Electronic Supplementary Material

Cardiomyocyte-targeted anti-inflammatory nanotherapeutics against myocardial ischemia reperfusion (IR) injury

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1. Experimental section

1.1 Material, cell, and animals

All chemicals and solvents were purchased from Energy Chemical (Shanghai, China) or Sinopharm (Shanghai, China), and were used as received unless otherwise indicated. Low-molecular-weight polyethylenimine (PEI 600, branched, MW = 600 Da) and high-molecular-weight polyethylenimine (PEI 25k, branched, MW = 25 kDa) were purchased from Sigma-Aldrich (St. Louis, MO, USA). RAGE-siRNA (siRAGE), siRNA with scrambled sequence (siScr), and primers were purchased from GenePharma (Shanghai, China), and their sequences were shown in Table S1 and S2. Rhodamine B isothiocyanate was purchased from Sangon Biotech (Shanghai, China). All antibodies were purchased from Abcam (Shanghai, China). BCA protein assay kit was purchased from Alfa (Shanghai, China). Lysotracker Red and Hoechst 33258 were purchased from Invitrogen (Carlsbad, CA, USA). Hydrogen peroxide (H_2O_2), tetraethyl orthosilicate (TEOS), and *N*-cetyltrimethylammonium bromide (CTAB) were obtained from Aladdin (Shanghai, China). Amine-poly(ethylene glycol)-carboxyl (NH_2-PEG_{2k}-COOH, MW = 2 kDa), poly(ethylene glycol)-carboxyl (PEG_{2k}-COOH, MW = 2 kDa), and prostaglandin E_2 (PGE_2) were obtained from Xirui Biotechnology Co., Ltd (Xi'an, China). APC-labeled anti-Cardiac Troponin T was purchased from Yanbo Biotechnology Co., Ltd (Shanghai, China).

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H9C2 (rat cardiomyocyte) cells were purchased from Bioleaf (Shanghai, China) and were cultured in DMEM containing 10% fetal bovine serum (FBS) at 37 °C in 5% CO_2 atmosphere.

Male Sprague-Dawley (SD) rats (250-300 g, 8-10 weeks) were obtained from Shanghai Slaccas Experimental Animal Co., Ltd. (Shanghai, China) and housed in a clean room, with access to water *ad libitum*, at a 12:12 h light-dark cycle (7:00 am-7:00 pm) and a temperature of 25 ± 1 °C. All the animal experimental protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Soochow University. The animal experimental protocols were performed in accordance with the NIH guidelines for the care and use of laboratory animals (NIH Publication No. 85-23 Rev. 1985).

1.2 Instrumentation

Nuclear magnetic resonance (NMR) spectra were recorded on an Agilent 400 MHz spectrometer. Gel permeation chromatography (GPC) analysis was performed on a Waters 1515 LC system (USA) equipped with PL aquagel-OH MIXED-Mx2 column and a differential refractometer (Model 2414). Acetate buffer (0.1 M, pH 2.8) was used as the eluent at a flow rate of 0.5 mL/min at 60 °C, and PEG standards were used for the calibration of the columns. The particle size and zeta potential of NCs were measured on a Malvern Zetasizer Nano ZS90 with a He–Ne laser (633 nm) with 90° collecting optics. Transmission electron microscopy (TEM, FEI Tecnai F20, acceleration voltage =200 kV) was used to observe the size and morphology of NCs. Gel documentation (GE Amersham Imager 600, Boston, USA) was used to observe the siRNA bands after agarose gel electrophoresis. High performance liquid chromatography (HPLC, Agilent 1200 Infinity Series, US) was used to analyze the Dex concentration. Flow cytometric analysis was recorded on the flow cytometer (FCM, FACS Calibur, BD, USA). Confocal laser scanning microscopy (CLSM, TCS SP5, Leica, Germany) was used to observe the endosomal escape and intracellular siRNA release. Echocardiographic measurement was performed on a Philips high-resolution ultrasound system.

1.3 Synthesis and characterization of ditellurium-crosslinked polyethylenimine (TP)

Sodium borohydride (3.8 g, 100 mmol) was dissolved in water (30 mL), into which tellurium powder (6.4 g, 50 mmol) was added under nitrogen atmosphere at room temperature (RT). The mixture was stirred for 30 min at 105 °C. An additional equivalent of tellurium powder (6.4 g, 50 mmol) was added, and the mixture was stirred for another 30 min at 105 °C. After the mixture was cooled to RT, 2-bromoethanol (12.5 g, 100 mmol) dissolved in tetrahydrofuran (50 mL) was added under nitrogen atmosphere and stirred for another 24 h. The final product (**compound 1**) was obtained as yellow liquid (6.2 g, yield 24.5%) after the mixture was extracted with chloroform (150 mL × 3), washed twice with water, dried over anhydrous Na_2SO_4, and purified by column chromatography on silica gel using ethyl acetate/hexane (1/1, v/v) as the eluent. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.88 (t, 4H, OCH_2), 3.28 (t, 4H, $TeCH_2$) (Fig. S1).

Compound 1 (1 g, 9.2 mmol) dissolved in dichloromethane (15 mL) was mixed with triethylamine (1.5 g, 14.5 mmol) on ice, into which acryloyl chloride (0.8 g, 8.7 mmol) was added dropwise under continuous stirring. The mixture was stirred overnight before the mixture was extracted with ethyl acetate, dried overnight with anhydrous Na_2SO_4, and purified *via* column chromatography on silica gel using hexane/ethyl acetate (20/1, v/v) as the eluent. The final product (**compound 2**) was obtained as deep reddish brown liquid (0.45 g, yield 25.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.58 (d, 2H, CH_2CH); 6.05 (d, 2H, CHCO); 5.79 (dd, 2H, CH_2CH); 4.57 (t, 4H, OCH_2); 3.50 (t, 4H, $TeCH_2$) (Fig. S2).

Compound 2 (0.10 g, 0.2 mmol) dissolved in dimethylformamide (2 mL) was mixed with PEI 600 (0.18 g, 0.3 mmol, molar ratio of primary amine/diacrylate = 4:1) dissolved in dimethylformamide (3 mL) under nitrogen atmosphere and stirred at 50 °C for 48 h. The final product (TP) was obtained after dialysis against distilled water (MWCO = 10 kDa) for 2 d and lyophilization (0.13 g, yield 46.4%).

CP, the non-responsive analogue of TP, was synthesized from PEI 600 (0.18 g, 0.3 mmol) and 1,6-hexylene glycol diacrylate (HGDA, 0.05 g, 0.2 mmol) using a similar method (0.07 g, yield 30.4%).

