Supplementary information

MnO2 nanoflowers as a multifunctional nano-platform for enhanced photothermal/photodynamic therapy and MR imaging

Wanru Sun [a,#], *Hui Yu* [c,#], *Deqiang Wang* [a,#], *Youjie Li* [b], *Baocheng Tian*[d], *Shuang Zhu*[a], *Ping-Yu Wang* [b], *Shuyang Xie* [b,*] *and Ranran Wang*[a,*]

[a]Institute of Rehabilitation Medicine, School of Rehabilitation Medicine, Binzhou Medical University, Yantai 264003, PR China

[b]Key Laboratory of Tumor Molecular Biology, Binzhou Medical University, Yantai 264003, PR China

[c]Binzhou Medical University Hospital, Binzhou, 256603 PR China

[d]School of Pharmacy, Binzhou Medical University, Yantai 264003, PR China

[#]Contributed equally

*To whom correspondence should be addressed.

E-mail addresses: shuyangxie@aliyun.com; (Shuyang Xie) and wangrr@bzmc.edu.cn (Ranran Wang)

Materials

Potassium permanganate ($KMnO_4$) and Trichloromethane ($CHCl_3$) were obtained from Sanhe Chemical Reagent Co. Ltd (Yantai). Oleic acid (OA) was provided by Fangzheng Chemical Reagent Factory (Tianjin). Hexadecyl trimethyl ammonium Bromide (CTAB) was purchased from Shanghai Yuanye Bio-Technology Co. Ltd. Treaethyl orthosilicate (TEOS) was purchased from Engrgy Chemical. Bis[3-(triethoxysilyl)propyl] tetrasulfide (BTES) was purchased from Shanghai Forneeds Biological Technology Co. Ltd. 3-Aminopropyltriethoxysilane (APTES) obtained from TCL (Shanghai). Sodium hydroxide (NaOH) was purchased from Tianjin Yongda Chemical Reagent Co. Ltd. Chlorin e6 (Ce6) was purchased from J&K Chemical. Singlet Oxygen Sensor Green Reagent was purchased from Meilunbio. Reactive

Oxygen Species Assay Kit was purchased from Beyotime. ROS-ID Hypoxia/Oxidative Stress Detection Kit was obtained from Enzo Life Sciences. Calcein- AM/propidium iodide (PI) was provided by Solarbio. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was purchased from Bioforxx. 4', 6'-diamidino-2-phenylindole (DAPI) was purchased from Ers. 4% paraformaldehyde solution was purchased from Biosharp. Anti-Hsp70 antibody was provided by Abcam.

Characterization

Transmission electron microscopy (TEM) was carried out with a JEOL microscope The Malvern zetasizer Nano ZS was performed to acquire size and zeta potential of the MSA. Shimadzu 2600 UV-vis-NIR spectrophotometer was carried out on UV absorption peak.Oxygen production capacity was record with an Ohaus Starter 400 D portable optical dissolved oxygen meter. The fluorescence spectrum of SO was measured with a Hitachi F-2700 fluorescence spectrometer. A Haobro medical ultrasonic therapy device was carried out SDT.

Cell culture

All experiments used lung cancer A549 cells, cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum. The cells were cultured in an incubator at 37 °C and 5% CO_2.

Animals

Healthy female BALB/c nude mice (5-7 weeks) were provided by GemPharmatech Co. Ltd., Nanjing,China. A549 cells were suspended in 100 μL of PBS and injected subcutaneously into the upper thigh of the mouse.

Antitumor effect in vitro

The cytotoxicity of MSA and MSA&C with or without laser irradiation was determined through MTT and Live/Dead fluorescence staining assay. Firstly, MTT assay was employed to quantitatively determine the cell viability. Briefly, A549 cells in a 96-well plate (1.5×10^4 cells per well) were cultured with MSA and MSA&C in a wide range of concentrations (from 0 to 50 μg/mL) for 24 h and standard MTT procedures was used to determine the survival rate of the cells. For light-induced cytotoxicity of MSA&C, A549 cells in a 96-well plate was co-incubated with different concentrations MSA&C for 12 h and exposed to 660 nm (0.1 W/cm^2), 1064 nm (1 W/cm^2) or dual laser for 5 min. After 2 h incubation, cell viability was measured by MTT method. For cell imaging, A549 cells in a CLSM-specific dish were incubated with MSA&C for 12 h. After 660 nm laser, 1064 nm laser or dual laser irradiation treatment for 5 min, the culture medium was discarded and washed with PBS for 3 times then the prepared Calcein AM (2 M) and PI (4.5 M) solutions were added to stain for 15 minutes in darkness. CLSM was used to detect fluorescence imaging after washed 3 times with PBS.

