Electronic Supplementary Information (ESI)

A New Strategy for Synthesis of Porous Magnetic Supraparticles with Excellent Biodegradability

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Experimental Section

Materials

Iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ammonium acetate (NH_4OAc), ethylene glycol (EG), anhydrous DMF and anhydrous ethanol were purchased from Shanghai Chemical Reagents Company (China) and used as received. Hydrazine ($NH_2NH_2 \cdot H_2O$) was purchased from Sinopharm Chemical Reagent Corp and used as received. Methyl mercaptoacetate was purchased from Aldrich. Agarose was purchased from Gene Tech (Shanghai) Company. Rhodamine B was purchased from Aladdin (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin G, streptomycin, and trypsinase were obtained from GIBCO BRL (Grand Island, NY). Deionized water was used in all experiments. All other chemicals were available commercially and used without further purifications.

Synthesis of magnetic supraparticles (MSPs)

The magnetic supraparticles (MSPs) were synthesized with agarose stabilizing by a facile solvothermal method.[1] $FeCl_3 \cdot 6H_2O$ (2.163 g) was dissolved in EG (70 mL) in a 150 mL three-necked bottle. The transparent yellow solution was mixed with agarose (0.5 g) and NH_4OAc

(3.854 g), which followed by vigorous stirring at 160°C for 1.5 h. Then the solution was transferred into a Teflon-lined stainless-steel autoclave (100 mL capacity). The autoclave was heated to 200°C rapidly and maintained at that temperature for 16 h. After cooled to room temperature, the black MSPs were rinsed several times with ethanol and water under ultrasonic conditions to effectively remove the surplus reagents, followed by drying in vacuum for 24 h.

Synthesis of porous magnetic supraparticles (p-MSPs)

Based on the pre-MSPs, the porous magnetic supraparticles (p-MSPs) was synthesized by a simple etching method. MSPs (50 mg) was dispersed ultrasonically in DMF (50 mL) in a 100 mL three-necked bottle, and then hydrazine (1.6 mL) and methyl mercaptoacetate (0.4 mL) were added. After N_2 protection for 30 min, the mixed solution reacted at 80 ^oC. The reaction was terminated by cold ethanol after 1 h. The product was collected by magnet and the black supernatant was removed. After washed by ethanol and water three times respectively, the p-MSPs was dried in vacuum for 24 h.

Acid-degradable experiment

The p-MSPs (5 mg) synthesized with different etching time (0, 30, 45, 60 min) were dispersed in 50 mL 0.1 M Na_3Cit/H_3Cit buffer (pH = 5.0), and then was shaken in table concentrator at 160 rpm. The samples (3 mL) were extracted with the help of an external magnetic field that removed the non-degraded p-MSPs and the concentration of Fe in the supernatants was detected by UV-vis and inductively coupled plasma (ICP) spectroscopy.

In vitro cytotoxicity study

In vitro cytotoxicity of p-MSPs were determined on HEK 293T cells using the CCK8 method. Specifically, 100 μL of cells were seeded in a 96-well flat culture plate at a density of $1*10^4$ cells per well and subsequently incubated for 24 h to allow attachment. Samples with different concentrations (1, 10, 100, 1000 μg/mL) of DMEM were then added to each group (three wells) for 24 h. After removing previous nutrient solution, the cells were incubated in 110 μL of DMEM containing 10 μL of CCK-8 solution for 1 h. The absorbance of the suspension was measured at 450 nm on an ELISA reader and the cell viability was calculated by means of the following

formula:

$$\text{Cell viability} = \frac{OD_{450(\text{sample})} - OD_{450(\text{blank})}}{OD_{450(\text{control})} - OD_{450(\text{blank})}} * 100\%$$

In vitro cell assay study

The HeLa cells were incubated in φ-15mm thin bottom culture chambers with 2 μg/mL p-MSPs and MSPs. The amount of Fe^{3+} dissolved from samples was detected by Fe^{3+}-selective fluorescence probe.[2] After 24 h incubation, the cells were washed with PBS three times followed by 0.01 mmol/mL Fe^{3+}-selective fluorescence probe stained for 30 min. After the surplus Fe^{3+}-selective fluorescence probe was removed by PBS washing, the samples were observed by CLSM with excitation wavelengths of 542 nm.

