration were co-incubated with red blood cells in PBS solution, as shown in Figure 4a, the hemolytic activity of MPCT@Li-R was less than 5% even at concentrations up to 400 μg/ml.

To evaluate the cytotoxicity of NPs, CCK8 assay was utilized to analyze the viability of HOS cells. As shown in Figure. S5, the cellular viability of HOS cells remained above 90% even after 48h co-incubation with 200 μg/ml MP@Li-R, which proves their excellent biocompatibility. Subsequently, we further explored the cytotoxicity of different treatment methods. As shown in Figure. 4b, the TH588 group and the Ce6 + laser group showed relatively low cell viability compared with the control group, indicating the killing effect of CHT or PDT on tumor cells. The cellular viability of MPC@Li-R + laser group was significantly lower than that of PDT group, which may be attribute to the Pt-related O₂-enhanced PDT effect or RGD targeting effect. Undoubtedly, the cellular viability of MPCT@Li-R + laser with dual amplification effect is the lowest, indicating that it has the best therapeutic effect. Based on the above findings, in order to explore whether the inhibition of the proliferation of tumor cells by MPCT@Li-R + laser is caused by inducing their apoptosis, we conducted a live (green)/dead (red) staining analysis on the cells. As shown in Figure. 4c, compared with the TH588 group and the Ce6 + laser group, the MPCT@Li-R + laser group exhibited stronger red fluorescence signal, which confirmed its unique advantages in killing tumor cells as a multifunctional nanotherapy platform.

Next, Western blot was used to assess the expression of some key marker proteins during combination therapy. As shown in Figure. 4d, e, MTH1 expression can be inhibited by TH588 or MPCT@Li-R. Moreover, MTH1 inhibitor can trigger p53-mediated apoptosis of cancer cells by inducing DNA damage. The expression of p53 protein was upregulated in all treatment groups, especially in the MPCT@Li-R group (Figure. S6). Bcl-2 can prevent the release of cytochrome c from mitochondria and has an anti-apoptotic effect, while BAX can interact with voltage-dependent ion channels on mitochondria to mediate the release of cytochrome c and have an apoptotic effect. As can be seen from Figure. S6, BAX and Bcl-2 exhibited an opposite trend. In the MPCT@Li-R + laser treatment group, the expression level of BAX was the highest, while the Bcl-2 was the lowest, which confirmed the efficacy of CHT-PDT combination therapy in mediating mitochondrial injury to kill tumor cells.

Fluorescence imaging and biodistribution of NPs in mouse

RGD peptide confers tumor targeting ability to MPCT@Li-R NPs through integrin receptor mediated endocytosis[41]. To further explore the appropriate irradiation time after NPs treatment, HOS tumor-bearing mice models were further utilized to investigate the tumor accumulation and biological distribution of MPCT@Li-R. Fluorescence images were collected at different time points (1h, 3h, 6h, 12h, 24h) after tail vein injection. As shown in Figure. 5a, a strong fluorescence signal appeared in the liver
region 1h after injection. With the EPR effect and the active targeting effect of RGD, fluorescence signals began to appear in the tumor region 3 h post injection, and reached the peak intensity 6 h post injection. It is noteworthy that we can still detect residual fluorescent signals in the tumor area 24h after injection. Moreover, mice were sacrificed 24h post injection, tumor tissues and organs were harvested. Quantitative analysis of fluorescence signals in tumors and major organs showed that the fluorescence intensity in tumor tissues was significantly higher than that in other major organs, confirming the excellent targeting ability, high uptake and retention ability of MPCT@Li-R NPs (Figure. 5b, c).