Anti-tumor effect in vivo

When the tumor volume grown to about 100 mm^3, the tumor-bearing mice were randomly divided into five groups containing 4 mice in each group：Control, MSA&C, MSA&C+660 nm, MSA&C+1064 nm, MSA&C+660 nm+1064 nm. A549 tumor mice were locally injected with PBS or MSA&C (100 μL, 1.2 mg/mL), and the laser groups were given 660 nm (0.1 W/cm^2 5 min), 1064 nm (1 W/cm^2 5 min) or double laser

irradiation. The body weight and tumor volume of the mice was monitored every other day during two weeks' treatment. The tumor volume was acquired by the formula: V_{tumor}=(tumor Length* tumor width^2/2). Finally, all organs and tumors were harvested and collected for H&E staining.

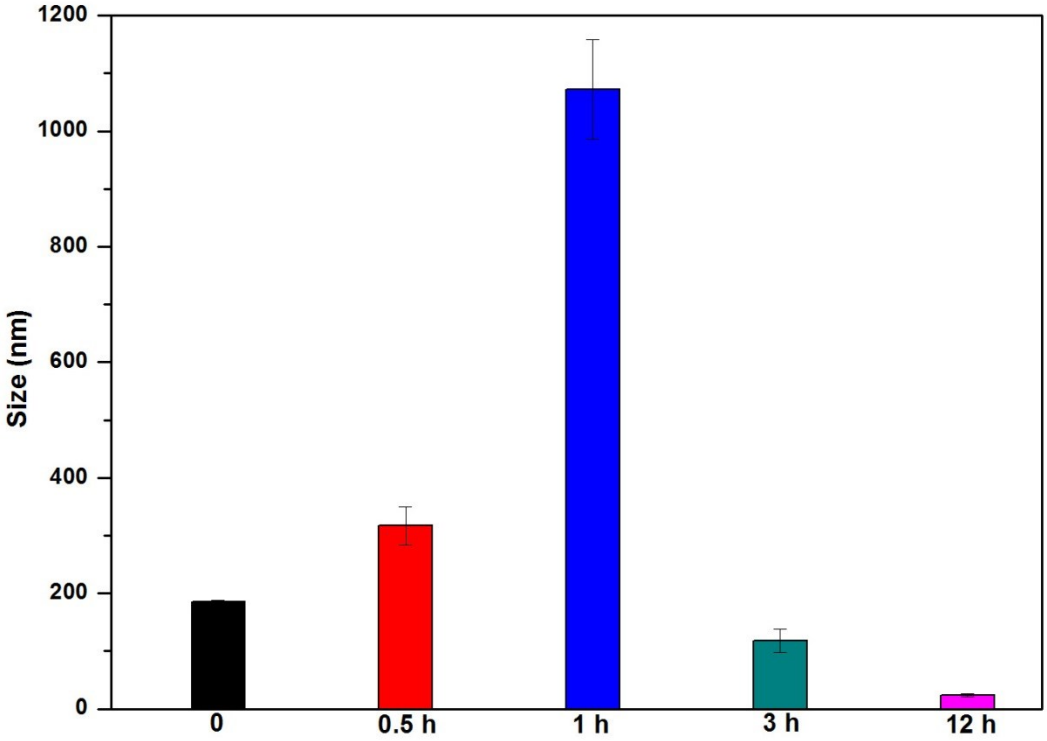

Fig. S1 Size of MSA after incubation in PBS with pH 6.5+H_2O_2 (100 μM) for different times.

