# Supporting Information

## Sustained ROS Generation from Percarbamide Nanomedicine via a Mechanism of X-Ray-Initiated Free Radical Chain Reactions

***Synthesis of HMSNs***

HMSNs were synthesized based on selective etching strategy.[1-3] In detail, TEOS (6.0 mL) and $NH_3 \cdot H_2O$ (3.1 mL) were dissolved in the mixture with ethanol (71.0 mL) and deionized water (10.0 mL) and stirred at 25℃ for 2 h to obtain the solid silica dioxide nanoparticles ($sSiO_2$). Then, the product was collected by centrifugation, washing, and drying. Next, $sSiO_2$ (0.50 g) was dispersed into deionized water (100 mL), followed by the mixture addition of CTAB (0.75 g), deionized water (150 mL), ethanol (150 mL) and $NH_3 \cdot H_2O$ (2.8 mL). After being stirred for 2 h at 25℃, TEOS (1.5 mL) was added to the solution quickly and kept stirring for an additional 6 h. The crude product ($mSiO_2@SiO_2$) obtained by centrifugation was redispersed into a $Na_2CO_3$ solution (40 mL, 0.4 M) and stirred at 50℃ for 10 h to obtain hollow cavity. Finally, the as-prepared product (1.0 g) was dispersed in an ethanol/HCl (200 mL, volume ratio 18:1) mixture solution and stirred at 60℃ for 8 h to remove the template CTAB. The final product (HMSNs) was collected by centrifugation, rinsed with deionized water several times and dried in the freeze dryer.

***Preparation of PCA@HMSNs, PCA@HMSNs-CTS, and PCA@HMSNs-CTS-FITC***

Firstly, PCA was synthesized in HMSNs by dispersing the as-prepared HMSNs (0.25 g) into a $CO(NH_2)_2$ solution (1.20 g, 50 mL) in a beaker. Secondly, $H_2O_2$ (2.7 mL, 30 wt%) and $(NaPO_3)_6$ (0.060 g) were added to the mixture and stirred for an additional 24 h at 25℃. The product was collected by centrifugation, rinsed with deionized water several times and dried in the freeze dryer. Finally, the as-prepared PCA@HMSNs (0.10 g) was dispersed in chitosan sol (20 mL) and stirred for 24 h under dark conditions at pH 5.0, followed by being stirred for additional 2 h at pH 7.4. The product (PCA@HMSNs-CTS) was collected by centrifugation, rinsed with deionized water several times and dried in the freeze dryer. Additionally, HMSNs-CTS was synthesized following a similar procedure.

Meanwhile, fluorescein isothiocyanate (FITC) (7.5 mg) and 3-Aminopropyltriethoxysilane (APTES) (20 μL) were added in methanol (3.0 mL) and stirred for 24 h under dark conditions. Subsequently, PCA@HMSNs-CTS (25.00 mg) was dispersed into the above solution. After stirring for 24 h in the dark, PCA@HMSNs-CTS-FITC was collected by centrifugation, rinsed with deionized water three times and dried in the freeze dryer.

***Evaluation of PCA loading capacity and releasing behavior in different PBS solutions***

The loading capacity of PCA and the release profiles of PCA@HMSNs and PCA@HMSNs-CTS in different PBS solutions were obtained by using a UV/Vis spectrophotometer (TECHCOMP, China) to detect absorbance of $KMnO_4$ at λ=525 nm which was in direct proportion with the content of PCA.[4] Initially, a standard curve between the concentration of $H_2O_2$ and the absorbance of $KMnO_4$ were determined by using a UV/Vis spectrophotometer. Subsequently, 5.00 mg of PCA@HMSNs or PCA@HMSNs-CTS were mixed with the $KMnO_4$

solution and the absorbance of mixtures at λ=525 nm was measured. The loading capacity of PCA in nanocomposites could be obtained by comparing to the standard curve. Drug loading efficiency was expressed as the drug capacity (%, w/w).[5] The PCA release behavior was also determined by UV/Vis spectrophotometer in the similar way. PCA@HMSNs and PCA@HMSNs-CTS were dispersed in PBS solutions (pH 6.5, 7.4), respectively. After a given time interval, 5.0 mL of above mixtures was added in the $KMnO_4$ solution and the absorbance of mixtures at λ=525 nm was monitored.

