Supporting Information

Bioreducible, branched poly(β-amino ester)s mediate anti-inflammatory ICAM-1 siRNA delivery against myocardial ischemia reperfusion (IR) injury

Xiao Wang,[a, #] Qiujun Liang,[a, #] Yiming Mao,[b, c] Rujing Zhang,[d] Qiurong Deng,[a] Yongbing Chen,*[, b] Rongying Zhu,[b] Shanzhou Duan,[b] Lichen Yin*[, a]

[a]Institute of Functional Nano and Soft Materials (FUNSOM), Jiangsu Key Laboratory for Carbon-Based Functional Materials and Devices, Collaborative Innovation Center of Suzhou Nano Science & Technology, Soochow University, Suzhou 215123, China.

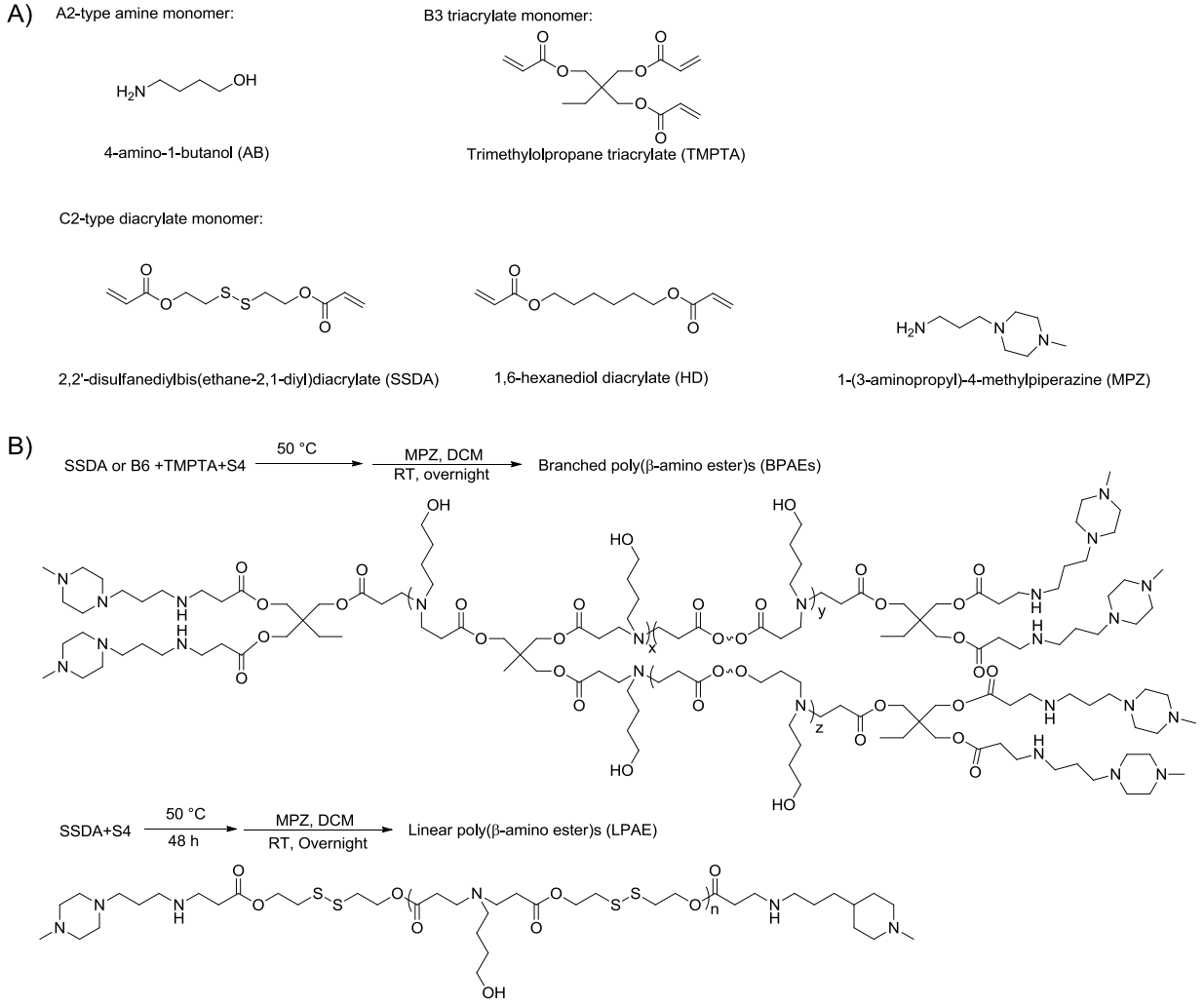
[b]Department of Cardiothoracic Surgery, the Second Affiliated Hospital of Soochow University, Suzhou 214804, China.

