Supporting Information

Enzyme Responsive Mesoporous Silica Nanoparticles for Targeted Tumor Therapy *in vitro* and *in vivo*

*Junjie Liu, Beilu Zhang, Zhong Luo, Xingwei Ding, Jinghua Li, Liangliang Dai, Jun Zhou, Xiaojing Zhao, Jingya Ye, Kaiyong Cai**

Key Laboratory of Biorheological Science and Technology, Ministry of Education, College of Bioengineering, Chongqing University, Chongqing 400044, PR China

* Corresponding author: Prof. Kaiyong Cai

College of Bioengineering

Chongqing University,

Chongqing 400044,

P. R. China

Tel: +86-23-65102507

Fax: +86-23-65102877

E-mail: kaiyong_cai@cqu.edu.cn

List of contents

Figure S1. FITR spectra of different MSNs.……..…...S3

Figure S2. TGA curves of different MSNs.…….....……..S5

Figure S3. Cleavage assay of the peptide detected by HPLC and MS……..S6

Figure S4. Cytotoxicity assay of bare MSNs……S8

Figure S5. Dose-dependent cytotoxicity of MSNs-HSA-PBA@DOX.....S9

Figure S6. Histological analysis for tumor tissues.…….....…….…….S10

Figure S7. Biodistribution of MSNs and MSNs-HSA-PBA……..…….S11

Table S1. BET and BJH parameters of different MSNs….....S12

Table S2. Zeta-potentials of different MSNs ….....S12

References for Supporting Information.....S13

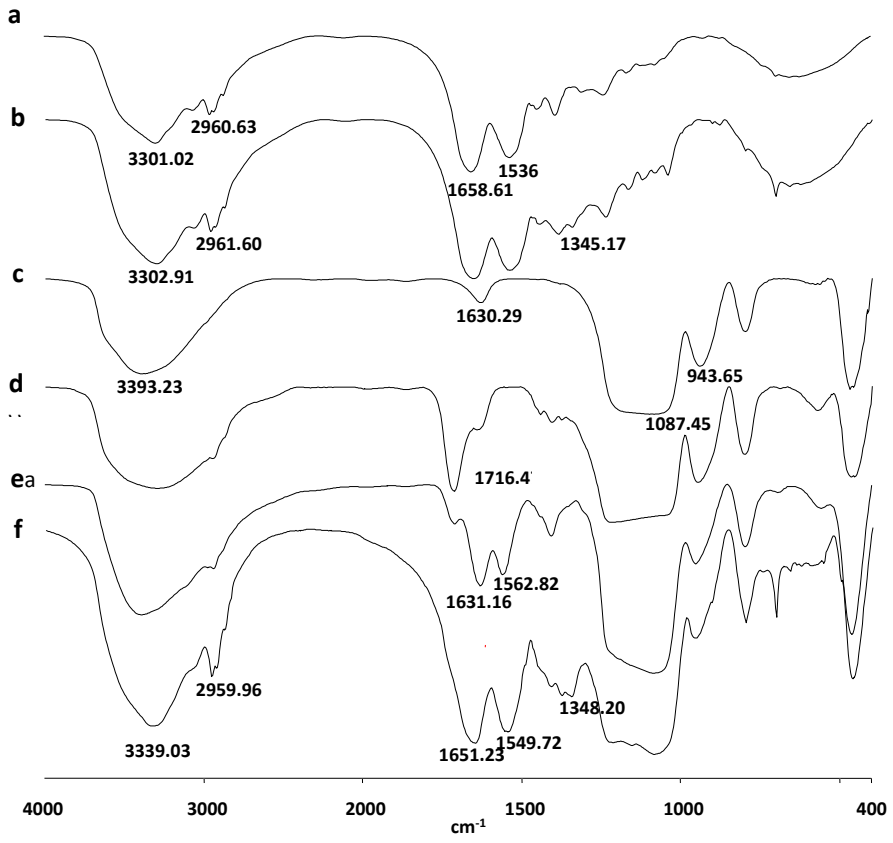


Figure S1. FI-TR spectra of (a) HSA, (b) PBA-HSA, (c) MSNs, (d) MSNs-COOH, (e) MSNs-polypeptide, and (f) MSNs-HSA-PBA, respectively.