Additionally, PCA@HMSNs-CTS (20 μg/mL) and the equal amount of PCA were dispersed in PBS (pH 6.5) solutions. The concentrations of $H_2O_2$ in solutions with or without soft-X-Ray initiation (60 kV, 8 mA) for 2 min were monitored by UV/Vis spectrophotometer.

***Detection of $O_2$***

PCA@HMSNs-CTS were dispersed in 50 mL of PBS (pH 6.5) with a concentration of 20 μg/mL. The real-time production of $O_2$ was measured via a Dissolved Oxygen Meter (JPSJ-605F). The same process for detecting $O_2$ was applied to PBS solutions with or without PCA@HMSNs-CTS (20 μg/mL) after soft-X-Ray initiation (60 kV, 8 mA). The whole initiation process lasted for 2 min. Notably, the PBS solution used in the experiment was deoxygenated.

***Detection of ROS generation***

The ROS production of PCA@HMSNs-CTS was examined by the free radical indicator (5 mM PTA, 10 mM NaOH). Non-fluorescent PTA can react with ROS to generate highly fluorescent 2-hydroxyterephthalic acid.[6] In brief, PTA-PBS (pH 7.4, 6.5) was prepared and PCA@HMSNs-CTS were dispersed in 50 mL of PTA-PBS (pH 6.5) with a concentration of

20 μg/mL. The fluorescence spectra of mixtures were measured by using a fluorescence spectrometer (HORIBA FlouoroMAX-4, France) at λ=310 nm after soft-X-Ray initiation (60 kV, 8 mA). In addition, the fluorescence spectra of PCA@HMSNs-CTS without soft-X-Ray initiation and PTA-PBS with soft-X-Ray initiation was obtained.

To further measure the ROS generation of PCA@HMSNs-CTS with soft-X-Ray initiation, PCA@HMSNs-CTS were dispersed in 10 mL of PTA-PBS (pH 7.4, 6.5) with the concentration of 5, 10 and 20 μg/mL, respectively. The mixtures were irradiated by various intensities of soft-X-Ray initiation (60 kV, 6 mA; 60 kV, 8 mA; 60 kV, 10 mA) for 2 min. The fluorescence spectra of mixtures were measured by using a fluorescence spectrometer at λ=310 nm with or without soft-X-Ray initiation, respectively.

***Cell culture***

4T1 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) with 10% FBS and 1% antibiotics (penicillin-streptomycin) at 37℃ in a humidified atmosphere containing 5% $CO_2$.

***Observation of intracellular uptake of PCA@HMSNs-CTS***

The fluorescence microscopy (Olympus IX71, Japan) was used to observe the intracellular uptake of nanoparticles according to the previous reports.[7] Initially, 4T1 cancer cells were inoculated into culture dishes and incubated for 12 h. Afterwards, the cells were treated with media containing PCA@HMSNs-CTS-FITC (20 μg/mL) for 12 h. Then, the media were removed and the cells were rinsed with PBS, followed by addition of 0.5 mL of 4,6-diamidino-2-phenylindole (DAPI, 10% in methanol) and incubated for 15 min to stain the nuclei. Finally, the cells were rinsed twice with PBS and observed by using the fluorescence microscopy.