[c]Department of Thoracic Surgery, Suzhou Kowloon Hospital Shanghai Jiao Tong University School of Medicine, Suzhou 215123, China.

[d]Department of Micro- and Nanotechnology, DTU Nanotech, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark.

* Corresponding author, Tel.: 86-0512-65882039; Email address: lcyin@suda.edu.cn (L. Yin)

[#] These authors contributed equally to this work.



Scheme S1. Synthetic route of BPAEs via an A2 + B3 + C2 Michael addition reaction. A) Structures of the monomers used. B) Synthetic route of BPAEs and LPAE.

Table S1. Sequences of siRNA.

	Sequence
siICAM-1 sense	GAA AGA UCA GGA UAU ACA AdTdT
siICAM-1 antisense	UUG UAU AUC CUG AUC UUU CdTdT
siNC sense	UUC UUC GAA CGU GUC ACG UTT
siNC antisense	ACG UGA CAC UUC GGA GAA TT

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

Primer	Sequence
ICAM-1 F	TCA AAC GGG AGA TGA ATG G
ICAM-1 R	TCT GGC GGT AAT AGG TGT AAA T
GAPDH F	CAT GCC GCC TGG AAA CCT GCC A
GAPDH R	TGG GCT GGG TGG TCC AGG GGT TTC

Table S3. Feed ratios of monomers for polymer syntheses.

Polymer	SSDA (mg)	B6 (mg)	TMPTA (mg)	S4 (mg)	MPZ (mg)
LPAE	314	0	0	89	157
BPAE-SS	217	0	74	89	157
BPAE-CC	0	187	74	89	157

Table S4. GPC results of polymers.

Polymer	M_n (Da)	M_w (Da)	PDI
LPAE	17700	21500	1.21
BPAE-SS	11200	18800	1.68
BPAE-CC	13400	19500	1.46

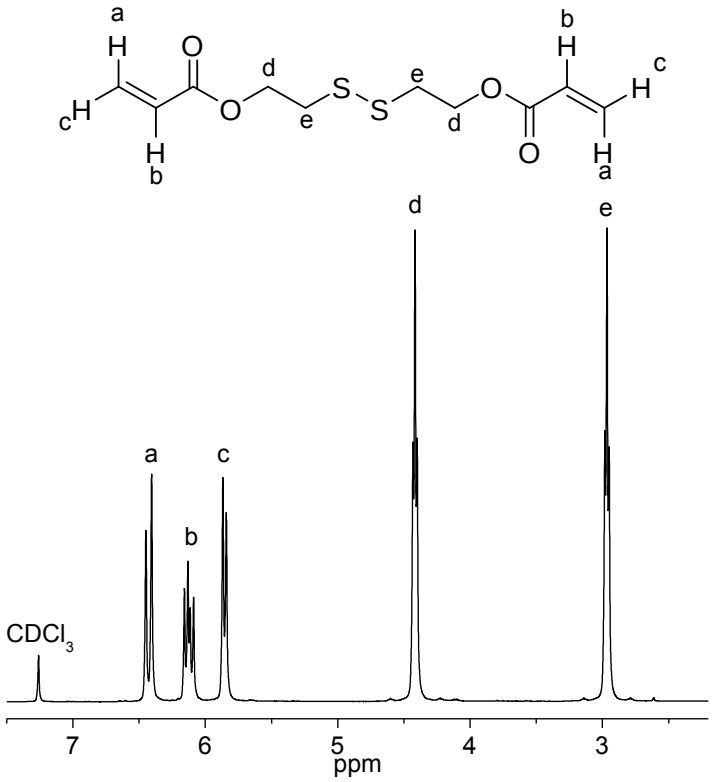


Figure S1. ^{1}H NMR spectrum of SSDA ($CDCl_3$, 400 MHz).