FTIR was further used to monitor the synthesis of PBA-HSA and verify the chemical reactions at each step of surface functionalization of MSNs (**Fig. S1**). HSA displayed strong peaks at 1658.6 cm^{-1} and 1536 cm^{-1}, which were attributed to amide Ⅰ and amide Ⅱ of -CO-NH- groups in HSA molecules (**Fig. S1**, **a**).[1] Comparing with

HSA, PBA-HSA displayed new characteristic absorption peak at 1345 cm^{-1}, which was assigned to the stretching vibration of B-O bond in phenylboronic acid molecules (**Fig. S1**, **b**).[2] The result suggests that PBA-HSA molecules were successfully synthesized. Bare MSNs displayed strong absorption peaks at 1087 cm^{-1} and 943 cm^{-1}, which were ascribed to the asymmetric stretching vibration of Si-O-Si bridges and stretching vibration of C-O bonds.[1] The peaks of 3393 cm^{-1} and 1630 cm^{-1} were contributed to silicon hydroxyl groups (**Fig. S1, c**). MSNs-COOH showed additional strong absorption peak at 1716 cm^{-1}, which was assigned to the carbonyl (C=O) in carboxyl groups (**Fig. S1**, **d**). The result shows that 3-(triethoxysilyl) propylsuccinic anhydride molecules have been grafted to MSNs and the propylsuccinic anhydride groups were converted into carboxyl groups. Compared with MSNs-COOH, the intensity of carboxyl absorption peak (around 1716 cm^{-1}) of MSNs-polypeptide sharply decreased. The result suggests that the amino groups in polypeptide molecules have reacted with carboxyl groups of MSNs-COOH. The surplus absorption at peak of 1716 cm^{-1} could be contributed to the carboxyl groups in polypeptide molecules. Furthermore, additional peaks at 1631.1 cm^{-1} and 1562.8 cm^{-1} could be observed, which were contributed to the amide I and amide II in the polypeptide molecules (**Fig. S1, e** vs **d**). The result indicates that polypeptide molecules were covalently grafted onto the surface of MSNs, resulting in MSNs-polypeptide. After further grafting with PBA-HSA molecules (MSNs-HSA-PBA), the amide I and amide II shifted from 1631.1 cm^{-1} to 1651.2 cm^{-1} and from 1562.8 cm^{-1} to 1549.7 cm^{-1}, respectively. More importantly, a new peak at 1348.2 cm^{-1} was observed, which was assigned to the vibration of B-O bonds in phenylboronic acid molecules (**Fig. S1, f** vs **e**).[2] All results again suggest that MSNs-HSA-PBA system was successfully constructed step by step.