Antitumor efficacy of NPs in vivo

Encouraged by the excellent targeting, in situ O$_2$ generation facilitated PDT effect and CHT effect of MPCT@Li-R, antitumor efficacy was further observed in HOS tumor xenograft mouse model. Tumor-bearing mice were randomly divided into five groups (n=3 each group) and received different prescriptions after tumor volume reached about 80-100mm$^3$. Based on the results of fluorescence imaging and biological distribution in vivo, 6h after intravenous injection was determined as the optimal time window for laser irradiation (660nm, 1 W cm$^{-2}$, 5 min). As shown in Figure. 5d, Tumors in the control group showed a faster growth trend and no inhibition trend was observed. TH588 and Ce6 + laser group had certain inhibitory effect on tumor growth. However, in the Ce6 + laser group, tumor growth volume began to accelerate after day 8, demonstrating that hypoxia within the larger solid tumors may impair the efficacy of PDT. Encouragingly, ROS generation based on Pt NPs catalytic properties can effectively inhibit tumor growth, while the addition of MTH1 inhibitor can bring out the best effect of this dual-amplification therapy model. Figure. 5e shows the general image of mice after 14 days of treatment, in which the change of tumor volume is consistent with the trend of Figure. 5d. Moreover, the tumor tissue images and weight further confirmed the changing trend of tumor volume (Figure. 5f, h).

Biosafety assessment

To assess the biosafety of MPCT@Li-R, body weight changes of mice were recorded every other day during each treatment period. Compared with the PBS treatment group, the other five treatment groups showed no significant weight fluctuations (Figure. 5g). Finally, tumor tissues and major organs of the mice were collected. No obvious inflammatory lesions or histological abnormalities were observed in the organs, which fully confirmed the excellent biosafety of MPCT@Li-R (Figure. S7). In contrast, TUNEL, Ki67 analysis and H&E staining showed that MPCT@Li-R + laser had a definite anti-tumor effect compared to the other five treatment groups. (Figure. 5i)

Conclusions

In summary, on the basis of MSN, we successfully realized the in-situ growth of Pt NPs by one-step reduction method, and finally prepared a dual-amplification effect nanotherapy platform MPCT@Li-R, which could elevate the ROS level during PDT by alleviating the hypoxia situation within the tumor, and simultaneously inhibited the purification process of oxidized nucleotides regulated by MTH1 through the
TME responsive release of TH588. Specifically, NPs with excellent biocompatibility and biodegradability can accumulate at the tumor site through EPR effect or RGD-mediated active targeting effect. Next, the liposome shell depolymerized in the slightly acidic environment of tumor cells to release Ce6 and TH588 to achieve the purpose of TME response release, and the exposed Pt NPs in the inner layer can decompose H$_2$O$_2$ to generate O$_2$. Under the dual action of 660nm laser and the generated O$_2$, sufficient ROS can be produced to cause oxidative damage of DNA, while TH588 can inhibit the scavenging of oxidative bases by MTH1 protein in dNTP pool, resulting in the accumulation of DNA oxidative damage. Both in vivo and in vitro experiments have confirmed that this dual amplification mode treatment strategy, which combines the elevating O$_2$-promoted PDT and inhibiting DNA oxidative damage repair pathway, exhibits more ideal anti-tumor efficacy than traditional PDT or CHT. In brief, MPCT@Li-R NPs take MTH1 protein as the target and combine with Pt NPs nanoenzyme to form a novel cancer therapeutic nanoplatform.