1.4 Synthesis and characterization of PGE_2-PEG_{2k}-TP (PPTP)

PGE_2 (3.5 mg, 0.01 mmol), EDC·HCl (2.3 mg, 0.01 mmol), and NHS (1.4 mg, 0.01 mmol) were dissolved in anhydrous dimethyl sulfoxide (DMSO, 5 mL). After the mixture was stirred at RT for 6 h, NH_2-PEG_{2k}-COOH (20 mg, 0.01 mmol) and 4-dimethylaminopyridine (DMAP, 0.2 mg, 0.002 mmol) were added. The mixture was stirred for another 24 h at RT before dialysis against distilled water (MWCO = 1 kDa) for 2 d and lyophilization. The final product (PGE_2-PEG_{2k}) was obtained as white powder (18 mg, yield 76.6%) (Fig. S3).

PGE_2-PEG_{2k} (12 mg, 0.006 mmol) dissolved in anhydrous DMSO (2 mL) was activated with EDC·HCl (1.4 mg, 0.006 mmol) and NHS (0.9 mg, 0.006 mmol) under nitrogen atmosphere at RT for 6 h. TP (0.1 g) was then added to the above mixture under nitrogen atmosphere. After 24-h reaction at RT, the mixture was dialyzed against deionized water (MWCO = 3.5 kDa) and lyophilized. The final product (PPTP) was obtained as light yellow powder (90 mg, yield 80.4%).

The non-targeted PEG_{2k}-TP (PTP) was similarly synthesized, wherein PEG_{2k}-COOH was used instead of the PGE_2-PEG_{2k}. The final product (PTP) was obtained as a light yellow powder (95 mg, yield 84.8%).

PPTP (10 mg) and rhodamine B isothiocyanate (RhB, 1 mg) were dissolved in PBS (0.1 M, pH = 8) and stirred overnight in the dark. The mixture was dialyzed against ethanol/deionized water (1/1, v/v, MWCO = 3.5 kDa) for 3 d and then against deionized water for 1 d in the dark. RhB-labeled PPTP (RhB-PPTP) was obtained as reddish powder after lyophilization.

1.5 Preparation and characterization of MSNs

MSNs were prepared *via* a typical sol-gel method [1, 2]. Briefly, CTAB (0.5 g, 1.37 mmol) and NaOH (0.14 g, 0.25 mmol) were dissolved in deionized water (200 mL) and stirred at 80 °C for 20 min. TEOS (2.5 mL) was then added dropwise to the mixture under vigorous stirring. After stirring for another 2 h at 80 °C, the mixture was centrifuged (12000 rpm, 10 min) and washed with deionized water (100 mL × 3) and ethanol (100 mL × 3). Subsequently, the crude MSNs were mixed with HCl/ethanol (1/20, v/v) and stirred for 24 h to remove CTAB. The final MSNs were collected as white powder (0.8 g, yield

34.3%) after centrifugation (12000 rpm, 10 min), washing with methanol (100 mL × 3), and drying under vacuum overnight. The pore size and the surface area of MSNs were characterized using the Brunauer-Emmett-Teller (BET) analysis (ASAP2050, Micromeritics, America) and Barret-Joyner-Halenda (BJH) method [3].

1.6 Preparation and characterization of Dex-loaded, PPTP-coated MSNs NCs (PPTP@MD NCs)

The PPTP stock solution (1 mg/mL in ultrapure water) was added to MSNs in ultrapure water (10 mg/mL) at the PPTP/MSNs weight ratios of 1:1, 1:5, 1:10, 1:15, 1:20, and 1:40. The mixture was stirred for 0.5 h (180 rpm, RT) followed by centrifugation (12000 rpm, 10 min) and washing with deionized water. The resulting PPTP@M NCs were subjected to zeta potential measurement.

To prepare the PPTP@MD NCs, MSNs (10 mg/mL in ethanol, 1 mL) and Dex (2.5, 5, or 10 mg/mL in ethanol, 1 mL) were mixed and stirred for 24 h in the dark. The mixture was centrifuged (10000 rpm, 10 min), and the supernatant was collected to determine the drug loading capacity (DLC) and drug loading efficiency (DLE) of Dex using HPLC [4]. The sediment was collected and resuspended with ultrapure water. PPTP (1 mg/mL in ultrapure water, 1 mL) was then added to the above solution and stirred for another 0.5 h at RT. The final PPTP@MD NCs were collected after centrifugation (12000 rpm, 5 min), washing twice with deionized water, and drying under vacuum. The DLC and DLE of Dex were calculated as follows: DLC (wt%) = $(W_{iniD} - W_{resD})/W_{finMD} \times 100\%$, DLE (wt%) = $(W_{iniD} - W_{resD})/W_{iniD} \times 100\%$. Where W_{iniD}, W_{resD}, and W_{finMD} represent the initial amount of Dex, the residual amount of Dex, and the final amount of PPTP@MD, respectively.

1.7 Preparation and characterization of siRAGE-loaded PPTP@MD NCs (PPTP@MD/R NCs)

PPTP@MD NCs (1 mg/mL in DEPC water) were mixed with siRAGE solution (0.1 mg/mL in DEPC water) at various PPTP/siRNA weight ratios (1, 2, 3, 4, 5, and 6). The mixture was vortexed for 10 s and incubated at 37 °C for 30 min to allow NCs formation. NCs containing siScr instead of siRAGE were similarly prepared (PPTP@MD/S NCs). The abbreviations of all tested NCs were listed in Table S4. Gel retardation assay was used to evaluate the siRNA encapsulation in NCs. Briefly, freshly prepared NCs were subjected to electrophoresis in 2% agarose gel at 90 mV for 20 min before

observation of the siRNA migration using gel documentation. The particle size and zeta potential of freshly NCs were evaluated using the Malvern Zetasizer, and the morphology was observed by TEM.

The serum stability of the PPTP@MD/R NCs was evaluated *via* determination of the particle size alteration after incubation in 10% FBS-containing DMEM for different time. In addition, the siRNA stability against hydrolytic degradation in mouse serum was also evaluated using gel electrophoresis. In brief, PPTP@MD/R NCs or naked siRAGE (5 μg siRAGE, 70 μL) was incubated with mouse serum (100 μL) at 37 °C for 4 h, and the mixture was then heated at 80 °C for 5 min to deactivate the nucleases. Heparin (1000 U/mL, 80 μL) was then added to dissociate the PPTP@MD/R NCs, and the mixture was subjected to electrophoresis in 2% agarose gel (400 ng siRNA/well) at 90 V for 20 min followed by observation of the siRAGE integrity using gel documentation.