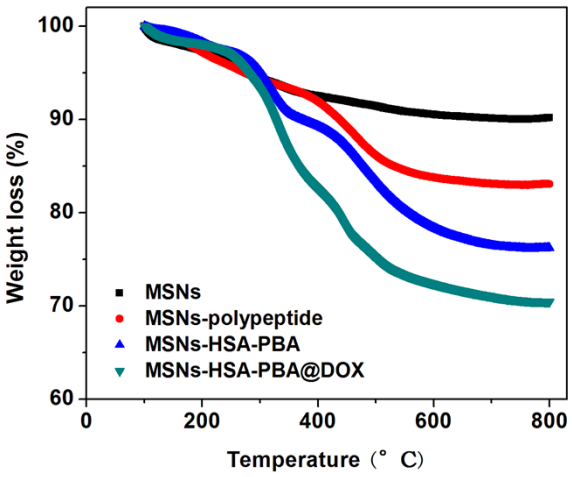
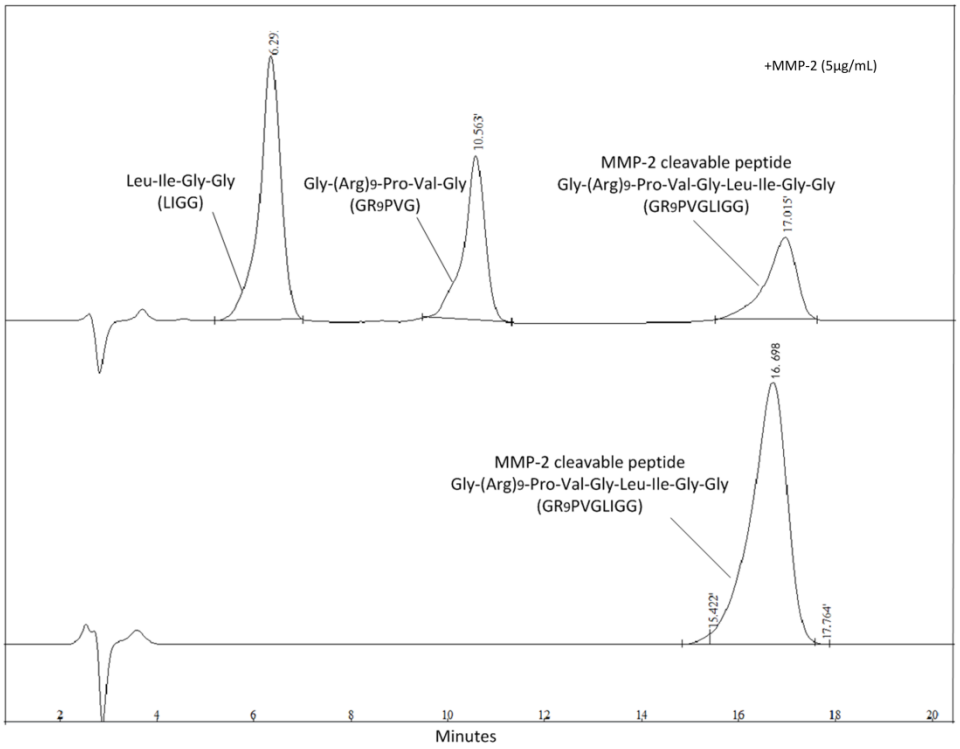
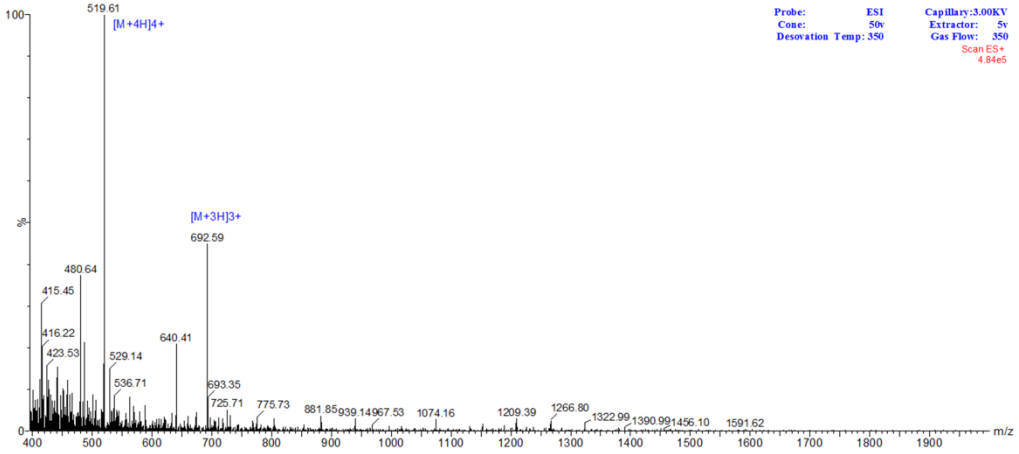


Figure S2. TGA curves of MSNs, MSNs-polypeptide, MSNs-HSA-PBA, and MSNs-HSA-PBA@DOX, respectively.