**Abbreviations**

PDT: Photodynamic therapy; O$_2$: Oxygen; Pt NPs: Platinum nanoparticles; MSNs: Mesoporous silica nanoparticles; Ce6: Chlorin e6; RGD: Arginine-glycine-aspartic acid; ROS: Reactive oxygen species; RBC: Red blood cell; H$_2$O$_2$: hydrogen peroxide; MnO$_2$: Manganese dioxide; CuO: Copper oxide; CHT: chemotherapy; PTT: Photothermal therapy; -NH$_2$: Amino group; dNTP: deoxynucleoside triphosphate; 8-oxo-dGTP: 8-oxo-2’-deoxyguanosine triphosphate; APTS: 3-Aminopropyltriethoxysilane; TEA: Triethanolamine; CTAC: cetyltrimethylammonium chloride; DSPE-PEG2K-NH$_2$: 1,2-Distearoyl-sn-glycero-3-phosphoethanolamine-N-(polyethylene glycol)-2000-carboxyl; DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; H$_2$PtCl$_6$·6H$_2$O: Hydrogen hexachloroplatinate (IV) hexahydrate; NaBH$_4$: sodium borohydride; TEOS: Tetraethoxysilane; NaOH: sodium hydroxide; DOTAP: 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt); PDI: Polydispersity index; CCK-8: Cell Counting Kit-8; TUNEL: Terminal deoxynucleotidyl transferase-mediated dUTP-digoxigenin nick-end labeling; TEM: Transmission electron microscopy; SEM: Scanning electron microscopy; DLS: Dynamic light scattering; -OH: hydroxyl groups; L: laser; SD: Standard deviation.

**Declarations**

**Acknowledgements**

We would like to thank the Department of Electron Microscopy Center, Hebei Medical University for TEM measurements and we would be grateful to Chenming Zhou for his help.

**Authors’ contributions**

QS, YZ and HH designed this study. QS, XD, WY and YZ performed the experiments. QS completed this manuscript. WL, WC and JL revised and proofread this manuscript. All authors read and approved the final manuscript.
Funding

This study was supported by the Non-profit Central Research Institute Fund of the Chinese Academy of Medical Sciences [2019PT320001].

Availability of data and materials

All data generated or analyzed during this study are included in this manuscript.

Ethics approval and consent to participate

All animal experiments were performed according to protocols approved by the Experimental Animal Ethics Committee of Hebei Ex & Invivo Biotechnology Co., Ltd.

Consent for publication

All authors gave their consent for publication.

Competing interests

The authors declare that they have no competing interests.

References

1. Liu Y, Bhattarai P, Dai Z, Chen X. Photothermal therapy and photoacoustic imaging via nanotheranostics in fighting cancer. Chemical Society reviews. 2019;48:2053–108.

2. Chen H, Zhang W, Zhu G, Xie J, Chen X. Rethinking cancer nanotheranostics. Nature reviews Materials 2017, 2.

3. Kwiatkowski S, Knap B, Przystupski D, Saczko J, Kędzierska E, Knap-Czop K, Kotlińska J, Michel O, Kotowski K, Kulbacka J. Photodynamic therapy - mechanisms, photosensitizers and combinations. Biomedicine pharmacotherapy = Biomedicine pharmacotherapie. 2018;106:1098–107.

4. Vankayala R, Hwang KC. Near-Infrared-Light-Activatable Nanomaterial-Mediated Phototheranostic Nanomedicines: An Emerging Paradigm for Cancer Treatment. Advanced materials (Deerfield Beach Fla). 2018;30:e1706320.

5. Ai X, Ho CJH, Aw J, Attia ABE, Mu J, Wang Y, Wang X, Wang Y, Liu X, Chen H, et al. In vivo covalent cross-linking of photon-converted rare-earth nanostructures for tumour localization and theranostics. Nature communications. 2016;7:10432.

6. Dolmans DEJGJ, Fukumura D, Jain RK. Photodynamic therapy for cancer. Nature reviews Cancer. 2003;3:380–7.

7. Cheng L, Wang C, Feng L, Yang K, Liu Z. Functional nanomaterials for phototherapies of cancer. Chemical reviews. 2014;114:10869–939.

8. Harris AL. Hypoxia—a key regulatory factor in tumour growth. Nature reviews Cancer. 2002;2:38–47.
9. Wang X-Q, Gao F, Zhang X-Z. Initiator-Loaded Gold Nanocages as a Light-Induced Free-Radical Generator for Cancer Therapy. Angew Chem. 2017;56:9029–33.