1.8 ROS-responsive siRNA/Dex release

The ROS-responsive degradation of PPTP was first monitored by determining the MW change of PPTP after treatment with H_2O_2 (0.1 mM, 24 h) *via* GPC analysis. The non-responsive PPCP was used as the control. Then, the siRNA condensation capacity of PPTP@M NCs and PPCP@M NCs upon ROS treatment was investigated. Freshly prepared PPTP@MD/R NCs and PPCP@MD/R NCs were treated with H_2O_2 (0.1 mM) for various time (0, 2, 4, and 12 h), and then subjected to electrophoresis in 2% agarose gel. Moreover, sizes of the PPTP@MD/R NCs and PPCP@MD/R NCs after treatment with H_2O_2 (0.1 mM, 12 h) were also monitored. To explore the *in vitro* Dex release, freshly prepared MD, PPTP@MD/R NCs, or PPCP@MD/R NCs (0.5 mg Dex/mL, 2 mL) were put into a dialysis tube (MWCO = 3.5 kDa) and immersed in PBS (50 mL, pH 7.4, with or without 0.1 mM H_2O_2). The release medium was kept in a constant temperature shaker (37 °C, 150 rpm). At predetermined time intervals (0.5, 1, 2, 4, 6, 8, 10, and 12 h), an aliquot of the release medium (1 mL) was withdrawn, and equal volume of fresh medium was added to keep a constant volume. The Dex content in the harvested medium was determined by HPLC.

1.9 *In vitro* cellular uptake in cardiomyocytes

H9C2 cells were seeded on 12-well plates (1.5×10^5 cells/well) and cultured for 24 h. Various FAM-

siScr-containing NCs were added (1 μg FAM-siScr/mL, PPTP/FAM-siScr= 6, PTP/FAM-siScr = 6, PEI/FAM-siScr = 1, w/w) and incubated with cells at 37 °C for 4 h under hypoxic condition (1% O_2, 5% CO_2, and 95% N_2). Cells were then washed with heparin-containing PBS (20 U/mL) for three times, and subjected to flow cytometric analysis (FACS Calibur, BD, USA). Cells without any treatment served as the blank control. In a parallel study, H9C2 cells were seeded on 96-well plates (2×10^4 cells/well) and incubated for 24 h. Cells were then treated with various FAM-siScr-containing NCs (1 μg FAM-siScr/mL) as described above. Cells were then washed with heparin-containing PBS (20 U/mL) for three times, and lysed with the RIPA lysis buffer (100 μL/well). The FAM-siScr and total protein contents in the lysate were determined by spectrofluorimetry (λ_{ex} = 492 nm, λ_{em} = 518 nm) and the BCA kit, respectively. The uptake level was defined as the amount of FAM-siScr per milligram of cellular protein.

1.10 Endosomal escape and intracellular siRNA release

To evaluate the endosomal escape of NCs, H9C2 cells were seeded on a culture dish (20 mm in diameter, 4×10^4 cells/dish) and cultured for 24 h. Cells were then incubated with FAM-siScr-containing NCs (1 μg FAM-siScr/mL) for 4 h under hypoxic condition (1% O_2, 5% CO_2, and 95% N_2), and washed with heparin-containing (20 U/mL) PBS for three times. After staining with Hoechst 33258 (5 μg/mL, for nucleus, 0.5 h) and Lysotracker Red (200 nM, for endosome/lysosome, 1.5 h), cells were washed with PBS and observed by CLSM.

Then, NCs comprised of FAM-siScr and RhB-PPTP (or RhB-PPCP) were used to evaluate the intracellular siRNA release. H9C2 cells were seeded on a culture dish (20 mm in diameter, 4×10^4 cells/dish) and cultured for 24 h. Cells were then incubated with NCs under normoxic (20% O_2, 5% CO_2, and 75% N_2) or hypoxic (1% O_2, 5% CO_2, and 95% N_2) conditions for 4 h, washed with heparin-containing PBS for three times, stained with Hoechst 33258 (5 μg/mL, 30 min), and observed by CLSM. The colocalization ratios between FAM-siScr and RhB-PPTP (or RhB-PPCP) were quantified using the ImageJ software (Version 1.34).

1.11 *In vitro* gene silencing in H9C2 cells

H9C2 cells were seeded on 6-well plates (2×10^5 cells/well) and cultured for 24 h. Cells were then incubated with various NCs (1 μg siRNA/mL) under hypoxic condition (1% O_2, 5% CO_2, and 95% N_2) at 37 °C for 4 h followed by incubation under normoxic condition (20% O_2, 5% CO_2, and 75% N_2) for 20 h. After cells were further incubated under hypoxic condition (1% O_2, 5% CO_2, and 95% N_2) for another 6 h, and the gene silencing efficiency was determined by real-time PCR. Total RNA was collected from cells using the Trizol reagent (Biosharp, Shanghai, China). Reverse transcription of mRNA was carried out using a PrimeScript RT reagent kit (Takara Bio, Shiga, Japan) to obtain cDNA from 500 ng of total RNA. Synthesized cDNA, RAGE or TNF-α primers (forward and reverse), and SYBR Premix Ex Taq (Takara Bio, Shiga, Japan) were mixed and run on the real-time PCR system (Bio-Rad CFX connect). All samples were analyzed for GAPDH expression in parallel in the same run. The gene silencing efficiency was denoted as the percentage of RAGE or TNF-α mRNA level of control cells without NCs treatment.

1.12 *In vitro* cytotoxicity

H9C2 cells were seeded on 96-well plates (2×10^4 cells/well) and cultured at 37 °C for 24 h. PPTP@M/R NCs or PPCP@M/R NCs with or without H_2O_2 pre-treatment (0.1 mM, 12 h) were added at various final concentrations. After 4-h incubation, the medium was replaced with fresh medium, and cells were cultured for another 20 h before viability assessment using the MTT assay. Results were represented as percentage viability of control cells without NCs treatment.

1.13 Biocompatibility

To evaluate the biocompatibility of NCs, healthy SD rats were *i.v.* injected with PBS or PPTP@MD/R NCs (300 μg siRNA/kg and 1.54 mg Dex/kg). Blood was collected from the orbital venous at 24 h post injection, and was subjected to hematological and biochemical analyses.