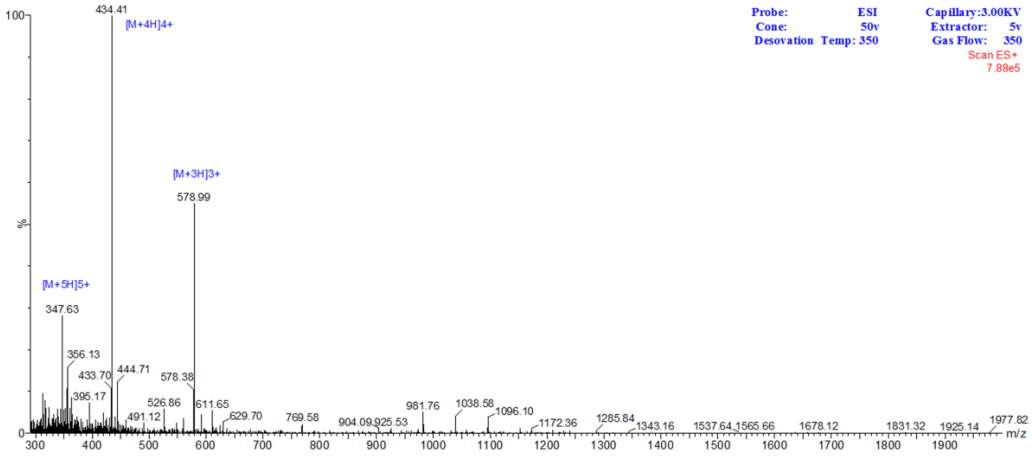
A.


+MMP-2 (5μg/mL)
6.29'
10.563'
17.015'
Leu-Ile-Gly-Gly
(LIGG)
Gly-(Arg)9-Pro-Val-Gly
(GR9PVG)
MMP-2 cleavable peptide
Gly-(Arg)9-Pro-Val-Gly-Leu-Ile-Gly-Gly
(GR9PVGLIGG)
16.698
MMP-2 cleavable peptide
Gly-(Arg)9-Pro-Val-Gly-Leu-Ile-Gly-Gly
(GR9PVGLIGG)
15.422'
17.764'
2
4
6
8
10
12
14
16
18
20
Minutes

B.


Probe: ESI
Cone: 50v
Desovation Temp: 350
Capillary:3.00KV
Extractor: 5v
Gas Flow: 350
Scan ES+
4.84e5
100
%
0
519.61
[M+4H]4+
[M+3H]3+
692.59
480.64
415.45
416.22
423.53
529.14
536.71
640.41
693.35
725.71
775.73
881.85
939.14
967.53
1074.16
1209.39
1266.80
1322.99
1390.99
1456.10
1591.62
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
m/z

C.



D.

