

Supporting Information

Robust magnetic double-network hydrogels with self-healing, MR imaging, cytocompatibility and 3D printability.

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S1. Experiment section

1.1 Materials.

3-Hydroxytyramine hydrochloride (DA-HCl), acrylic acid (AA) and tetramethylethylenediamine (TEMED) were purchased from Sigma-Aldrich. Triethylamine (Et₃N), acrylyl chloride (AC), methanol (MeOH), tetrahydrofuran (THF), ethyl acetate (EA), sodium hydroxide (NaOH), ammonium persulfate (APS), Ferric chloride hexahydrate (FeCl₃·6H₂O), ethylene glycol (EG), diethylene glycol (DEG), dodecylamine (DDA), hydrochloric acid (HCl, 36%–38%) acrylamide (AM) and chitosan (CS) were purchased from Sinopharm Chemical Reagent Co., Ltd. All chemicals were of analytical grade without further purification. Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS) and penicillin-streptomycin solution were purchased from HyClone. Cell Counting Kit-8 (CCK-8) was purchased from Dojindo. L929 cells were obtained from American type culture collection.

1.2 Preparation of magnetic double network hydrogel.

Preparation of Fe₃O₄ nanoparticles. Fe₃O₄ nanoparticles with a diameter of about 50 nm were successfully synthesized using a one-pot solvothermal method.¹⁻³ In brief, to a solution of NaOH in EG/ DEG binary system was added DDA as the template agent. The reaction mixture was stirred at 200 °C for 12 h in an electric oven. Then, the obtained nanoparticles were added to HCl solution (pH ≈ 1) with stirring for 1 h. Finally, stand-Fe₃O₄ nanoparticles were collected with the help of a magnet and washed alternately with deionized water and ethanol, respectively.

Synthesis of dopamine acrylamide (DAm). To a solution of DA-HCl and Et₃N in MeOH was alternately added AC (dissolved in THF) and Et₃N (dissolved in MeOH) dropwise under ice-cooling. The reaction was stirred a room temperature for 1 h and then evaporated under reduced pressure, redissolved in EA, washed with HCl (1 M) and brine, dried over Na₂SO₄, and collected the pure product from recrystallization in EA. The structure of the compound was identified by NMR, see Fig. S4. ¹H NMR and ¹³C NMR were recorded utilizing a Bruker AMX-500 spectrometer at 500 and 125 MHz in methanol-*d*₄ with tetramethylsilane (TMS) as internal standard.

Preparation of double network (DN) hydrogels. Stirred AM, DAm, Fe₃O₄ nanoparticles, CS and TEMED in deionized water at room temperature until a uniform mixture solution, followed by addition of AA and APS, then transferred into a mold and put it into 60 °C environment for 4 hours to fully gel. Finally, the synthesized hydrogels were immersed in saturated NaCl solution for 2 h to obtain the hydrogel with enhanced mechanical properties. The compositions of the different hydrogels are listed in Table S1.

1.3 Characterize of magnetic hydrogel.

Mechanical tests. Cylindrical hydrogel Samples with 10 mm height, 10mm diameter were tested by an electronic universal testing machine (EUTM, ZWICKZ020, Germany). The compressive strain speed was maintained at 5 mm/min. Compressive strength is defined as the corresponding stress at which the sample breaks. The compressive modulus is defined as the slope of the strain from 25 to 40 %.⁴ The tensile tests were performed on an electronic universal testing machine (EUTM, WDW 3020, China). Strain rate was constant at 50 mm/min. The hydrogels had a long-strip shape (length 40 mm, width 9 mm, thickness 3 mm). Three parallel experiments were performed for each sample.

Dynamic rheological. Rheological experiments of different hydrogels were performed at room temperature using a dynamic rheometer (DR, MCR301, Anton-Paar, Austria) equipped with 25 mm parallel plates. The storage modulus (G') of the hydrogel were determined as a function of frequency ranging from 0.01 to 10 Hz at 1.0% strain amplitude.