***Cytotoxicity by MTT assay***

The cytotoxicity of the nanoparticles was investigated on 4T1 cells. In brief, 4T1 cells were inoculated into a 96-well plate at a cell density of $5\times10^4$ cells per mL and allowed to adhere for 24 h. Then, the media were replaced with fresh media containing different concentrations (5, 10, 20, and 50 μg/mL) of PCA or PCA@HMSNs-CTS, and the cells were incubated for 24 h. Subsequently, the above media were replaced by 20 μL of MTT (5 mg/mL in PBS). After incubated for 4 h, the MTT solution was removed and 150 μL of DMSO was successively added. The optical density (OD) was measured at λ=492 nm by a microplate reader. Meanwhile, the cytotoxicity of HMSNs and HMSNs-CTS with different concentrations (20, 40, 80, and 100 μg/mL) were measured with the same procedure.

For cytotoxicity assays with soft-X-Ray initiation, 4T1 cells were inoculated into a 96-well plate at a cell density of $5\times10^4$ cells per mL and allowed to adhere for 24 h. Then, the media were replaced with fresh media containing PCA@HMSNs-CTS at different concentrations (0, 5, 10, 20, and 50 μg/mL). After incubated for 2 h, the cells were irradiated by various intensities of soft-X-Ray initiation (60 kV, 6 mA; 60 kV, 8 mA; 60 kV, 10 mA) for 2 min. Afterward, the cells were incubated for 24 h and the OD was recorded in the same way. For comparison, cytotoxicity assays without soft-X-Ray initiation were performed by the same procedure. The cell viability was calculated as the percentage of OD relative to the control group.

***Detection of ROS in cancer cells***

The ROS generation in 4T1 cells was examined by fluorescence microscopy using 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) as the ROS indicator, which could be

rapidly oxidized by intracellular ROS to emit green fluorescence.[8] Firstly, 4T1 cells were inoculated into culture dishes and cultured with serum-free media with PCA@HMSNs-CTS (5, 10 and 20 μg/mL). After incubated for 2 h, the cells were irradiated by soft-X-Ray initiation (60 kV, 8 mA) for 2 min and incubated for another 4 h. Secondly, the media were removed and the cells were rinsed with PBS, followed by DCFH-DA (150μL, 10 μM) addition and incubated for 30 min. Thirdly, the cells were rinsed twice with PBS and the fluorescence images were monitored by the fluorescence microscopy. Finally, the cells above were collected and dispersed in media without serum. The fluorescence spectra of DCF in cancer cells at λ=488 nm was obtained by using the fluorescence spectrometer.

***Statistical Analysis***

Quantified data are presented as means ± SDs (n = 6). Significance of differences was analyzed by Student's t-test. $p < 0.05$ and $p < 0.01$ are considered as significant difference and extremely significant difference, respectively.