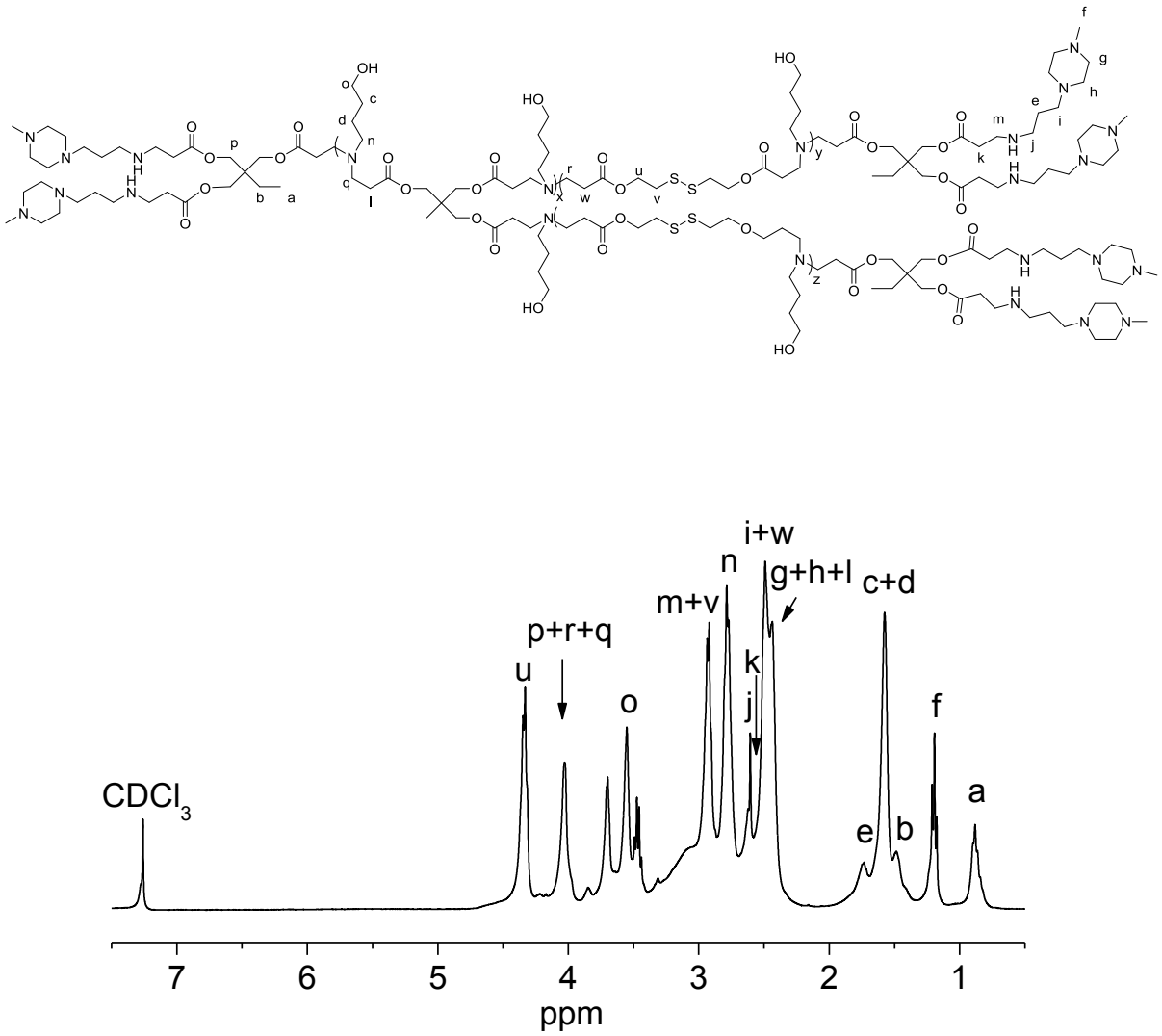


Figure S2. ^{1}H NMR spectrum of BPAE-SS ($CDCl_3$, 400 MHz).