1.14 Establishment of rat myocardial ischemia reperfusion (IR) injury model

Rat myocardial IR injury was induced *via* transient ligation of the left coronary artery as described

previously [5]. Briefly, rats were anesthetized *via* intraperitoneal injection of pentobarbital sodium (80 mg/kg), and were intubated and ventilated mechanically using a rodent ventilator (2 mL/100 g). A left intercostal thoracotomy was performed to expose the heart through a 2-cm incision, and the left anterior descending (LAD) artery was ligated using a 6-0 prolene suture for 30 min. The ischemic condition was confirmed by the evidence of immediate changes, including sudden pallor, distinct dilatation, and paralysis. Then the coronary perfusion was restored by loosening the suture and the chest was closed after expelling the air from the chest cavity using a rubber tube needle. In sham-operated rats, the same procedure was carried out except the LAD ligation in the artery of rats.

1.15 Cardiomyocyte targeting *in vivo*

Rats were *i.v.* injected with FAM-siScr-containing NCs (300 μg siRNA/kg) at 10 min post myocardial IR injury. The hearts were harvested at 4 h post injection, and the infarcted tissue with white color was separated, minced with scissors, digested with collagenase II (450 U/mL), DNase I (60 U/mL), trypsin (60 U/mL), and hyaluronidase (60 U/mL) at 37 °C for 1 h to form single-cell suspension. The suspension was filtered with nylon mesh and cells (2×10^6) were stained with APC-labeled Cardiac Troponin T antibody (1:200), and subjected to flow cytometric analysis. Data were analyzed using the FCS EXPRESS V3 software.

1.16 *In vivo* RAGE silencing and anti-inflammatory efficiency

Rats were *i.v.* injected with saline or various NCs at 1.54 mg Dex/kg and 300 μg siRNA/kg at 10 min post myocardial IR injury. Rat hearts were harvested at 24 h post *i.v.* administration, and the total RNA was collected from the ischemic myocardium using the Trizol reagent (Biosharp), and relative RAGE and TNF-α mRNA levels were determined *via* real-time PCR as described above.

The RAGE and TNF-α protein expression levels in the ischemic myocardium were determined by Western blot analysis, and the concentrations of each antibody used were 1:1000 for anti-RAGE, 1:500 for anti-TNF-α, and 1:5000 for anti-GAPDH.

1.17 Infarct size analysis

Rat hearts were collected on day 3 post myocardial IR injury, cross-sectioned into 2-mm slices, soaked in 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) solution (1%, 20 min, 37 °C), and fixed in paraformaldehyde (4%). The heart sections were scanned using a digital scanner (Cannon, Shanghai, China) to differentiate the normal area (deep red) and infarcted area (white). The infarct size was calculated as the ratio (%) of the cumulative weight of infarcted tissues to that of the entire left ventricle.

1.18 Fibrosis analysis

In a parallel study, rats hearts were harvested on day 7 post myocardial IR injury, fixed in formalin (10%), embedded in paraffin, sectioned (8 μm in thickness), and stained with hematoxylin & eosin (H&E) and Masson's trichrome (MT) followed by histological observation using optical microscopy (Laica, DM4000 M, Germany). The fibrotic area (%) was calculated using the ImageJ software and results were denoted as MT-stained fibrotic area (blue) normalized by the total area.

1.19 Apoptosis analysis

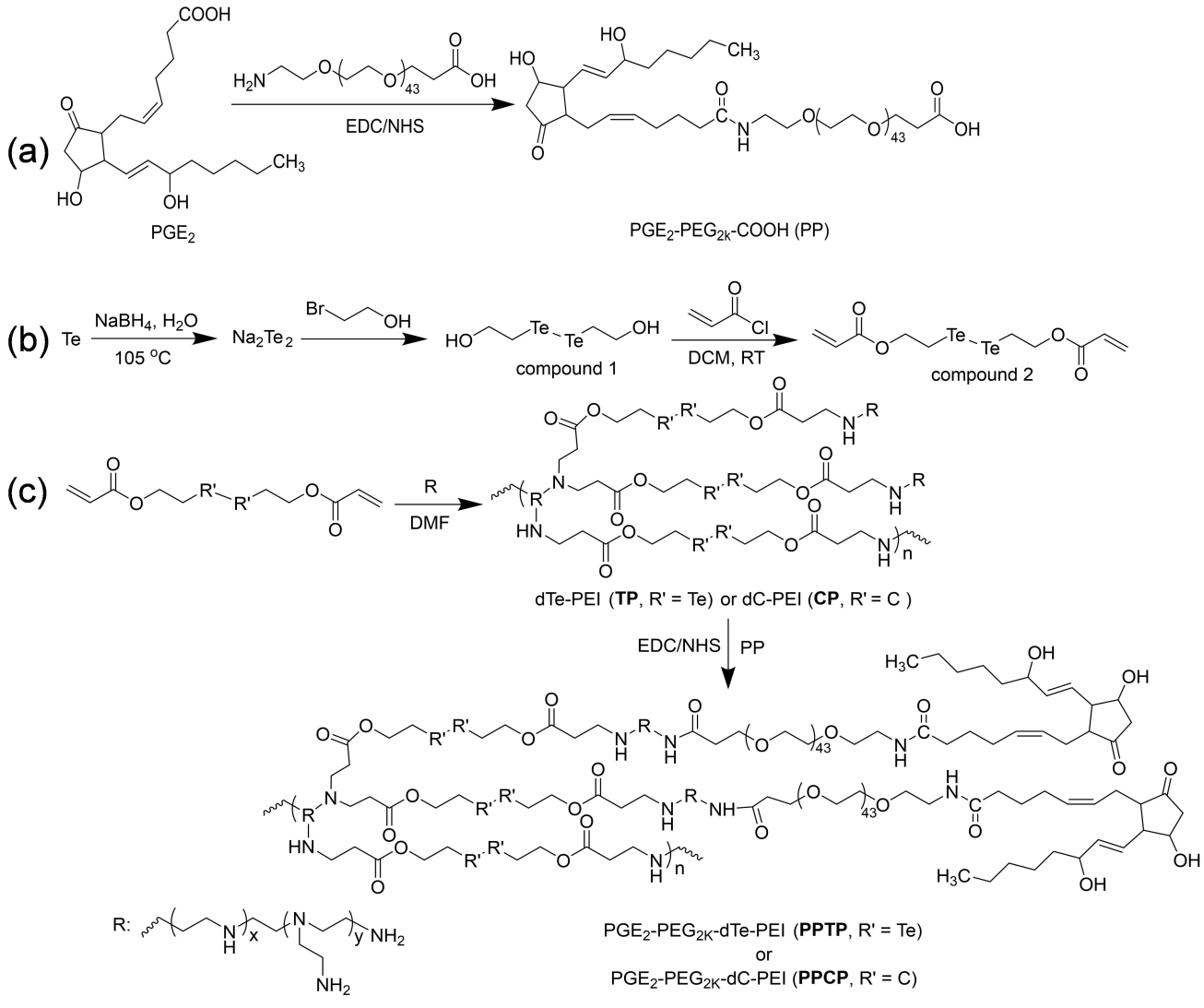
Paraffin tissue slices of heart tissues harvested on day 7 post myocardial IR injury were stained with the Colorimetric TUNEL Apoptosis Assay Kit® (Beyotime Biotechnology, China) and observed by optical microscopy. Three sample slides were prepared per animal, and cell apoptotic ratio (%) was examined over a total of five high-power fields (HPF) per slide.