SEM morphology of the hydrogels. The internal structure of the freeze-dried hydrogels was investigated by High-resolution field emission scanning electron microscope (HR FE-SEM, GEMINISEM 500, Zeiss, Germany). Specifically, the dried hydrogel was cut to expose its internal structure and its cross section was observed.

Swelling tests. The swelling ratios of the hydrogel were measured in phosphate buffered saline (PBS) at 25 °C. The initial weight (W_0) and swelling weight (W_s) of the hydrogels at different time points were recorded. Three parallel experiments were performed for each sample. The swelling ratio is calculated by the following formula:

$$\text{Swelling ratio} = W_s / W_0 \quad (1)$$

Cytocompatibility tests. L929 fibroblasts were cultured on the AAD-CS, AA-CS-Fe, AAD-Fe, AAD-C-Fe and AAD-CS-Fe hydrogels. Before cell culture, the hydrogels with diameters 6 mm and thicknesses 2 mm were first purified in deionized water, sterilized with 75% alcohol for 24 h, and then immersed in Dulbecco's modified Eagle's medium (DMEM) until equilibrium was reached. L929 cells at a density of 8×10^3 cells per hydrogel were seeded onto the hydrogels, followed by RPMI-1640 supplemented with 10% FBS and 1% penicillin–streptomycin after adhering for 3 hours at 37 °C in a 5% CO₂ incubator. The cytocompatibility of the various hydrogels were assessed by CCK-8 assay after 3 and 5 days of culture. Representative CLSM images of L929 cells stained with Calcein-AM and PI were observed by a confocal microscope (Ultraview VOX, PerkinElmer, UK). Cells

morphologies on the surface of the hydrogel were observed by HR FE-SEM, (GEMINISEM 500, Zeiss, Germany).

1.4 Multifunction of magnetic hydrogel.

Self-healing. The original hydrogels were cut into four portions and immediately brought the sections into contact, stored in a sealed container and placed in an oven at 70 ° C for 12 hours to allow complete self-healing.

Magnetothermal property. Magnetothermal property of magnetic hydrogels were tested by placing 0.25 g hydrogel (8 mg/mL Fe₃O₄) and 0.5 mL deionized water in a sealed vessel under alternative magnetic field (AMF) for 10 min. The temperature monitored by fiber optic probes was recorded constantly by a computer.

MR Imageability. T₂-weighted MRI relaxation of hydrogels with different concentrations of nano-Fe₃O₄ was performed in a 3T clinical MRI scanner (Philips, Achieva 3.0T, Netherlands). The hydrogel was fixed in a test dish containing an aqueous solution of 2% agarose. After obtaining the T₂-weighted MR image, the signal intensity was measured in the manual drawing of the region of interest.

3D printability. The AAD-CS-Fe DN hydrogel containing 8 mg/mL nano-Fe₃O₄ was printed using a GeSim Bioscaffolder 3.2 BioPrinters (Germany) while the diameter of needle was 0.25mm, the applied pressure was 100 kPa, the speed of nozzle was 3 mm/s. APS-contained saturated saline solution was prepared as a pre-solution to receive a custom-printed hydrogel at room temperature.

1.5 Statistical analysis.

All data are expressed as means standard deviation (SD) values for at least three independent experiments. One-way analysis of variance (ANOVA) followed by post hoc test using SPSS 22.0 software to determine the significant difference between two groups. Values of *(p < 0.05) were considered statistically significant.

S2. Results and discussion

2.1 Characterizations of the nano-Fe₃O₄.

Fe₃O₄ nanoparticles were synthesized following an improved solvothermal method [1]. These Fe₃O₄ nanoparticles have relatively few surface groups and have been proved very suitable for in situ surface coating and in situ reaction [2, 3]. The synthesized Fe₃O₄ nanoparticles were about 40~50 nm (Figure S1a) and good single crystallization (Figure S1b), and the lattice stripes are clearly visible in local HRTEM image (Figure S1c). The single-crystalline structure was further conformed by SAED in Figure S1c. XRD spectra further showed the obtained Fe₃O₄ nanoparticles have

excellent crystallinity, and all peaks can perfectly match pure Fe_3O_4 (JCPDS Card No. 39-1346). The synthesized Fe_3O_4 nanoparticles also possessed super-paramagnetism and a relatively high saturation magnetization (M_s) of ~ 87.4 emu/g (Figure S1f), suitable for further biomedical applications.