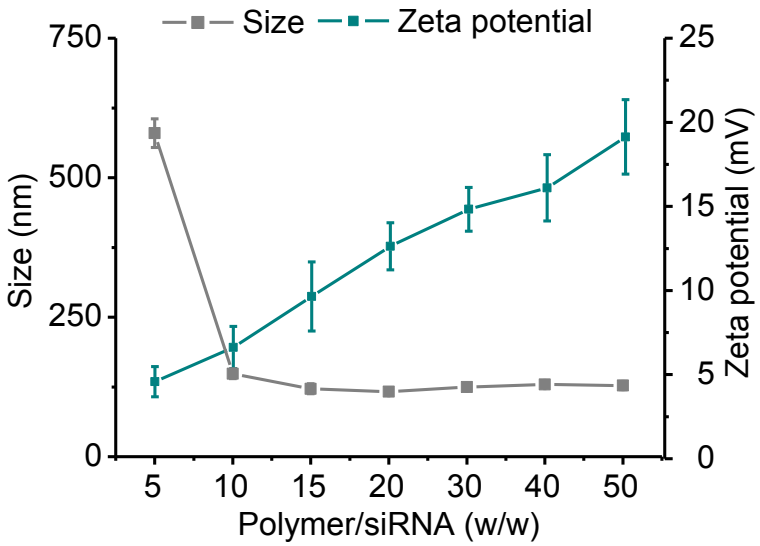


Figure S3. Size and zeta potential of BPAE-SS/siRNA polyplexes at various BPAE-SS/siRNA weight ratios (n = 3).