## Supporting Figures

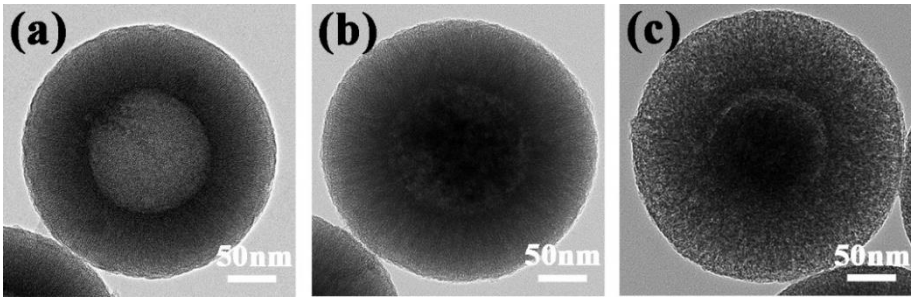


**Figure S1.** TEM images of (a) HMSNs. (b) PCA@HMSNs and (c) PCA@HMSNs-CTS.

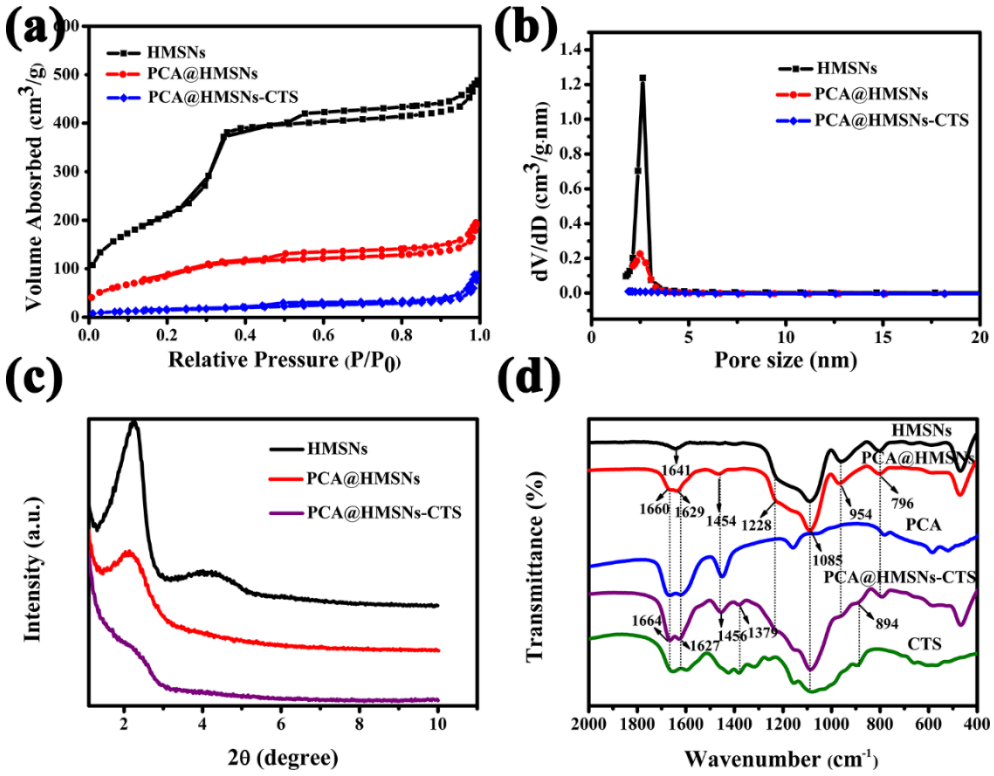


**Figure S2.** (a) Adsorption-desorption isotherms of HMSNs, PCA@HMSNs, and PCA@HMSNs-CTS. (b) Pore size distributions of HMSNs, PCA@HMSNs, and PCA@HMSNs-CTS. (c) Low-angle XRD patterns of HMSNs, PCA@HMSNs, and PCA@HMSNs-CTS. (d) Fourier transform infrared (FTIR) spectra of HMSNs, PCA@HMSNs, PCA, PCA@HMSNs-CTS, and CTS.

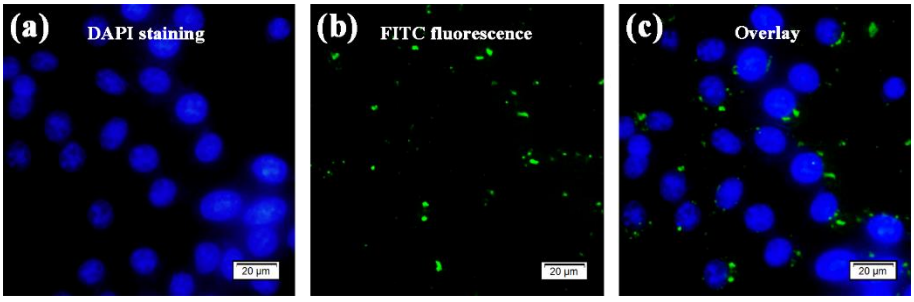


**Figure S3.** The fluorescence microscopy image of 4T1 cells incubated with PCA@HMSNs-CTS-FITC. (a) Blue fluorescence image of cell nuclei stained with DAPI. (b) Green fluorescence of PCA@HMSNs-CTS-FITC in cells. (c) Merged fluorescence image.