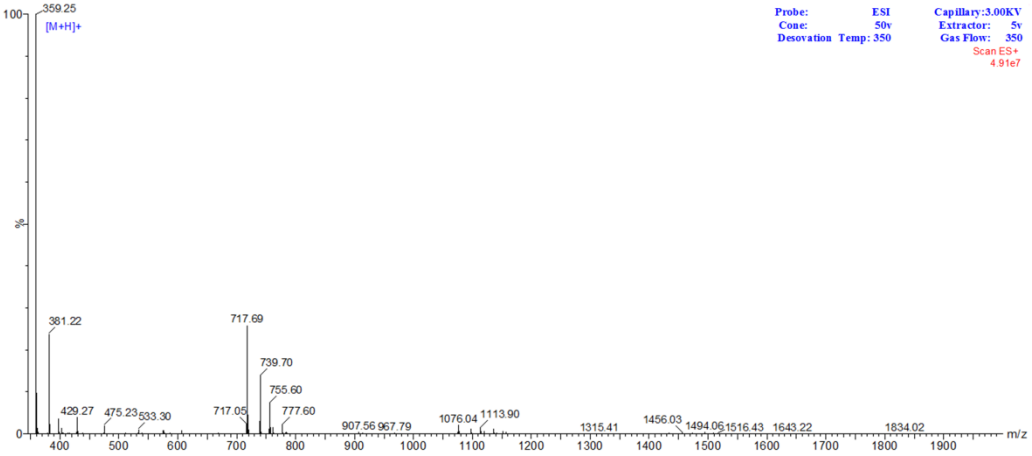


Figure S3. Cleavage reaction assays of the peptide. HPLC of peptide linker treated with or without MMP-2 at 37°C for 12h (**A**). Mass spectrum of peptide linker (GR9PVGLIGG, 519.61 ($[M+4H]^{4+}$),692.59 ($[M+3H]^{3+}$)) (**B**)，peptide fragment (GR9PVG, 347.63($[M+5H]^{5+}$), 434.41 ($[M+4H]^{4+}$), 578.99($[M+3H]^{3+}$)) (**C**) and peptide fragment (LIGG, 359.25($[M+H]^{+}$)) (**D**).

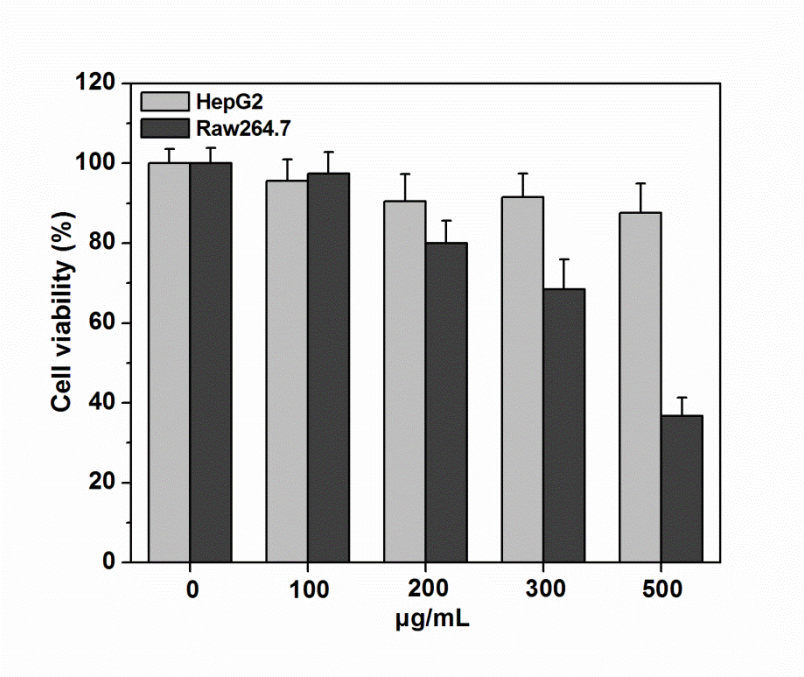


Figure S4. Cytotoxicity assays of MSNs. Cell viabilities of HepG2 and Raw264.7 cells treated with different concentrations of MSNs (0, 100, 200, 300 and 500 μg/mL) for 24 h, respectively (n=6).

To evaluate the cytotoxicity of MSNs, HepG2 and Raw264.7cells were co-cultured with different concentrations of MSNs for 24 h. MSNs at concentrations as high as 500μg/mL almost not has effect on the viability of HepG2 cells after 24 h incubation. But for Raw264.7 macrophages, MSNs lead to dramatic toxicity after 24 h exposure, only remaining about 30-40% cell activity (**Figure S4**). The results indicated that toxicity of MSNs was cell type and particle concentration dependent.