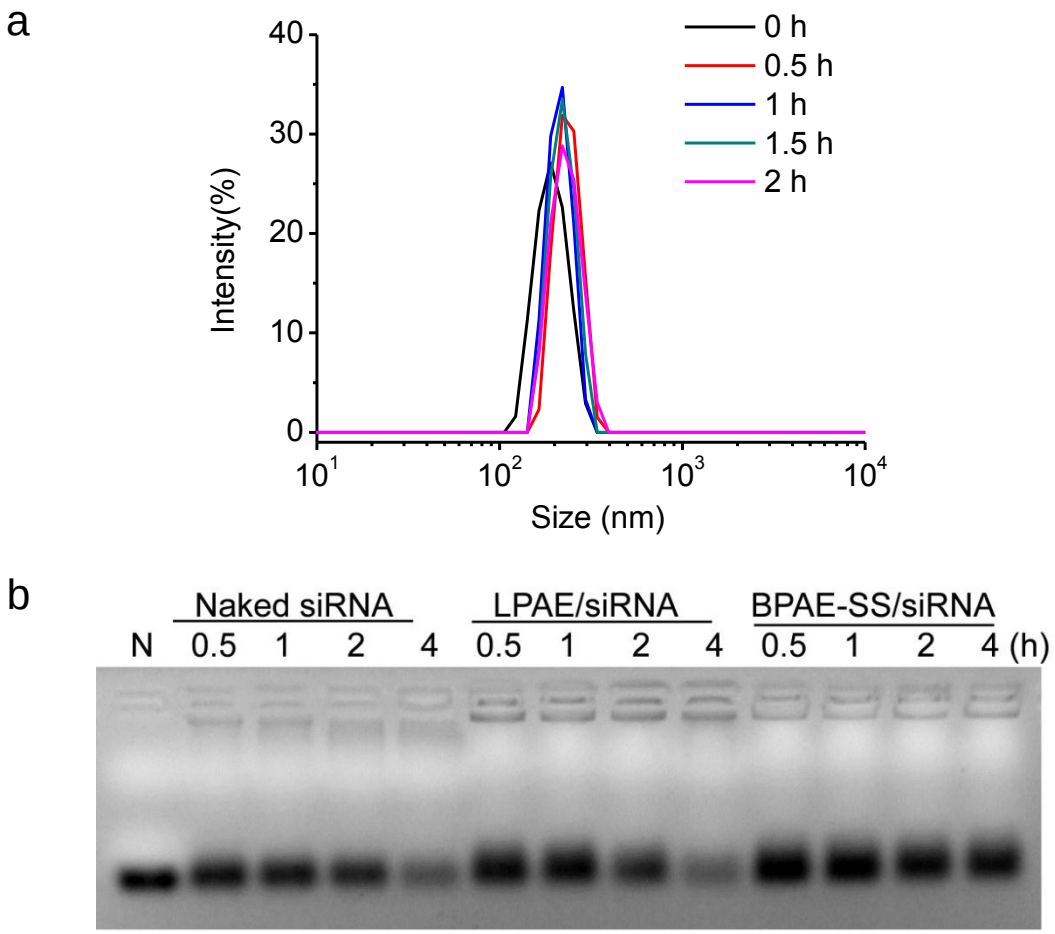


Figure S4. Serum stability of polyplexes. A) Particle size of BPAE-SS/siRNA polyplexes (w/w = 30) after dilution with DMEM containing 10% FBS and incubation for up to 2 h. B) siRNA stability in BPAE-SS/siRNA polyplexes (w/w = 30) or LPAE/siRNA polyplexes (w/w = 30) following treatment with rat serum for different time. N represents naked siRNA without serum treatment. Naked siRNA treated with serum for different time served as a control.

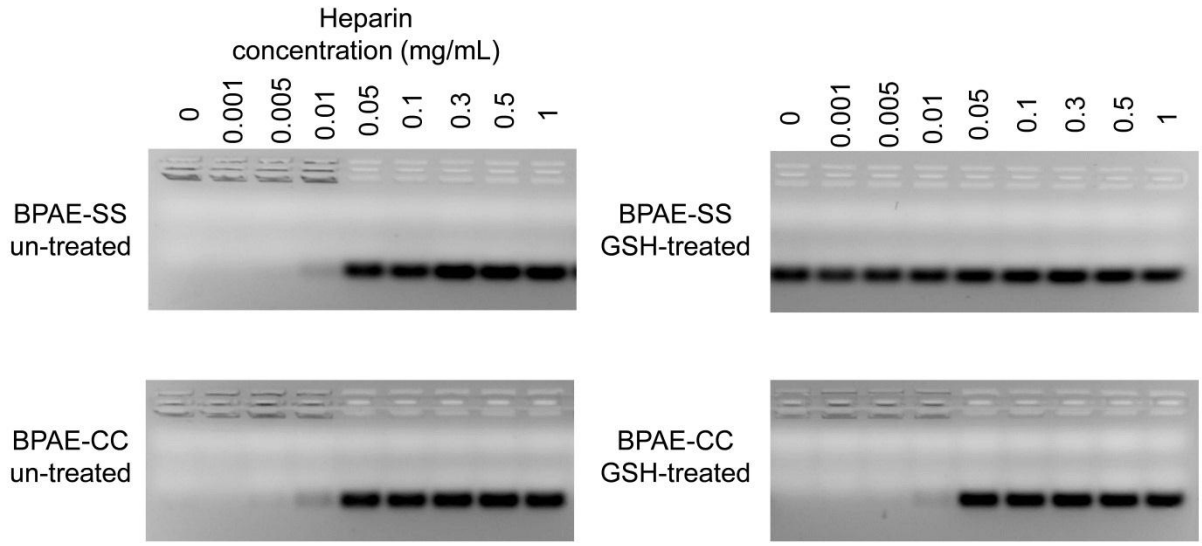


Figure S5. siRNA release from BPAE-SS/siRNA and BPAE-CC/siRNA polyplexes (w/w = 30) in the presence of heparin at various concentrations before and after GSH treatment (5 mM, 1 h).