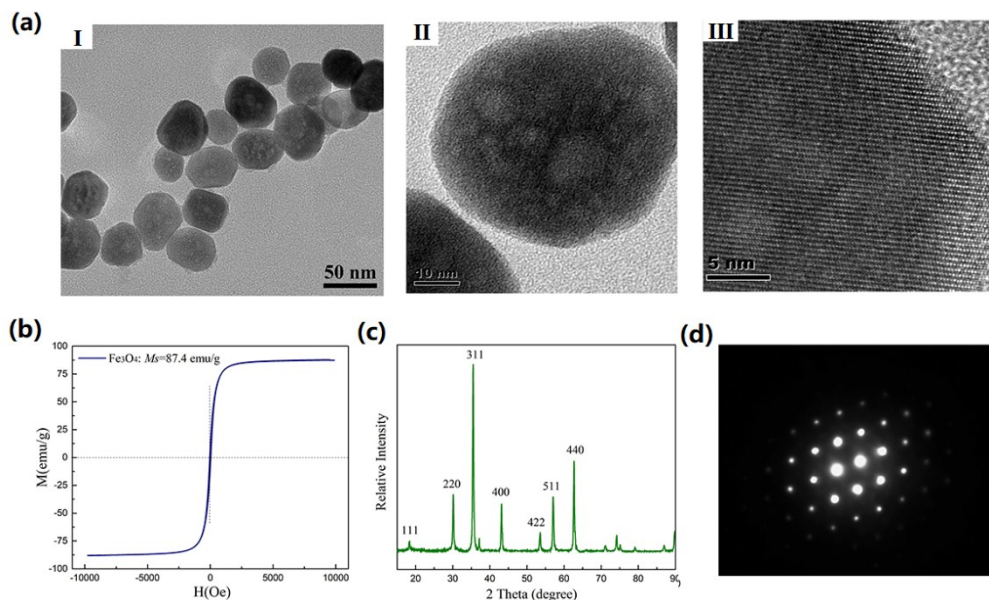


Fig. S1 Characterizations of the nano- Fe_3O_4 with a diameter of 50 nm. a) TEM image (I), HRTEM image (II) and local magnification of HRTEM image (III); b) Room-temperature magnetization curve of the nano- Fe_3O_4 ; c) XRD patterns of the nano- Fe_3O_4 ; d) Selected Area Electron Diffraction (SAED) of one Fe_3O_4 nanoparticle.