Characterization

High-resolution transmission electron microscopy (HRTEM) images were taken on a JEM-2010 (JEOL, Japan) transmission electron microscope at an accelerating voltage of 200 kV. Samples dispersed at an appropriate concentration were cast onto a carbon-coated copper grid. Field-emission scanning electron microscopy (FE-SEM) was performed on a Hitachi S-4800 Scanning electron microscope at an accelerating voltage of 20 kV. Sample dispersed at an appropriate concentration was cast onto a glass sheet at room temperature and sputter-coated with gold. The magnetic properties were carried out on a Model 6000 physical property measurement system (Quantum Design, USA) at 300 K. X-ray photoelectron spectrum (XPS) data were obtained on an RBD upgraded PHI-5000C (Perkin Elmer, USA) ESCA system with Mg Kα radiation (hν = 1253.6 eV) at 250 W and 14.0 kV with a detection angle at 54^{o}. Powder X-ray diffraction (XRD) patterns were obtained using a X'Pert Pro (Panalytical, Netherlands) diffraction meter with Cu Kα radiation at λ=0.154 nm operating at 40 kV and 40 mA. Fourier transform infrared (FT-IR) spectra of different samples were collected on a Magna-550 (Nicolet, USA) spectrometer using KBr pellets. Thermogravimetric analysis (TGA) data was obtained on a Pyris-1 (Perkin Elmer, USA) thermal analysis system under a flowing nitrogen atmosphere and at a heating rate of 20 ^{o}C/min from 100 to 800 ^{o}C. Ultraviolet-visible (UV-vis) absorption spectra were measured using a UV-3150 (Shimadzu, Japan) ultraviolet-visible spectrophotometer. Zeta potentials and DLS

hydrodynamic sizes were collected on the ZEN 3600 (Malvern, UK) Nano ZS instrument, where the laser wavelength was 633 nm. The confocal laser scanning microscope (CLSM) images were achieved by a P-4010 (Hitachi, Japan) spectrometer.

Experimental results

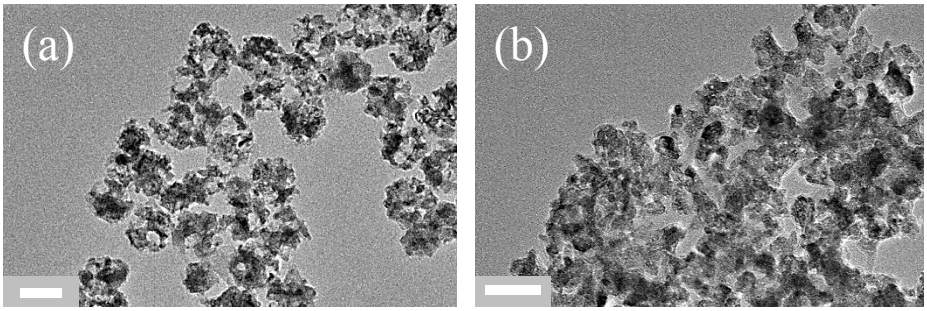

Fig. S1. The TEM images of p-MSPs by (a) 75 min and (b) 120 min etching. All the bars are 150 nm.

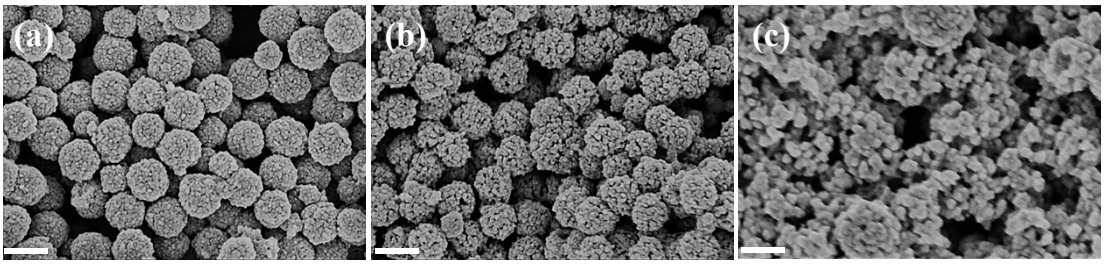

Fig. S2. The SEM images of (a) MSPs and p-MSPs that etched by (b) 60 min and (c) 120 min. all the bars are 200 nm.