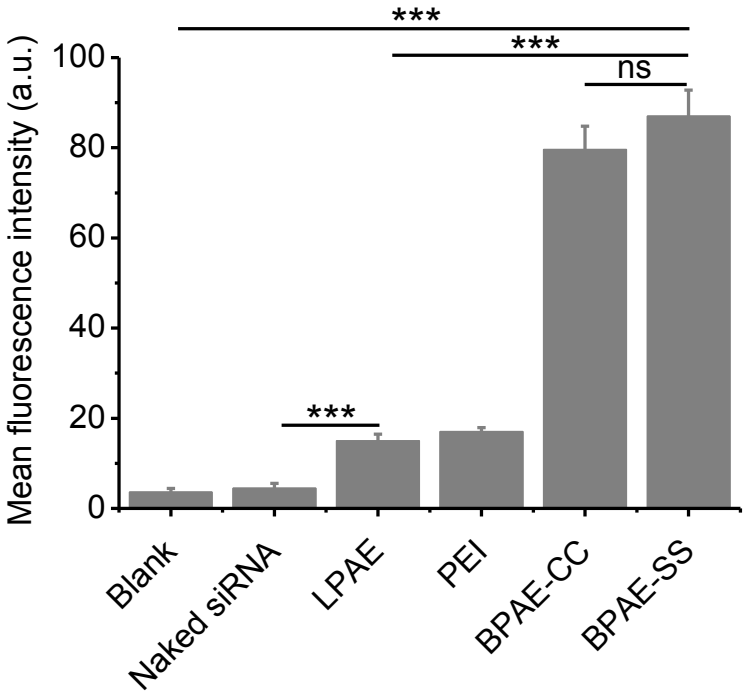


Figure S6. Calculated mean fluorescence intensity per cell from flow cytometric analyses in Figure 2a (n = 3).

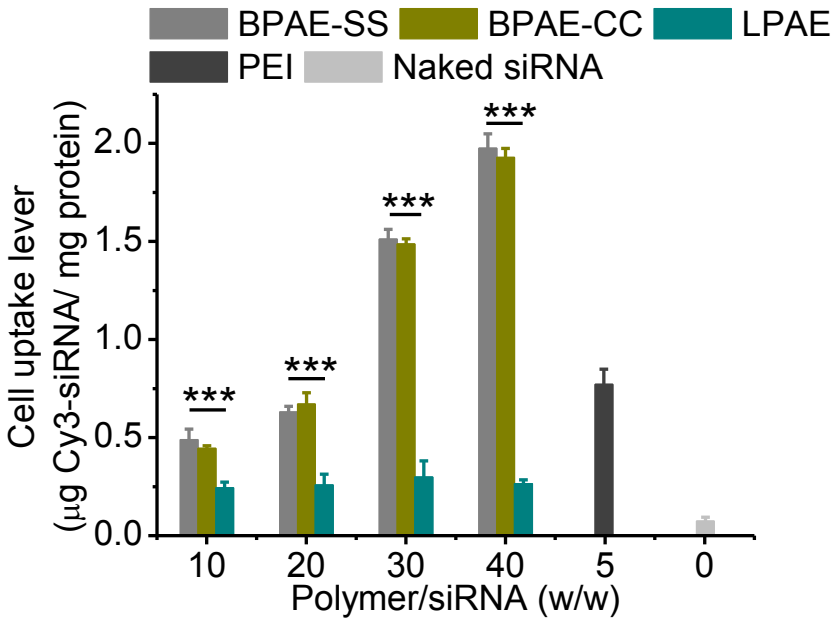


Figure S7. Cell uptake level of polyplexes at various polymer/Cy3-siRNA weight ratios in RCMECs after incubation at 37 ^{o}C for 4 h (n = 3).