1.20 Echocardiography

The echocardiographic measurement was performed to evaluate the cardiac function of rats on day 3 post myocardial IR injury. Ejection fraction (EF, %) and fractional shortening (FS, %), two critical parameters, were calculated to evaluate the left ventricular (LV) systolic function. An average of three consecutive cardiac cycles was used for each measurement.

1.21 Statistical analysis

All the experimental data were presented as the mean ± standard deviations, and statistical analysis was performed using one-way ANOVA or Student's t-test. The differences between two experimental groups were assessed to be significant at $*p < 0.05$ and very significant at $**p < 0.01$ and $***p < 0.001$.



Scheme S1 Synthetic routes of PP (a), **compound** 2 (b), PPTP, and PPCP (c).

Table S1 Sequences of siRNA

Name	Sequence
siRAGE sense	CAC UCU ACG AUC CCA AUU CAA dTdT
siRAGE anti-sense	UUG AAU UGG GAU CGU AGA GUG dTdT
siScr sense	UUC UCC GAA CGU GUC ACG UTT
siScr anti-sense	ACG UGA CAC GUU CGG AGA ATT

Table S2 Forward (F) and reverse (R) primer sequences

Name	Sequence
RAGE F	GAA TCC TCC CCA ATG GTT CA
RAGE R	GCC CGA CAC CGG AAA GT
TNF-α F	GCC ACC ACG CTC TTC TGT CT
TNF-α R	CTT GGT GGT TTG CTA CGA CG
GAPDH F	CAT GCC GCC TGG AAA CCT GCC A
GAPDH R	TGG GCT GGG TGG TCC AGG GGT TTC

Table S3 MW and M_w/M_n of polymers with or without treatment with H_2O_2 as determined by GPC

Polymer	M_n (g mol^{-1})	M_w/M_n
PPTP	12500	1.45
PPCP	8200	1.44
PPTP (H_2O_2, 0.1 mM, 12 h)	3000	1.16
PPCP (H_2O_2, 0.1 mM, 12 h)	7800	1.50

Table S4 Full names and abbreviations of various NCs

Abbreviation	Full name
MD	Dex-loaded MSNs
TP@MD NCs	TP-coated MD NCs
PPTP@M NCs	PPTP-coated MSNs NCs
PPTP@MD NCs	PPTP-coated MD NCs
PPCP@MD NCs	PPCP-coated MD NCs
PTP@M/S NCs	siScr-loaded PTP@M NCs
PTP@M/R NCs	siRAGE-loaded PTP@M NCs
PPTP@M/S NCs	siScr-loaded PPTP@M NCs
PPTP@MD/R NCs	siRAGE-loaded PPTP@M NCs
PPTP@MD/S NCs	siScr-loaded PPTP@MD NCs
PPCP@MD/S NCs	siScr-loaded PPCP@MD NCs
PPTP@MD/R NCs	siRAGE-loaded PPTP@MD NCs
PPCP@MD/R NCs	siRAGE-loaded PPCP@MD NCs

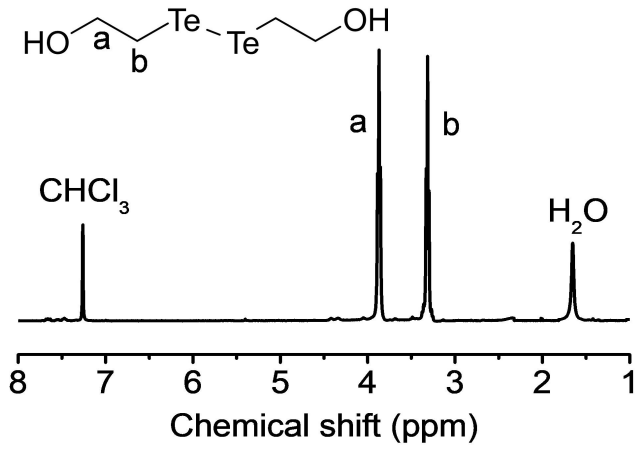


Figure S1 ^{1}H NMR spectrum of **compound 1** ($CDCl_3$, 400 MHz).

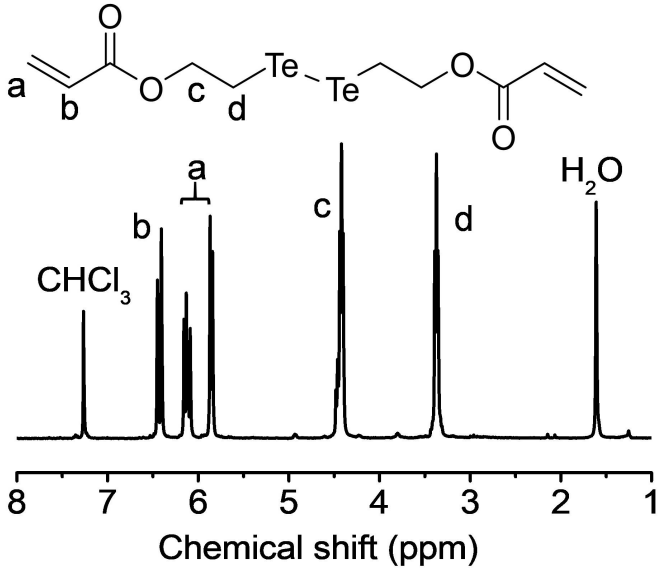


Figure S2 ^{1}H NMR spectrum of **compound 2** ($CDCl_3$, 400 MHz).

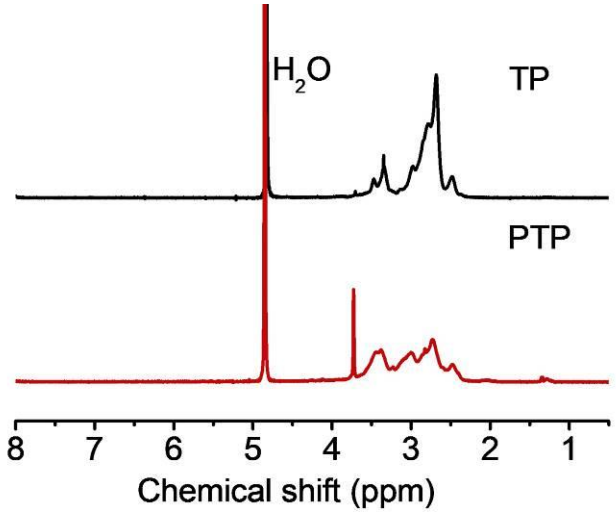


Figure S3 ^{1}H NMR spectra of TP and PTP ($CDCl_3$, 400 MHz).