2.2 Characterizations of magnetic double network hydrogel.

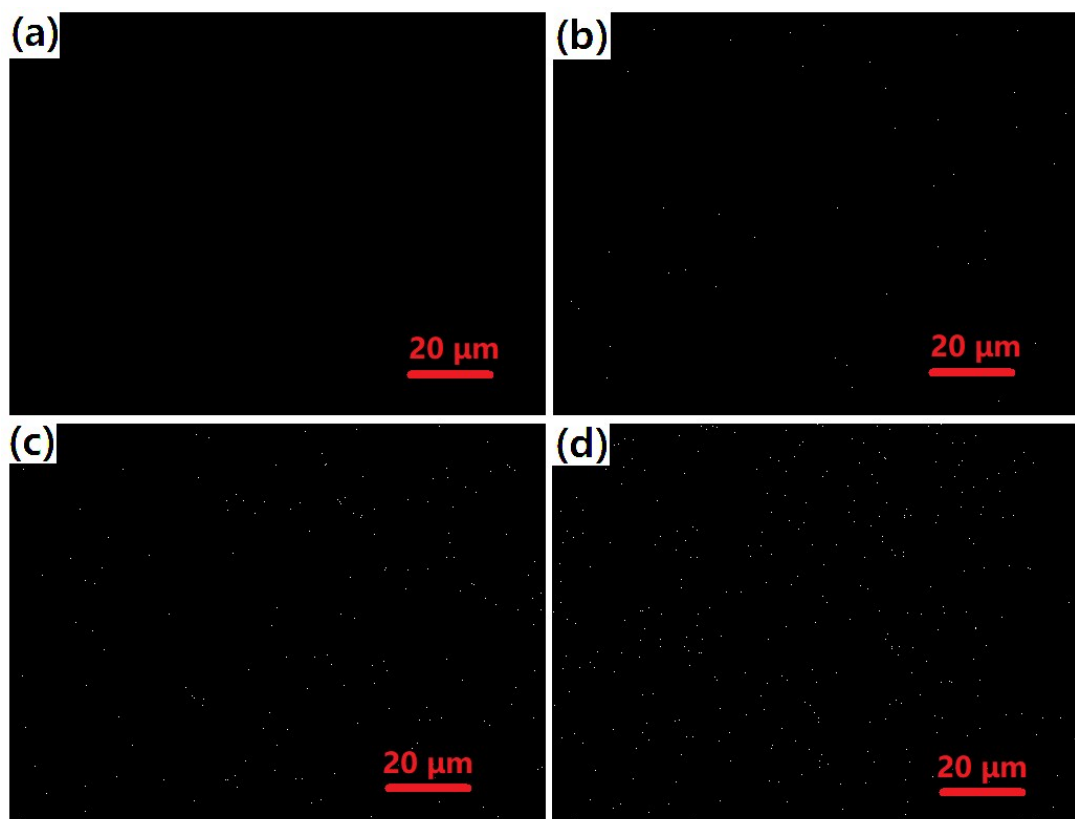


Fig. S2. Distribution of iron in hydrogels. a) $0 \text{ mg}\cdot\text{mL}^{-1}$; b) $2 \text{ mg}\cdot\text{mL}^{-1}$; c) $16 \text{ mg}\cdot\text{mL}^{-1}$; d) $32 \text{ mg}\cdot\text{mL}^{-1}$.

Table S1. Compositions of different hydrogels.

Hydrogel ^a	Fe	CS	AM + AA	DAm	Treated with brine
AAD-CS	×	√	√	√	√
AAD-CS-Fe	Fe ₃ O ₄	√	√	√	√
AAD-CS-Fe ³⁺	Fe ³⁺	√	√	√	√
AAD-CS-nano	Fe ₃ O ₄ ^b	√	√	√	√
AA-CS-Fe	Fe ₃ O ₄	√	√	×	√
AAD-Fe	Fe ₃ O ₄	×	√	√	√
AAD-C-Fe	Fe ₃ O ₄	√	√	√	×

^a AA (11 wt. %), AM (11 wt. %), DAm (0.01 g), H₂O (1.1 mL), Fe (8 mg•mL⁻¹), CS (7 wt. %), APS/(AA+AM+DAm) (2.8 wt. %), TEMED/(AA+AM+DAm) (1.4 wt. %).

^b Non-hydrochloric acid treated nano Fe₃O₄.