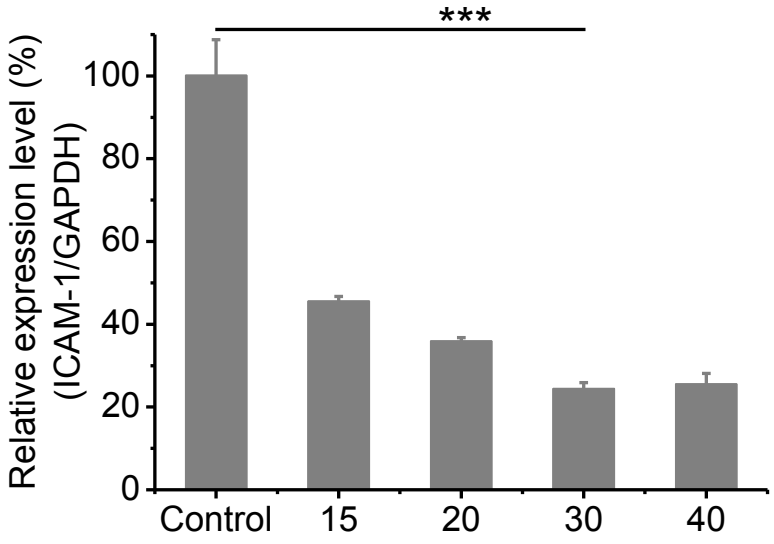


Figure S8. Relative ICAM-1 mRNA levels in RCMECs treated with BPAE-SS/siICAM-1 polyplexes at various polymer/siRNA weight ratios and challenged with LPS (300 ng/mL) (n = 3).

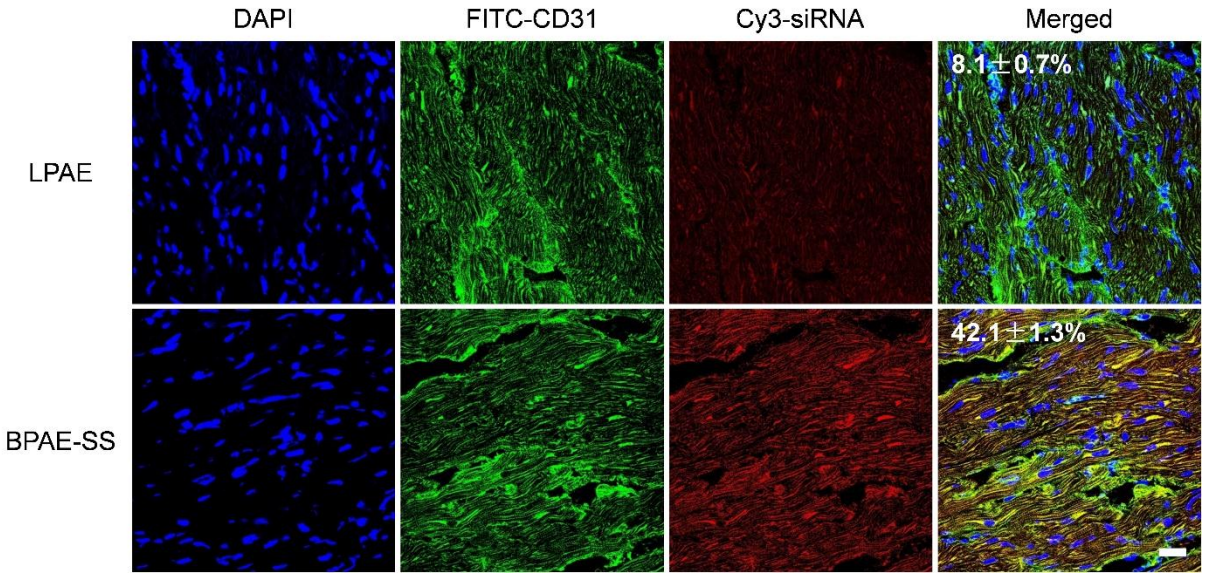


Figure S9. CLSM images of rat heart sections stained with FITC-CD31 (RCMECs, green) at 2 h post *i.v.* administration with BPAE-SS/Cy3-siRNA polyplexes (w/w = 30) or LPAE/Cy3-siRNA polyplexes (w/w = 30) at 400 μg siRNA/kg. Bar represents 25 μm. Colocalization ratios between Cy3-siRNA and FITC-CD31-labeled RCMECs were listed (n = 20).