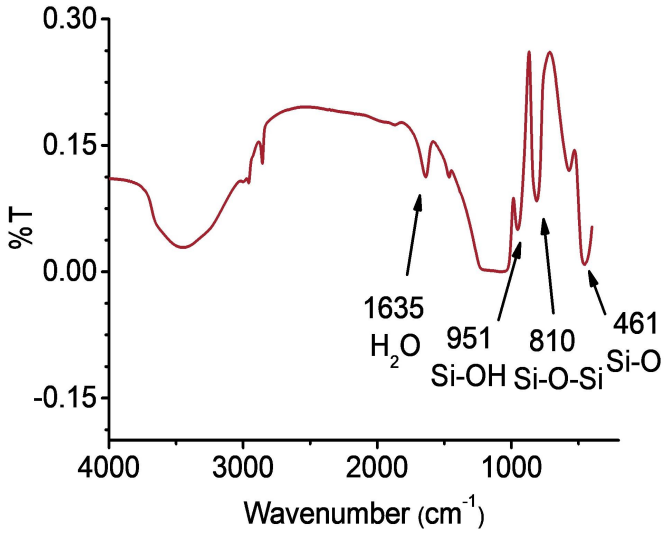


Figure S4 FTIR spectrum of MSNs.

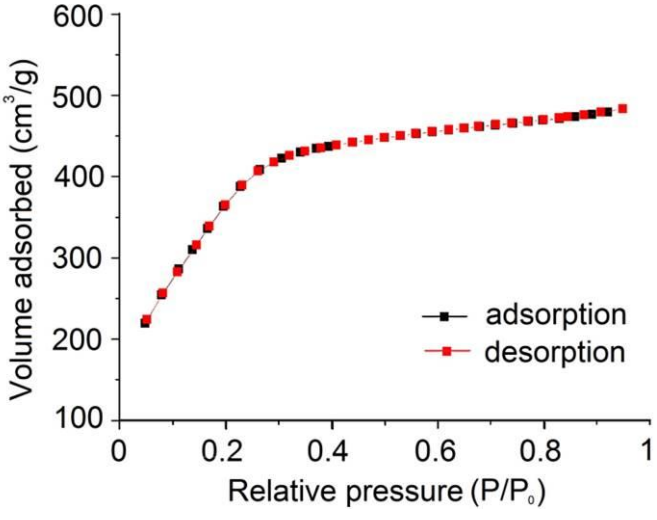


Figure S5 N_2 adsorption/desorption isotherm of MSNs.

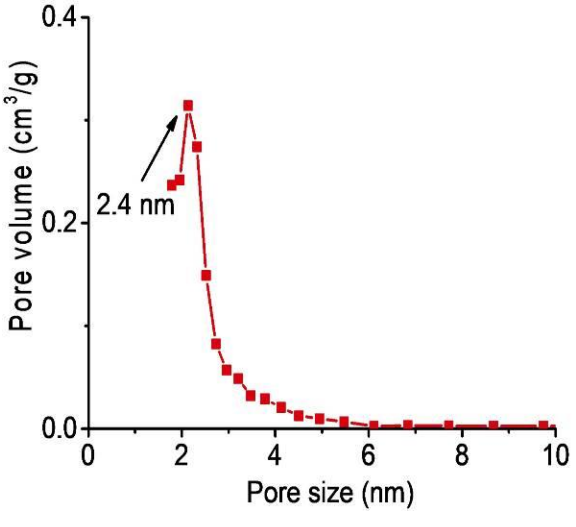


Figure S6 The pore size distribution of MSNs.

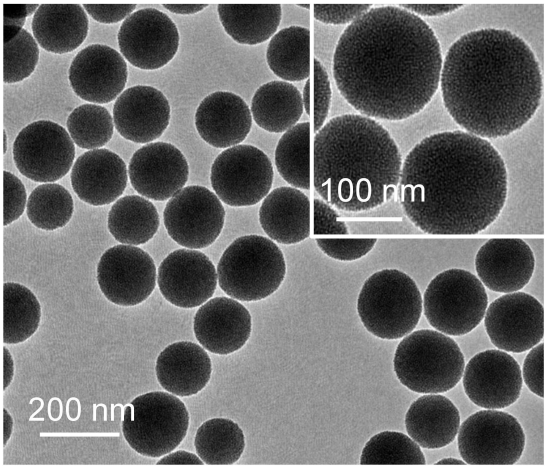


Figure S7 TEM image of MSNs.

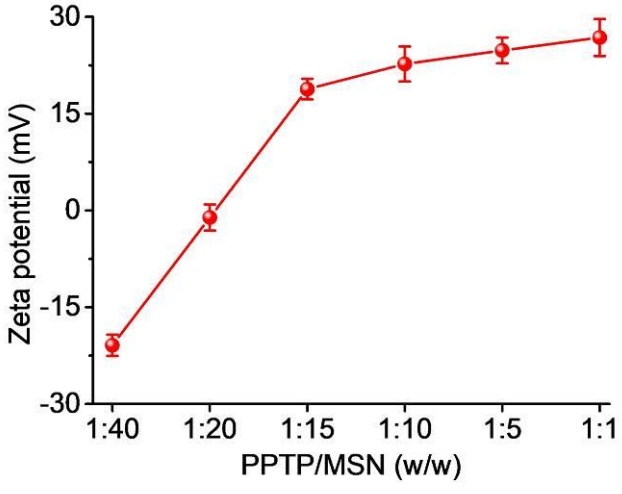


Figure S8 The zeta potential of PPTP@M NCs at various PPTP/MSNs weight ratios (n = 3).

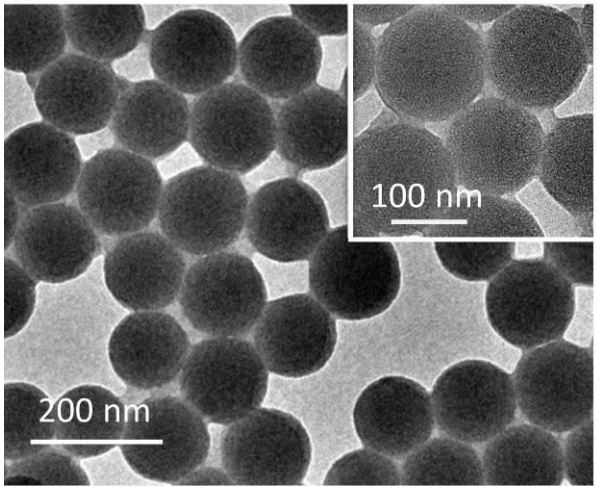


Figure S9 TEM image of PPTP@M NCs (PPTP/MSNs = 1/10, w/w).