10. Song G, Chen Y, Liang C, Yi X, Liu J, Sun X, Shen S, Yang K, Liu Z. Catalase-Loaded TaOx Nanoshells as Bio-Nanoreactors Combining High-Z Element and Enzyme Delivery for Enhancing Radiotherapy. Advanced materials (Deerfield Beach Fla). 2016;28:7143–8.

11. Yang G, Xu L, Chao Y, Xu J, Sun X, Wu Y, Peng R, Liu Z. Hollow MnO as a tumor-microenvironment-responsive biodegradable nano-platform for combination therapy favoring antitumor immune responses. Nature communications. 2017;8:902.

12. Bristow RG, Hill RP. Hypoxia and metabolism. Hypoxia, DNA repair and genetic instability. Nature reviews Cancer. 2008;8:180–92.

13. Tang W, Zhen Z, Wang M, Wang H, Chuang Y-J, Zhang W, Wang GD, Todd T, Cowger T, Chen H, et al. Red Blood Cell-Facilitated Photodynamic Therapy for Cancer Treatment. Adv Funct Mater. 2016;26:1757–68.

14. Allison RR. Photodynamic therapy: oncologic horizons. Future oncology (London England). 2014;10:123–4.

15. Zhao L, Li J, Su Y, Yang L, Chen L, Qiang L, Wang Y, Xiang H, Tham HP, Peng J, Zhao Y. MTH1 inhibitor amplifies the lethality of reactive oxygen species to tumor in photodynamic therapy. Sci Adv. 2020;6:eaaaz057.

16. Zhang BB, Sun H, Zhan Y, He QF, Zhu Y, Wang YK, Luo CF. Reliability and repeatability of tibial plateau fracture assessment with an injury mechanism-based concept. Bone Joint Res. 2019;8:357–66.

17. Li Q, Ren J, Chen Q, Liu W, Xu Z, Cao Y, Kang Y, Xue P. A HMCuS@MnO nanocomplex responsive to multiple tumor environmental clues for photoacoustic/fluorescence/magnetic resonance trimodal imaging-guided and enhanced photothermal/photodynamic therapy. Nanoscale. 2020;12:12508–21.

18. Zhao J, Fei J, Du C, Cui W, Ma H, Li J. Assembly of catalase-based bioconjugates for enhanced anticancer efficiency of photodynamic therapy in vitro. Chem Commun (Cambridge England). 2013;49:10733–5.

19. Zeng W, Zhang H, Deng Y, Jiang A, Bao X, Guo M, Li Z, Wu M, Ji X, Zeng X, Mei L. Dual-response oxygen-generating MnO2 nanoparticles with polydopamine modification for combined photothermal-photodynamic therapy. Chemical Engineering Journal 2020, 389.

20. Cao H, Yang Y, Qi Y, Li Y, Sun B, Li Y, Cui W, Li J, Li J. Intraparticle FRET for Enhanced Efficiency of Two-Photon Activated Photodynamic Therapy. Advanced healthcare materials. 2018;7:e1701357.

21. Abbas M, Zou Q, Li S, Yan X. Self-Assembled Peptide and Protein-Based Nanomaterials for Antitumor Photodynamic and Photothermal Therapy. Advanced materials (Deerfield Beach, Fla) 2017, 29.

22. Xu M, Wang P, Sun S, Gao L, Sun L, Zhang L, Zhang J, Wang S, Liang X. Smart strategies to overcome tumor hypoxia toward the enhancement of cancer therapy. Nanoscale. 2020;12:21519–33.
23. Liang J-H, Zheng Y, Wu X-W, Tan C-P, Ji L-N, Mao Z-W. 
A Tailored Multifunctional Anticancer Nanodelivery System for Ruthenium-Based Photosensitizers: Tumor Microenvironment Adaption and Remodeling. 
Advanced science (Weinheim, Baden-Wurttemberg, Germany) 2020, 7:1901992.