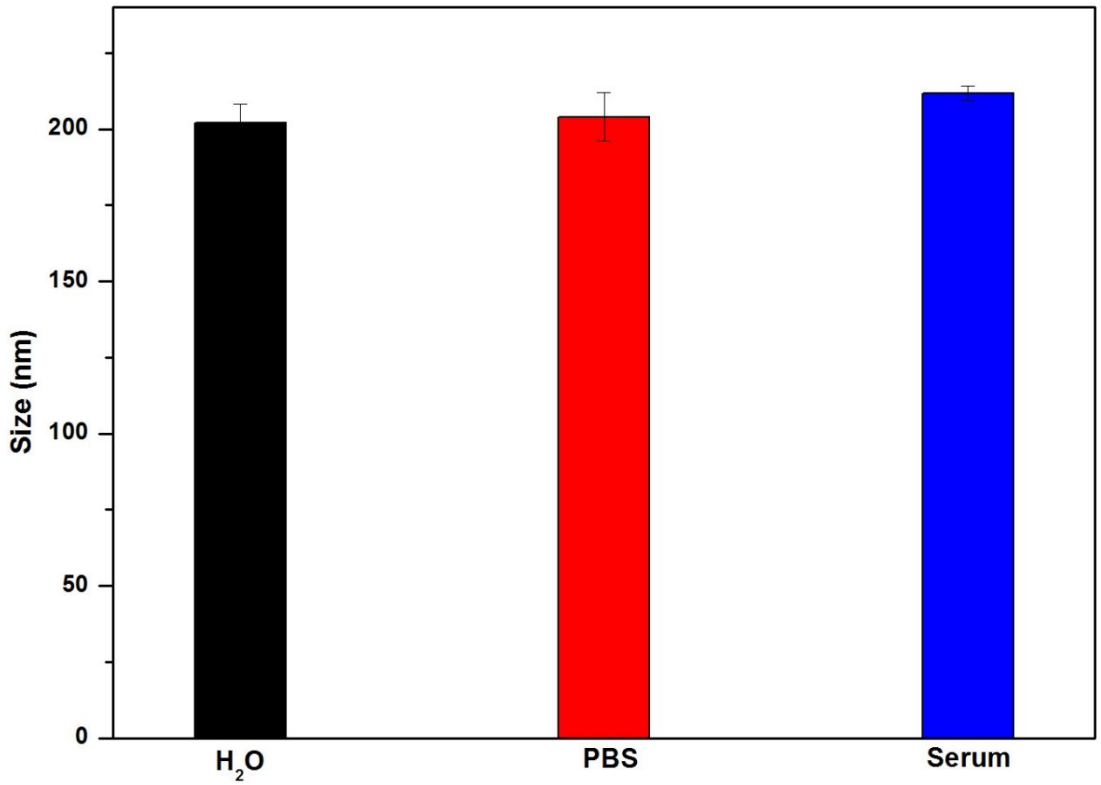

Fig. S2 Size of MSA&C in water, PBS and serum.