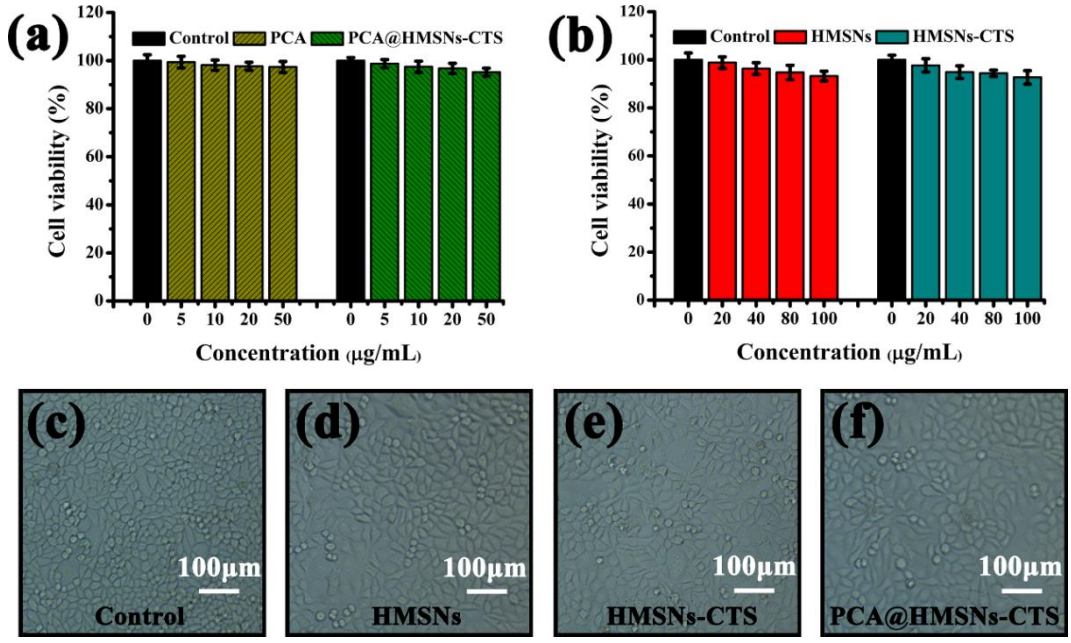


**Figure S4.** (a) The cytotoxicity of PCA and PCA@HMSNs-CTS against 4T1 cells after 24 h incubation. (b) The cytotoxicity of HMSNs and HMSNs-CTS against 4T1 cells after 24 h incubation. HMSNs, HMSNs-CTS, free PCA and PCA@HMSNs-CTS showed good biocompatibility with negligible cytotoxicity. (c-f) Microscopy images of 4T1 cells cultured with different samples. The morphology of 4T1 cell in experimental groups was well maintained with favorable adherence.