24. Li Y, Jian X, Zhou S, Lu Y, Zhao C, Gao Z, Song Y-Y. Protein Shell-Encapsulated Pt Clusters as Continuous O-Supplied Biocoats for Photodynamic Therapy in Hypoxic Cancer Cells. ACS Appl Mater Interfaces. 2019;11:17215–25.

25. Cao H, Yang Y, Liang M, Ma Y, Sun N, Gao X, Li J. Pt@polydopamine nanoparticles as nanozymes for enhanced photodynamic and photothermal therapy. Chem Commun (Camb). 2021;57:255–8.

26. Li Z, Zhang Y, Feng N. Mesoporous silica nanoparticles: synthesis, classification, drug loading, pharmacokinetics, biocompatibility, and application in drug delivery. Expert Opin Drug Deliv. 2019;16:219–37.

27. Nguyen TL, Choi Y, Kim J. Mesoporous Silica as a Versatile Platform for Cancer Immunotherapy. Advanced materials (Deerfield Beach Fla). 2019;31:e1803953.

28. Mai Z, Hu Y, Huang P, Zhang X, Dong X, Fang Y, Wu C, Cheng J, Zhou W. Outside-in stepwise bifunctionalization of magnetic mesoporous silica incorporated with Pt nanoparticles for effective removal of hexavalent chromium. Powder Technol. 2017;312:48–57.

29. Pan H, Li X, Zhang D, Guan Y, Wu P. Pt nanoparticles entrapped in mesoporous metal–organic frameworks MIL-101 as an efficient and recyclable catalyst for the asymmetric hydrogenation of α-ketoesters. J Mol Catal A: Chem. 2013;377:108–14.

30. Oleinick NL, Morris RL, Belichenko I. The role of apoptosis in response to photodynamic therapy: what, where, why, and how. Photochemical & photobiological sciences: Official journal of the European Photochemistry Association and the European Society for Photobiology 2002, 1.

31. Buytaert E, Dewaele M, Agostinis P. Molecular effectors of multiple cell death pathways initiated by photodynamic therapy. Biochimica et biophysica acta 2007, 1776.

32. Zhou X, Wang Y, Si J, Zhou R, Gan L, Di C, Xie Y, Zhang H. Laser controlled singlet oxygen generation in mitochondria to promote mitochondrial DNA replication in vitro. Scientific reports. 2015;5:16925.

33. Nakabeppu Y. Cellular levels of 8-oxoguanine in either DNA or the nucleotide pool play pivotal roles in carcinogenesis and survival of cancer cells. Int J Mol Sci. 2014;15:12543–57.

34. Sakumi K, Furuichi M, Tsuzuki T, Kakuma T, Kawabata S, Maki H, Sekiguchi M. Cloning and expression of cDNA for a human enzyme that hydrolyzes 8-oxo-dGTP, a mutagenic substrate for DNA synthesis. J Biol Chem. 1993;268:23524–30.

35. Gad H, Koolmeister T, Jemth A-S, Eshtad S, Jacques SA, Ström CE, Svensson LM, Schultz N, Lundbäck T, Einarsdottir BO, et al. MTH1 inhibition eradicates cancer by preventing sanitation of the dNTP pool. Nature. 2014;508:215–21.

36. Claassen I, Van Rooijen N, Claassen E. A new method for removal of mononuclear phagocytes from heterogeneous cell populations in vitro, using the liposome-mediated macrophage 'suicide' technique. J Immunol Methods. 1990;134:153–61.
37. Reddy BS, Banerjee R. 17Beta-estradiol-associated stealth-liposomal delivery of anticancer gene to breast cancer cells. Angew Chem. 2005;44:6723–7.

38. Chen H, Jiang J-H, Li Y-F, Deng T, Shen G-L, Yu R-Q. A novel piezoelectric immunoagglutination assay technique with antibody-modified liposome. Biosens Bioelectron. 2007;22:993–9.