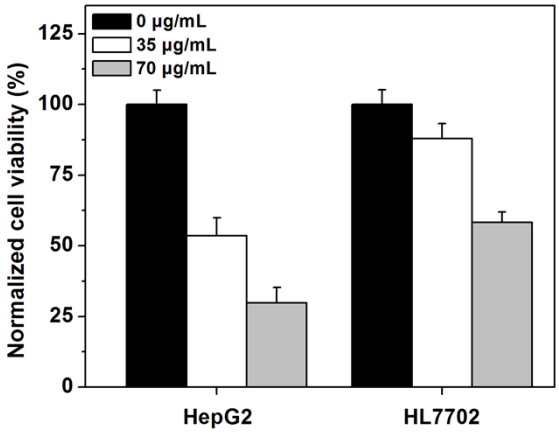


Figure S5. Cell viabilities of HepG2 and HL-7702 cells treated with different concentrations of MSNs-HSA-PBA@DOX (0, 35, 70 μg/mL), respectively (n=6).

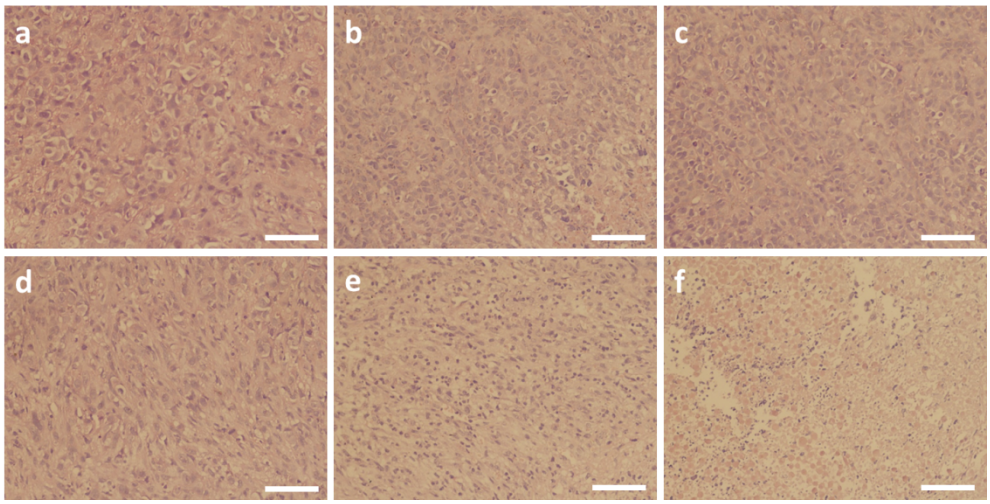


Figure S6. Histological analysis for tumor tissues of nude mice treated with (a) saline, (b) MSNs, (c) MSNs-HSA-PBA, (d) DOX, (e) MSNs@DOX, and (f) MSNs-HSA-PBA@DOX, respectively(scale bar: 100 μm).