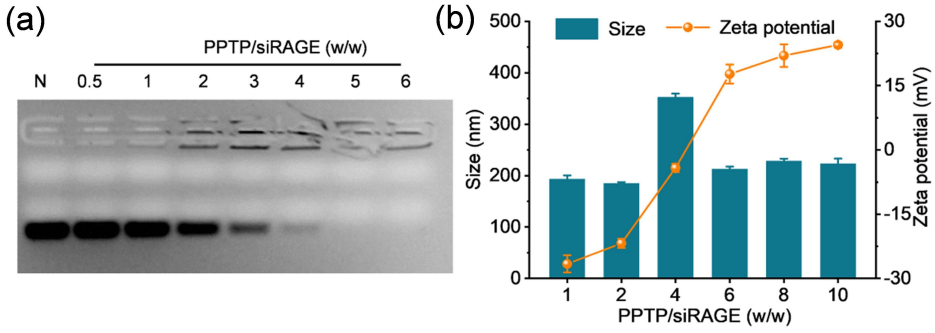


Figure S10 Characterization of PPTP@M/R NCs. (a) siRAGE condensation of PPTP@M NCs at various PPTP/siRAGE weight ratios as evaluated by the gel retardation assay. N represents naked siRAGE. (b) Zeta potential and particle size of PPTP@M/R NCs at various PPTP/siRAGE weight ratios (n = 3).

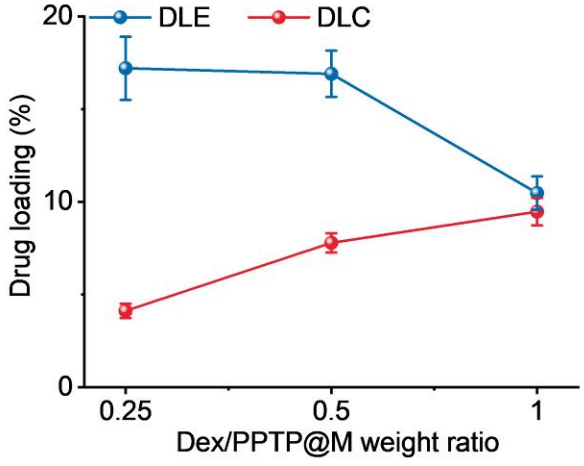


Figure S11 Drug loading content (DLC) and drug loading efficiency (DLE) of PPTP@M NCs at various Dex/PPTP@M weight ratios (n = 3).

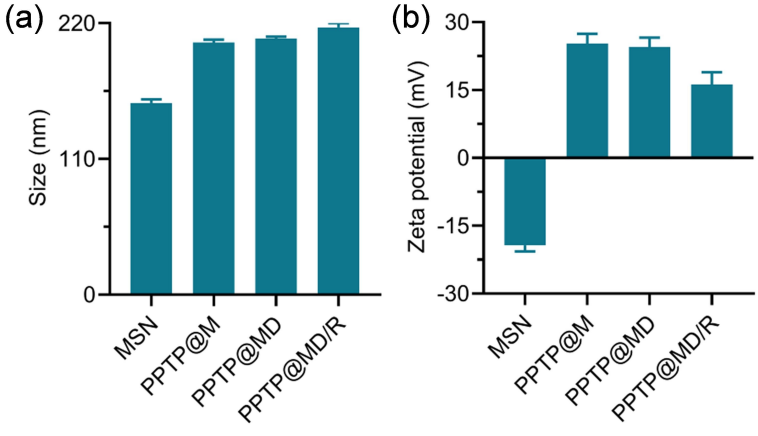


Figure S12 Particle size (a) and zeta potential (b) of MSNs, PPTP@M NCs, and PPTP@MD/R NCs (n = 3).

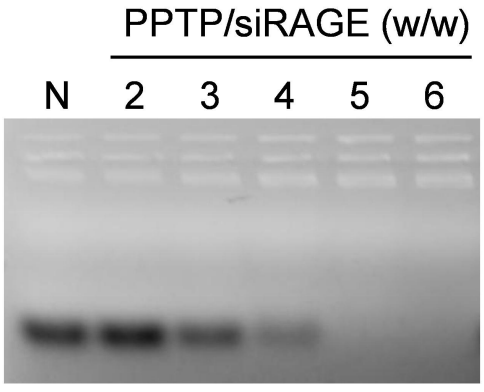


Figure S13 siRAGE condensation by PPTP@MD NCs at various PPTP/siRAGE weight ratios as evaluated by the gel retardation assay. N represents naked siRAGE.

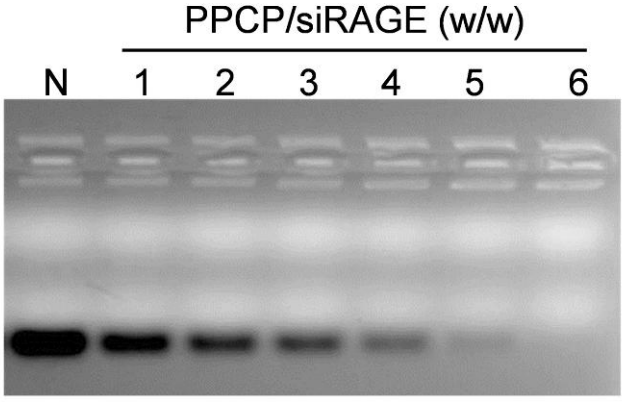


Figure S14 siRAGE condensation by PPCP@MD NCs at various PPCP/siRAGE weight ratios as evaluated by the gel retardation assay. N represents naked siRAGE.