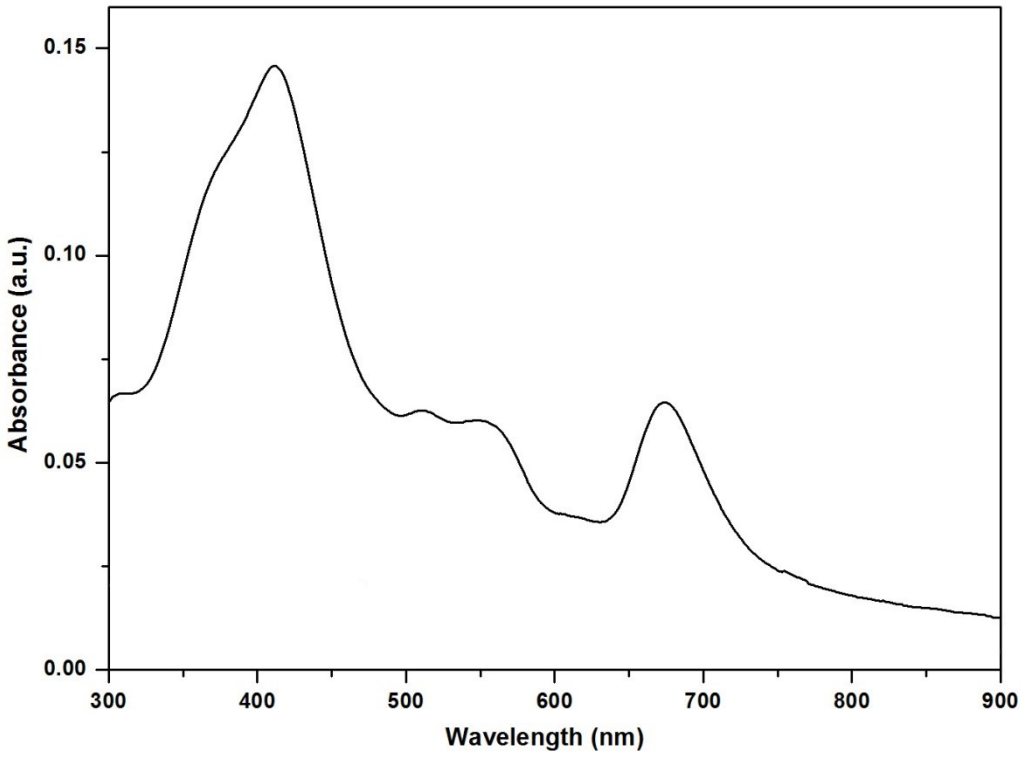

Fig. S3 UV–vis absorption of MSA&C in serum.

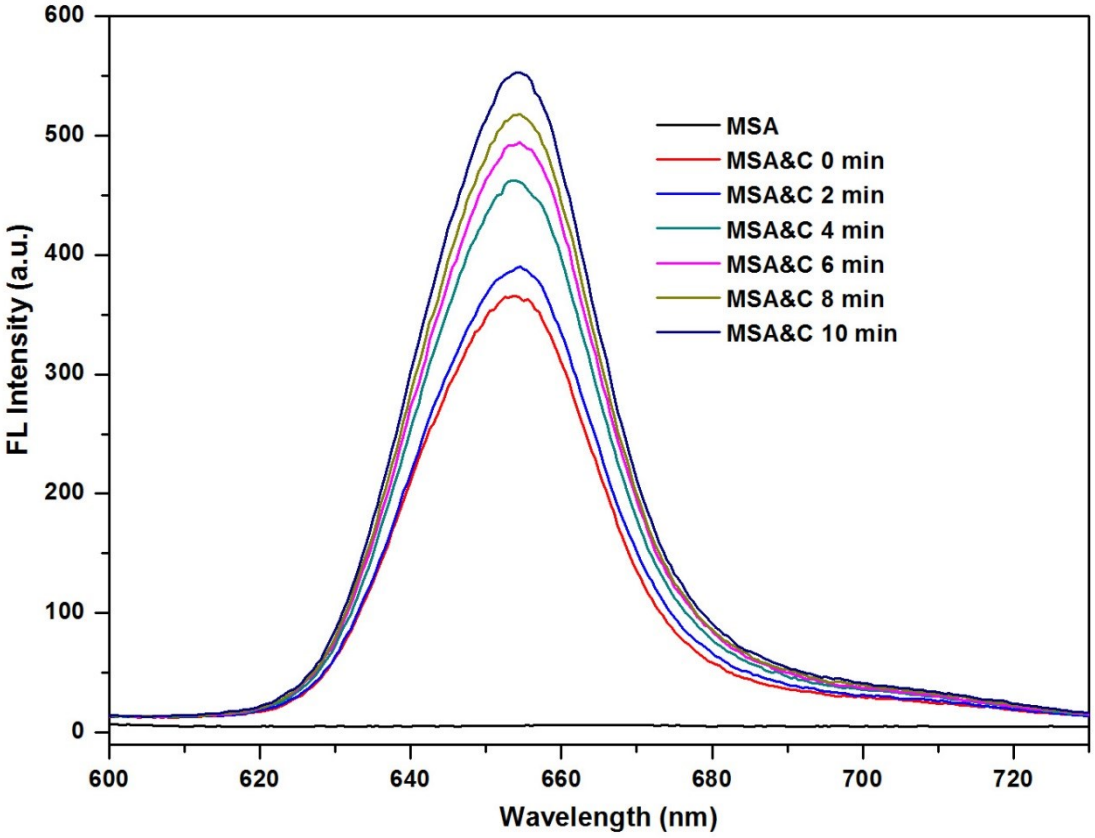

Fig. S4 Fluorescence spectra of MSA and MSA&C after incubation in PBS with pH 6.5+H_2O_2 (100 μM) for different times.