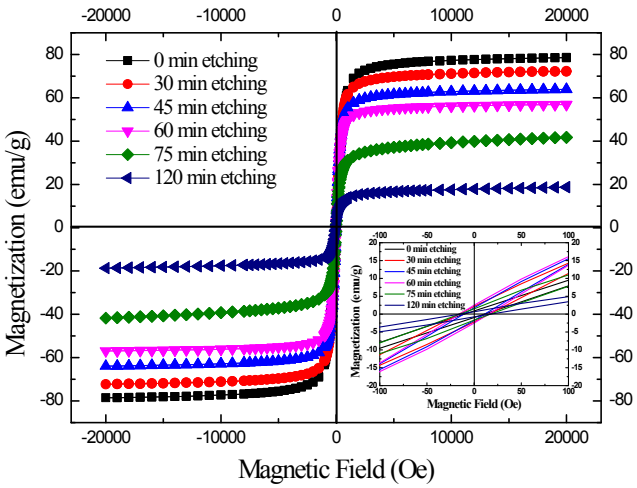

Fig. S3. The magnetization curves (inset: enlarged magnetic hysteresis curves) of p-MSPs by different time etching.

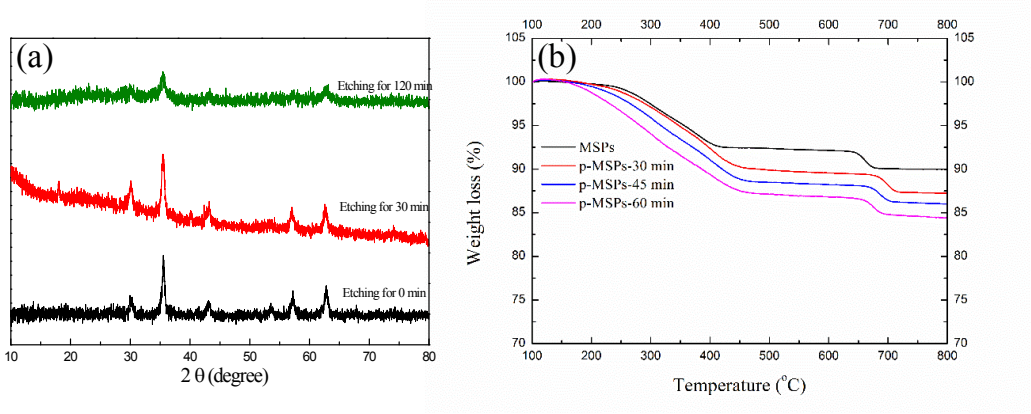

Fig. S4. The (a) XRD curves and (b) TGA curves of p-MSPs by different time etching.

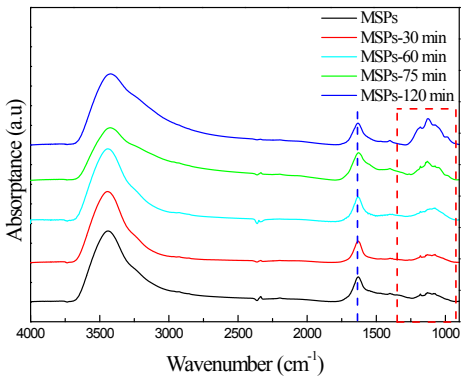

Fig. S5. The FT-IR spectra of p-MSPs by different time etching. The peaks in red frame should be

ascribed to the C-O bond in agarose and the peaks in 1600 cm^{-1} (blue line) should be ascribed to C=O bond coming from the carbonyl group stabilizing the Fe_3O_4.[1]

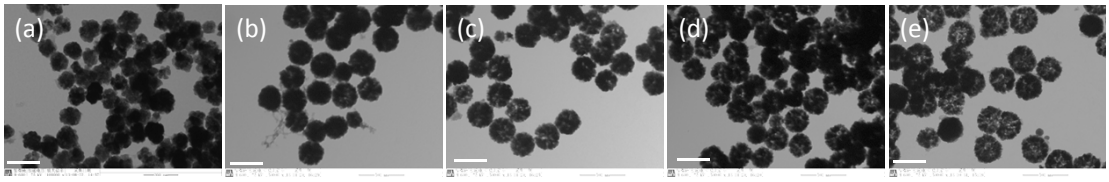

Fig. S6. The TEM images on synthesis of p-MSPs with (a) 0, (b) 400, (c) 800, (d) 1200 and (e) 1600 μL hydrazine at reaction time 1 h, where the methyl mercaptoacetate was kept in 400 μL. All the bars are 200 nm.