39. Gan Q, Dai D, Yuan Y, Qian J, Sha S, Shi J, Liu C. Effect of size on the cellular endocytosis and controlled release of mesoporous silica nanoparticles for intracellular delivery. Biomed Microdevices. 2012;14:259–70.

40. Tong L, Chuang C-C, Wu S, Zuo L. Reactive oxygen species in redox cancer therapy. Cancer letters. 2015;367:18–25.

41. Shi K, Li J, Cao Z, Yang P, Qiu Y, Yang B, Wang Y, Long Y, Liu Y, Zhang Q, et al. A pH-responsive cell-penetrating peptide-modified liposomes with active recognizing of integrin αvβ3 for the treatment of melanoma. Journal of controlled release: official journal of the Controlled Release Society. 2015;217:138–50.

Figures
Figure 1

TEM images of (a) MSN NPs, (b) MSN-Pt NPs and (c), (d) MPCT@Li-R NPs. Particle size distributions of (e) MSN, (f) MPCT@Li-R. (g) EDS analysis of MPCT@Li-R. HAADF-STEM image (h), and corresponding element mapping image and content of the MPCT@Li-R (i).
Figure 2

(a) Zeta potential of MSN, MSN-NH2, MSN-Pt, MPCT and MPCT@Li-R. (b) UV-vis absorption spectra of Ce6, MP, MPC, MPCT@Li-R. The concentration-fluorescence intensity standard curve of Ce6 (c) and TH588 (d). Drug release properties of MPCT (e) and MPCT@Li-R (f) in PBS solution at different pH values. (g) Determination of catalase properties of different materials. (h) Quantitatively determination of the O2 content in the solution after the reaction of MPCT@Li-R with H2O2 at different concentrations. (i) Catalytic durability of MPCT@Li-R after repeated addition of H2O2 solution.
Figure 3

(a) ROS fluorescence imaging of DCFH-DA stained cells after co-incubated with medium, free Ce6, TH588, MPC@Li-R and MPCT@Li-R NPs under 660 nm laser irradiation and corresponding surface plot images.

(b) Immunofluorescence images of 8-oxo-dGTP and corresponding surface plot images. Blue and red colors represent DAPI and 8-oxo-dGTP fluorescence, respectively. (c) Fluorescence images and
corresponding surface plot images of HOS cells incubated with free Ce6, MPCT@Li and MPCT@Li-R at 37°C for 4 h. Blue: Hoechst 33342, red: Ce6.

**Figure 4**

(a) Hemolysis image and rate of red blood cells incubated with different concentrations of MPCT@Li-R. (b) Relative viability of HOS cells treated with various formulations at a series concentration of MSN (1, 2, 4, 6, 12 μg/ml). (c) Live/dead fluorescence staining images of HOS cells after different treatments. Red:
PI (dead cells). Green: calcein AM (live cells). (d) Western blotting analysis of MTH1 protein and apoptosis signaling protein (p53, BAX, Bcl-2) in tumor tissues after different treatments. (e) Quantitative analysis of MTH1 protein expression by measuring the chemiluminescence intensity of protein band.

Figure 5

(a) In vivo fluorescence images of tumor-bearing mice at different times after injection of MPCT@Li-R NPs. (b) Ex vivo fluorescence images of major organs and tumor tissues 24 h after injection. (c) Semi-
quantitative analysis of fluorescence images of major organs and tumor tissues. (d) Change curves of relative tumor volume. (e) Images of HOS tumor-bearing mice after receiving different treatments for 14 days. Tumor weight (f) and tumor images (h) after treatment for 14 days. (g) Body weight changes of mice during treatment. (i) H&E, Ki67 and TUNEL staining images of tumor tissues after different treatments.

**Supplementary Files**

This is a list of supplementary files associated with this preprint. Click to download.

- Scheme1.jpg
- FigureS1.jpg
- FigureS2.jpg
- FigureS3.jpg
- FigureS4.jpg
- FigureS5.jpg
- FigureS6.jpg
- FigureS7.jpg