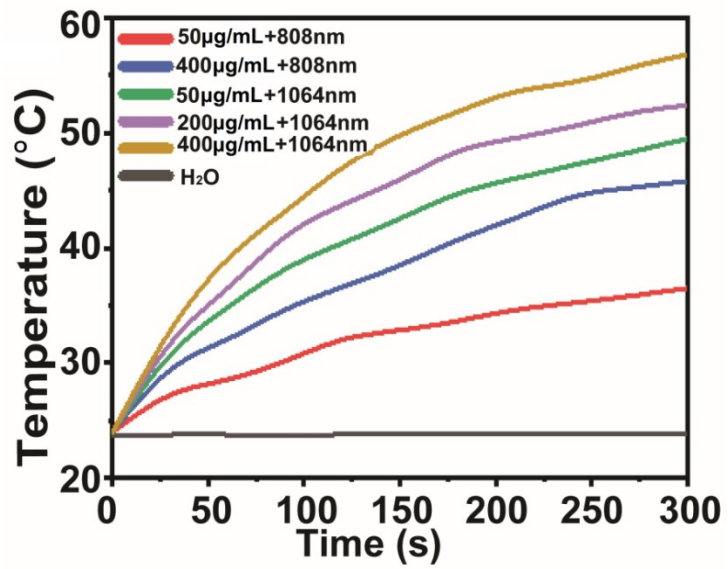

Fig. S5 Temperature rise curves of different concentrations of MSA under 808nm and 1064nm laser irradiation (1W/cm^2, 5min).

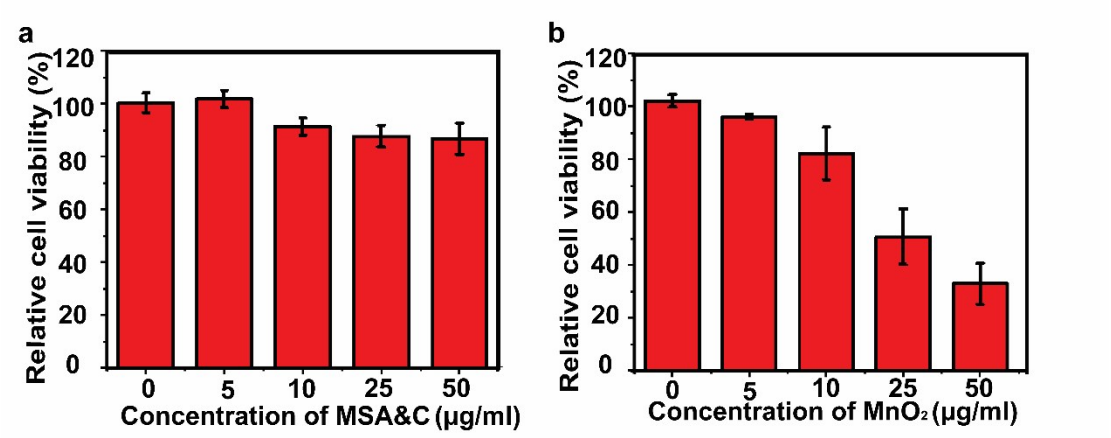

Fig. S6 a) MTT viability evaluate of 293T cells treated with MSA&C. b) and A549 cells treated with MnO_2 under 1064nm (1W/cm^2, 5min) laser.

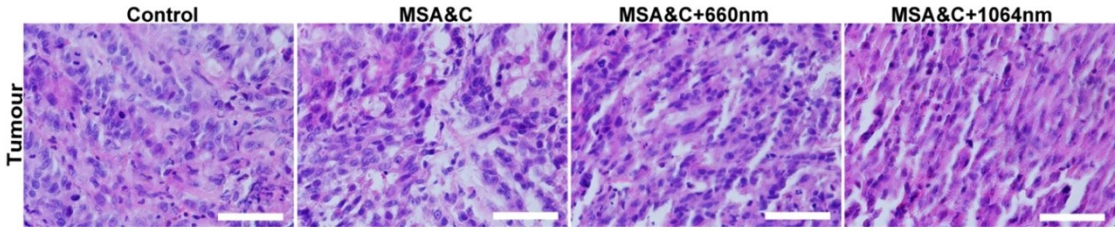

Fig. S7 H&E staining of tumor slices in each group after two weeks of treatment (Scale bar = 50 μm).