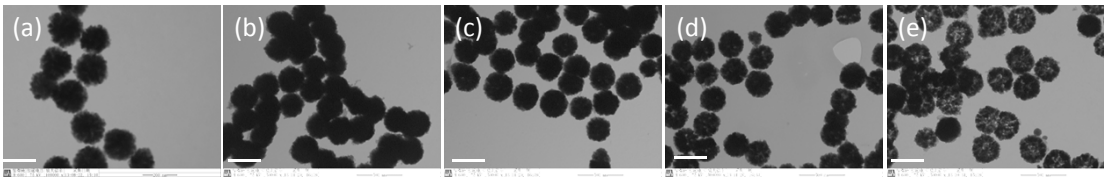

Fig. S7. The TEM images on synthesis of p-MSPs with (a) 0, (b) 50, (c) 100, (d) 200 and (e) 400 μL methyl mercaptoacetate at reaction time 1 h, where the hydrazine was kept in 1600 μL. All the bars are 200 nm.

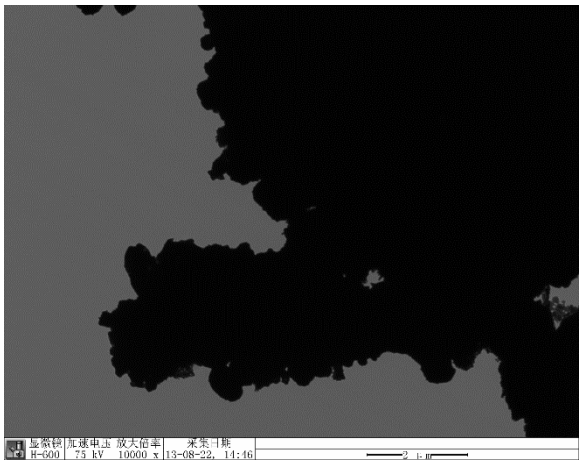

Fig. S8. The TEM images on synthesis of p-MSPs with ethanediamine substituting hydrazine at reaction time 1 h, where the methyl mercaptoacetate was kept in 400 μL

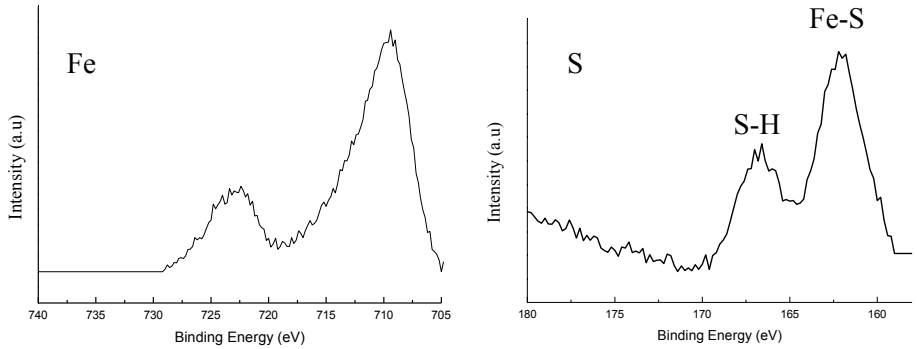

Fig. S9. The XPS curves of Fe element and S element in the black powder that was separated from supernatant.