2.3 Tunable mechanical properties of magnetic double network hydrogel.

The AAD-CS-Fe hydrogel with the best cell compatibility was further adjusted by components to prepare a series of hydrogels with different properties for different tissue engineering. Fig. 3a and 3d displayed the typical stress-strain curves of DN hydrogels with different concentrations of Fe₃O₄ nanoparticles. With the increase of nano-Fe₃O₄ content, the compressive strength and tensile strength of hydrogels tend to increase first and then decrease. The DN hydrogel has the highest strength (compressive strength is 1.75 MPa, tensile strength is 184.50 KPa) when the nano-Fe₃O₄ content is 16 mg/mL. This may be caused by the excessive crosslinking of the high concentration of nano-Fe₃O₄ with different hydroxyl groups and carboxyl groups, which affects the polymerization of the AAD terpolymer. Moreover, it can be seen from the iron distribution map (Fig. S2) that iron is well distributed in the hydrogel due to the coordination between iron ions on the surface of nano-Fe₃O₄ and different carboxyl/hydroxyl groups. In order to obtain a wider range of mechanical properties to expand the biological application of the system, we further adjusted the content of other components. It can be seen from Figures S3c and 3f that the strength of the hydrogel can be significantly affected by varying the CS content from 4 to 8 wt. %, especially the compressive strength range can be adjusted from 0.25 to 2.29 MPa. Although the increase in the content of the AAD terpolymer is less pronounced against the compressive strength, it can significantly reduce the compressive modulus of the hydrogel. In other words, the increase in the flexible composition can increase the toughness of the hydrogel while maintaining its compressive strength.

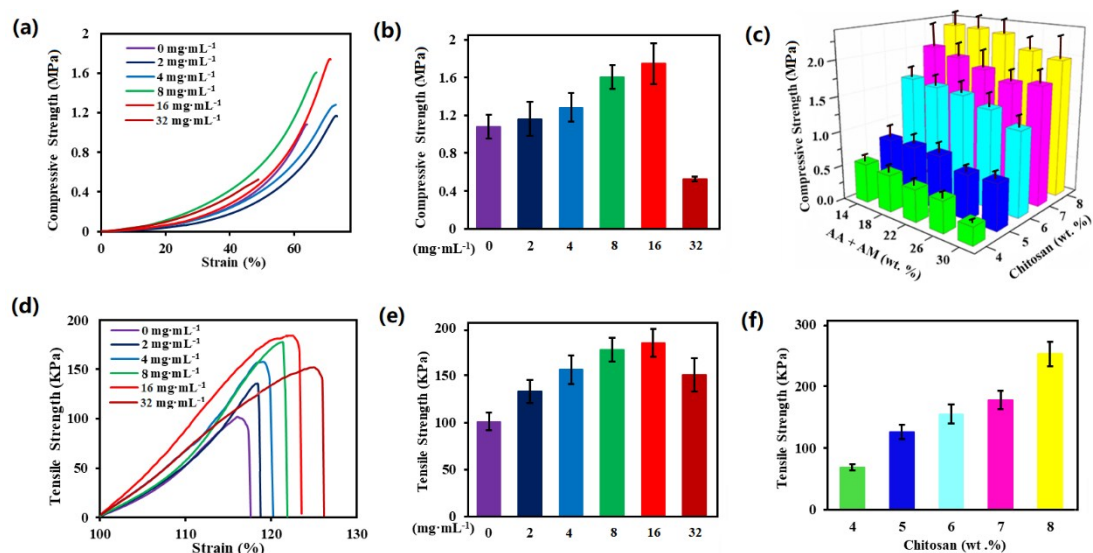


Fig. S3 a, d) Typical compression/tensile stress–strain curve of the hydrogels at different concentrations of Fe₃O₄ nanoparticles in water. b, c) Maximum compression/tensile strength of various hydrogels. c) Compressive/Tensile strength of hydrogels corresponding to different weight ratios of chitosan and AA + AM.

2.4 Cytocompatibility.

The ability of the hydrogels to interact with cells was assessed by culturing L929 fibroblasts on the surface of prepared hydrogels. Representative confocal laser scanning microscopy (CLSM) of LIVE/DEAD (Fig. 3a-c) assay showed that cell viability on different hydrogels varied after being cultured *in vitro* for 3 days. It was observed that there were more living cells on the AAD-Fe and AAD-CS-Fe hydrogels than AA-CS-Fe hydrogel, indicating that the addition of adhesion-promoting DAM in the hydrogels facilitated cells-gels favorable interaction. The SEM images further demonstrated that DAM monomer promoted cell adhesion and spreading on the hydrogels (Fig. 3d-f). In addition, the fibroblasts had adhered and spread more after CS and nano-Fe₃O₄ incorporation, which indicated that formation of DN hydrogels enhanced the cell affinity of hydrogels (Fig. 3e-f).