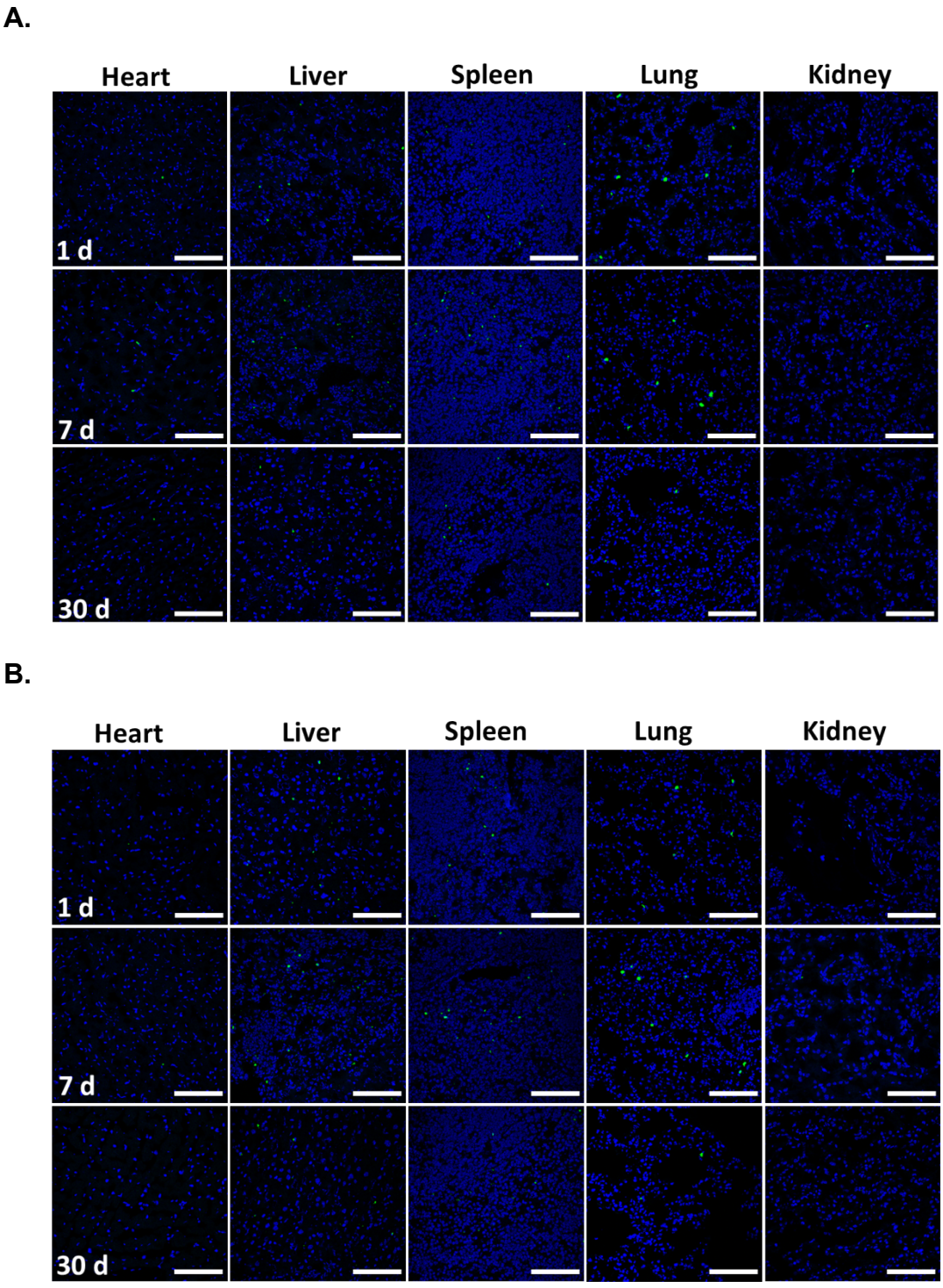


Figure S7. CLSM images of biodistribution of MSNs (**A**) and MSNs-HSA-PBA (**B**) in major organs after injection for once at different time points. Green: FITC-labeled MSNs or MSNs-HSA-PBA; blue: cell nuclei (scale bar: 100 μm).

Table S1. BET and BJH parameters of MSNs, MSNs-polypeptide and MSNs-HSA-PBA.

Materials	BET surface area $S_{BET}(m^2/g)$	BET pore volume $V_p(cm^3/g)$	BJH pore diameter $V_{BJH}(Å)$
MSNs	888.55	0.80	36.09
MSNs- polypeptide	474.83	0.39	30.03
MSNs-HSA-PBA	205.36	0.14	28.56

Table S2. Zeta-potentials of MSNs before and after each step of modification.

Materials	ζ-potential (mV)
MSNs	-26.2±5.65
MSNs-COOH	-40.5±7.45
MSNs-polypeptide	38.4±8.37
MSNs-HSA-PBA	19.7±6.5

References

(1) Z. Luo, K. Cai, Y. Hu, B. Zhang and D. Xu, *Adv. Healthc. Mater.,* 2012, **1**, 321-325.

(2) P. Rodríguez-Cuamatzi, O. I. Arillo-Flores, M. I. Bernal-Uruchurtu and H. Höpfl, *Cryst. Growth Des.,* 2005, **5**, 167-175.