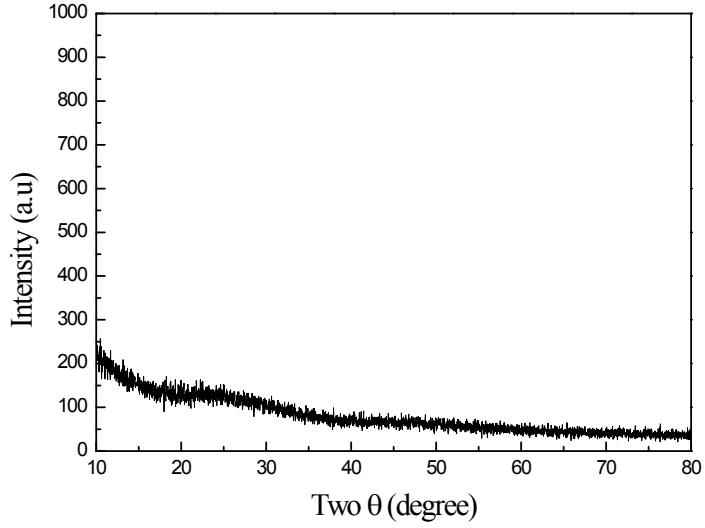

Fig. S10. The XRD curve of the black powder that was separated from supernatant.

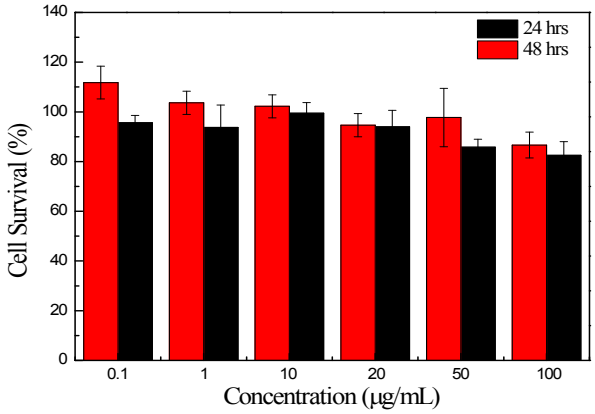

Fig. S11. The graph of HEK 293T cells survival situation in different concentration of p-MSPs for 24 h and 48 h co-incubation.

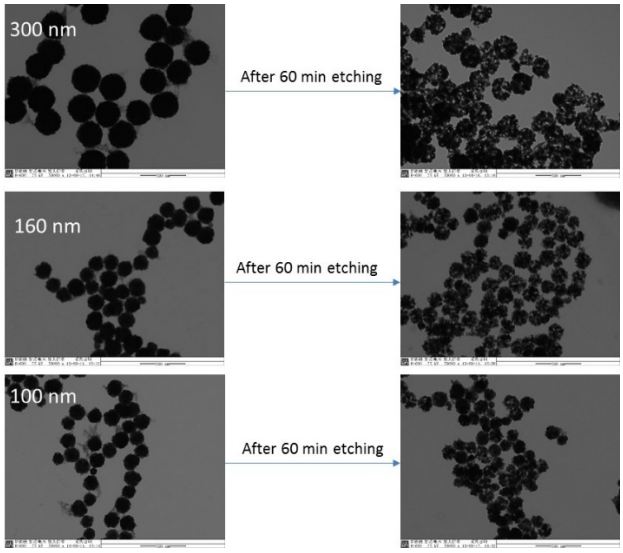

Figure S12. The TEM images of porous supraparticles with different sizes of 100 nm, 200 nm and 300 nm before and after etching.

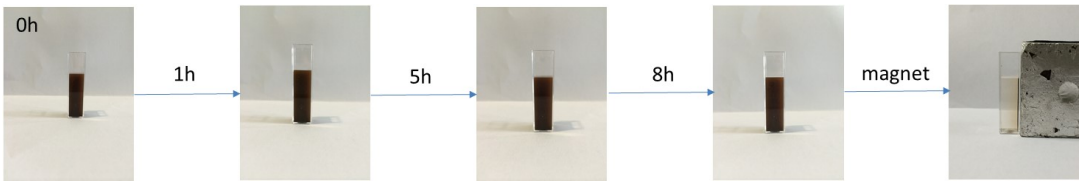

Figure 13. The test results of the colloidal stability for the porous supraparticles with 100 nm. Each image is the porous supraparticle dispersion with 1 mg/mL in 7.4 PBS buffer.

References

(1) D. Li, J. Tang, C. Wei, J. Guo, S. L. Wang, D. Chaudhary and C. C. Wang, *Small,* 2012, **8**, 2690.

(2) D. Li, Y. T. Zhang, P. Yang, M. Yu, J. Guo, J. Q. Lu and C. C. Wang, *ACS Appl. Mater. Interfaces,* 2013, **5**, 12329.