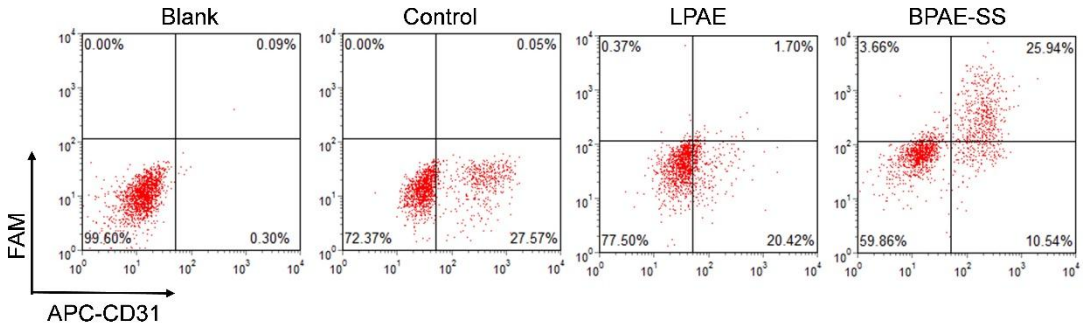


Figure S10. Flow cytometric analyses of cellular internalization in vivo at 2 h post i.v. administration with BPAE-SS/FAM-siRNA polyplexes (w/w = 30) or LPAE/FAM-siRNA polyplexes (w/w = 30) at 400 μg siRNA/kg. RCMECs cells were labelled by APC-CD31.

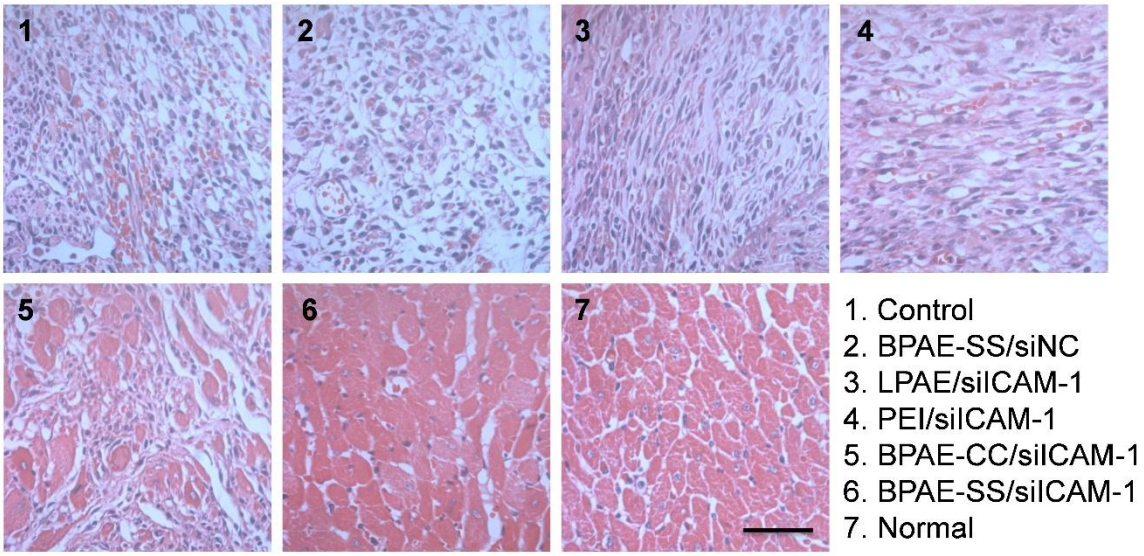


Figure S11. Hematoxylin/eosin (HE)-stained rat ischemic myocardium on day 7 after IR injury and siICAM-1 delivery. Bar represents 50 μm.