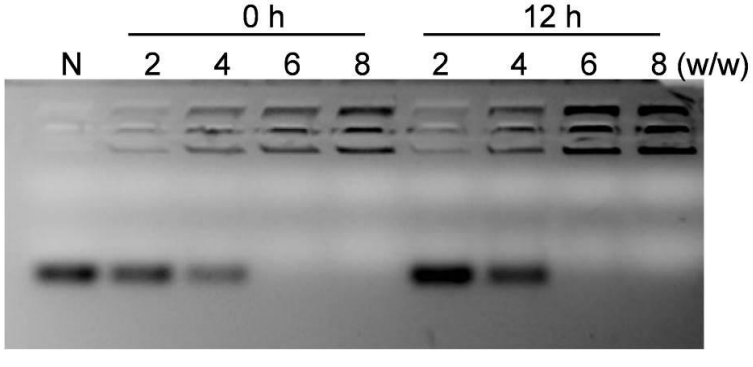


Figure S15 siRAGE release from PPCP@MD/R NCs at various PPCP/siRAGE weight ratios before and after H_2O_2 treatment (0.1 mM, 12 h). N represents naked siRAGE.

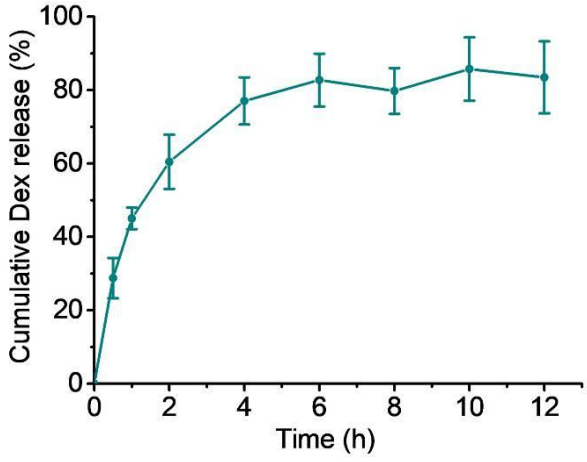


Figure S16 *In vitro* Dex release from MD in PBS (pH = 7.4, n = 3).

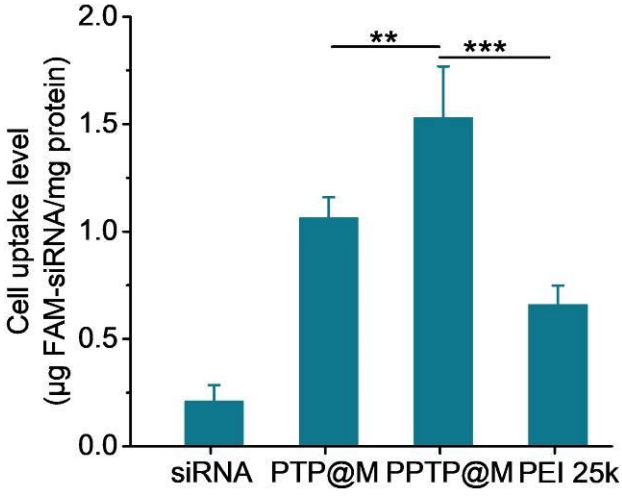


Figure S17 Cell uptake level of naked FAM-siScr and various FAM-siScr-containing NCs (PPTP/FAM-siScr = 6, PTP/FAM-siScr = 6, PEI/FAM-siScr = 1, w/w) in H9C2 cells following 4-h incubation (n = 3).

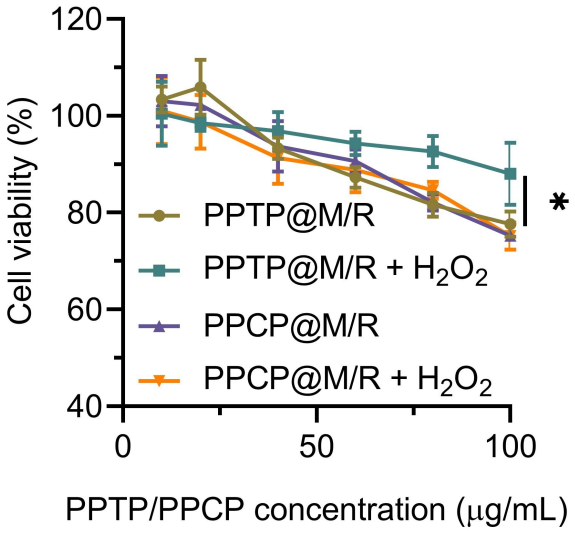


Figure S18 Cytotoxicity of NCs at various PPTP or PPCP concentrations in H9C2 cells (n = 3). For H_2O_2 treatment, NCs were pre-treated with H_2O_2 (0.1 mM) for 24 h before addition to cells.

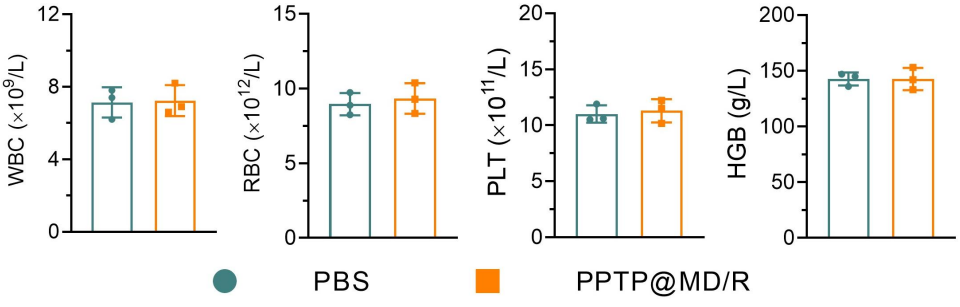


Figure S19 Hematological assessment of rats after *i.v.* injection of PBS or PPTP@MD/R NCs (300 μg siRNA/kg and 1.54 mg Dex/kg, n = 3).

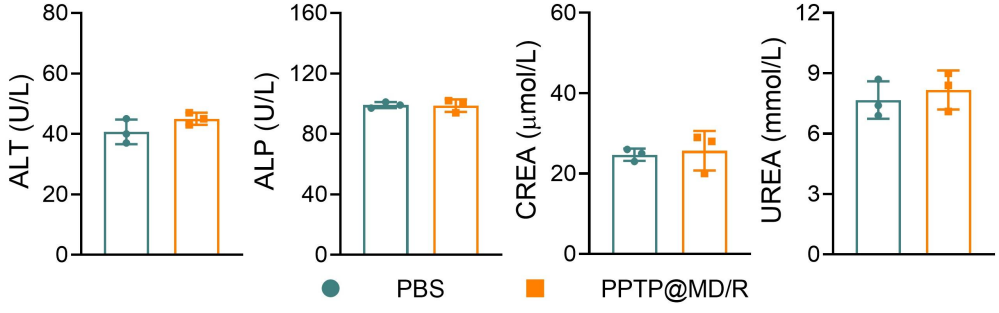


Figure S20 Blood biochemical parameters of rats after *i.v.* injection of PBS or PPTP@MD/R NCs (300 μg siRNA/kg and 1.54 mg Dex/kg, n = 3).

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