dopamine acrylamide (DAm)

^1H NMR (500 MHz, Methanol- d_4) δ 6.71 (d, $J = 8.0$ Hz, 1H), 6.68 (d, $J = 2.1$ Hz, 1H), 6.55 (dd, $J = 8.0, 2.1$ Hz, 1H), 6.22 (d, $J = 1.9$ Hz, 1H), 6.21 (s, 1H), 5.64 (dd, $J = 6.9, 5.1$ Hz, 1H), 3.43 (t, $J = 7.4$ Hz, 2H), 2.68 (t, $J = 7.4$ Hz, 2H). ^{13}C NMR (125 MHz, Methanol- d_4) δ 166.1, 144.1, 142.7, 130.0, 129.9, 124.5, 118.9, 114.8, 114.3, 40.3, 33.8.

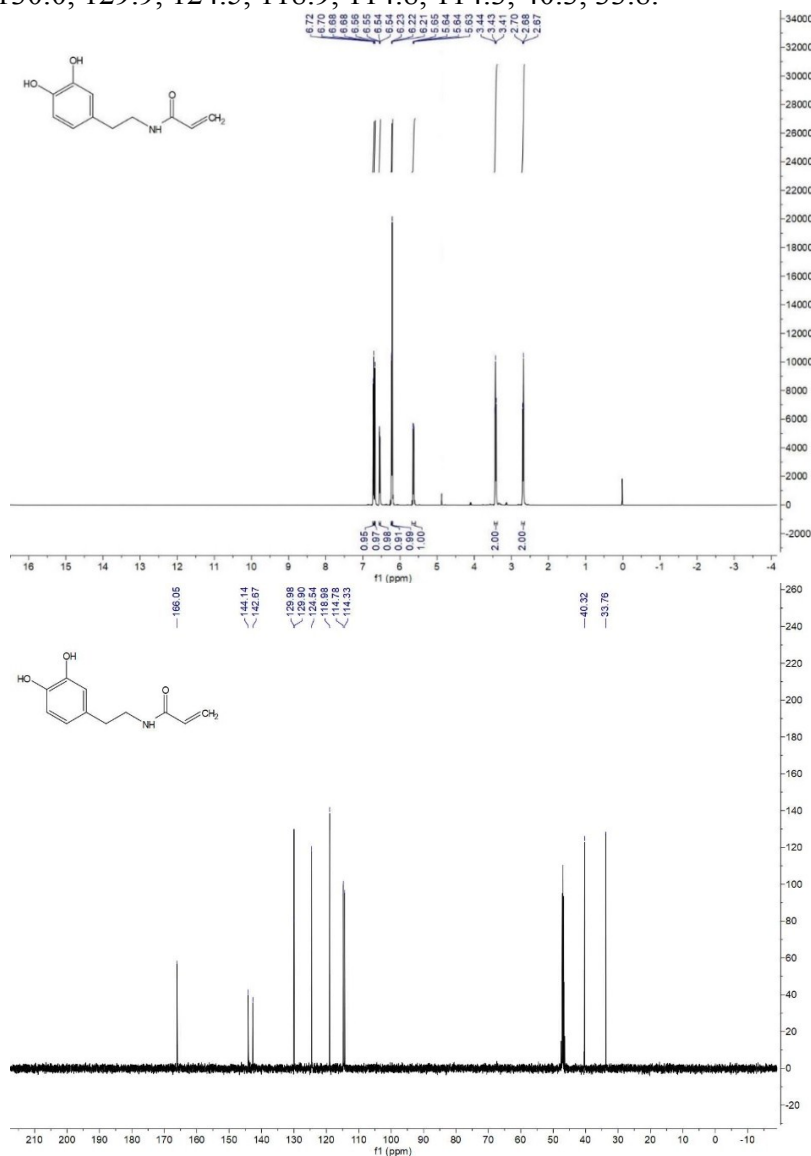


Fig. S4. ^1H and ^{13}C NMR spectra of DAm.

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