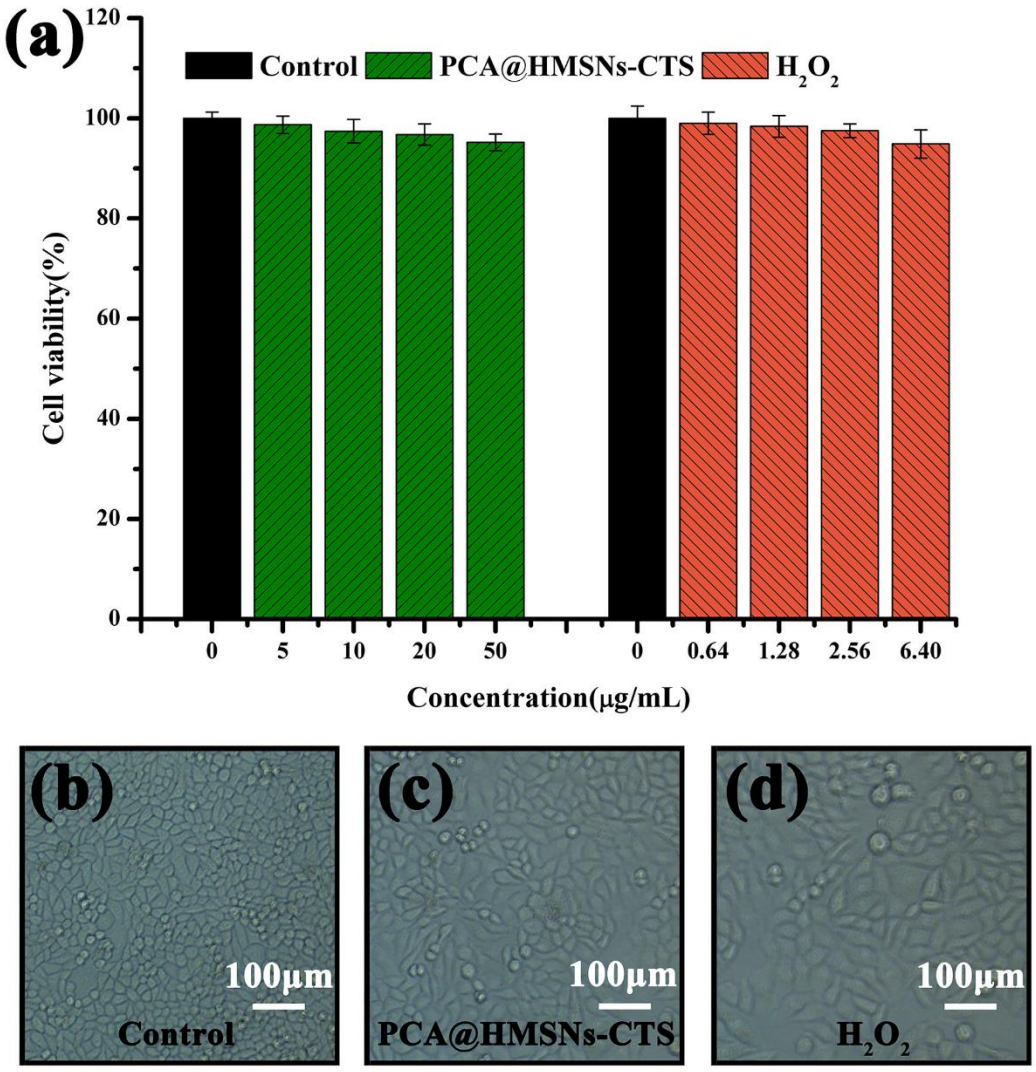


**Figure S5.** (a) The cytotoxicity of PCA@HMSNs-CTS and equivalent amount of $H_2O_2$ against 4T1 cells after 24 h incubation. Microscopy images of 4T1 cells cultured with media solution alone (b), PCA@HMSNs-CTS (20 μg/mL) (c) and $H_2O_2$ (2.56 μg/mL) (d).

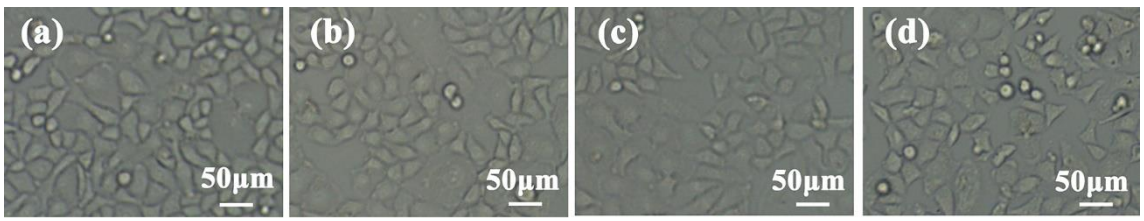


**Figure S6.** (a-d) Bright field images of 4T1 cells incubated with PCA@HMSNs-CTS (0, 5, 10 and 20 μg/mL) and irradiated by soft-X-Ray (60 kV, 8 mA) in the experiment to measure the fluorescence density in cells.

# References


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