Supplementary Information

Ligand biodegradation induced surface reconstruction of magnetite nanoparticles: Potential overlooked toxicity

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Materials and Methods

Elemental analysis of various nanoparticles (NPs)

Fe_3O_4-HA_H, Fe_3O_4-HA_L and Fe_3O_4-HA_R (20 mg Fe/L) were suspended in 1 mL of ultrapure water. Two microliter of each suspension was dropped onto a transmission electron microscopy (TEM) grid (Zhongjing Keyi, China) and the samples were air dried overnight. The elemental analysis was performed by energy dispersive spectrometer (EDS) coupled with TEM (JEM-2800F, Hitach, Japan) at 200 kV accelerating voltage.

Prussia blue stain Assay

J774A.1 cells were incubated with 20 mg-Fe/L of Fe_3O_4-HA_H, Fe_3O_4-HA_L and Fe_3O_4-HA_R in dulbecco's modified eagle medium (DMEM, pH 7.4) for 12 h at 37 °C, respectively. The cells were fixed by 4% paraformaldehyde for 30 min. Then the cells were rinsed with phosphate buffered solution (PBS) and stained with 1 mL of 2% potassium ferrocyanide in 6% hydrochloric acid (Perl reagent for Prussian blue staining, Yisheng BioTECH, Shanghai, China) for 30 min. After wash, the cells were examined with a confocal laser scanning microscope (CLSM, LSM880 with Airyscan, Zeiss, Germany).

Table S1. The wavelength of peaks in FTIR spectra and corresponding functional groups

Functional Group \ Sample	HA (cm^{-1})	Fe_3O_4-HA_H (cm^{-1})	Fe_3O_4-HA_L (cm^{-1})	Fe_3O_4-HA_R (cm^{-1})
O-H	3393	3421	3400	3490
C=O	1616	1614	1616	1647
COO^-	1407	1411	1406	1384
C-O	1047	1045	1043	1024

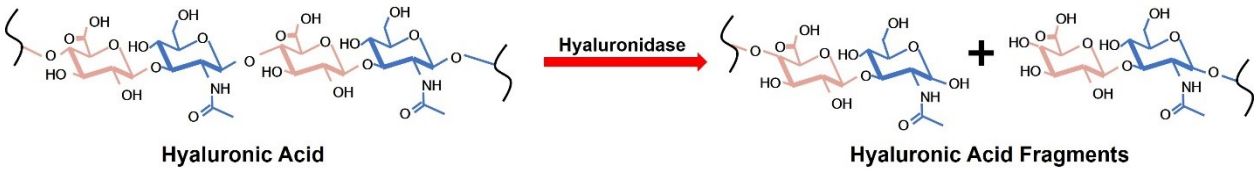

Fig. S1. Hydrolysis of hyaluronic acid by hyaluronidase.

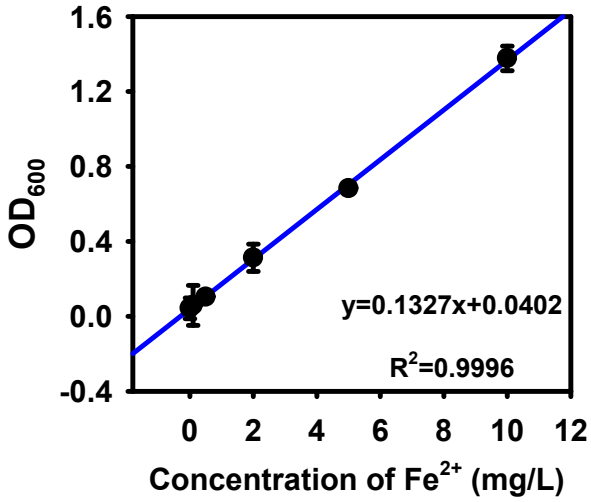

Fig. S2. A calibration curve of Fe^{2+}-phenanthroline complex.

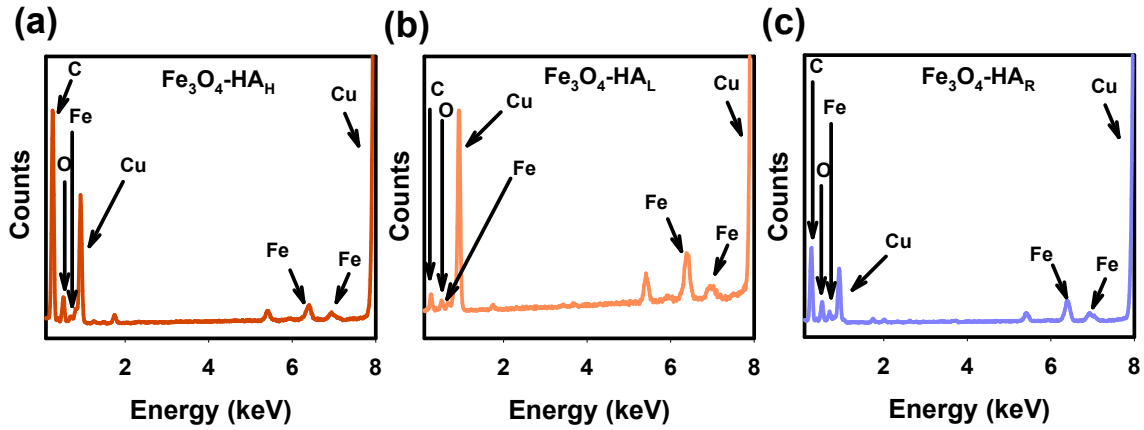

Fig. S3. EDS spectra of (a) Fe_3O_4-HA_H, (b) Fe_3O_4-HA_L and (c) Fe_3O_4-HA_R.

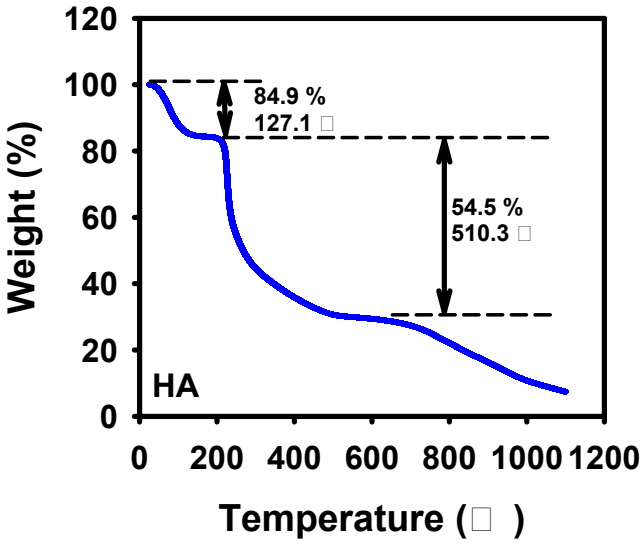

Fig. S4. TGA thermogram of HA.

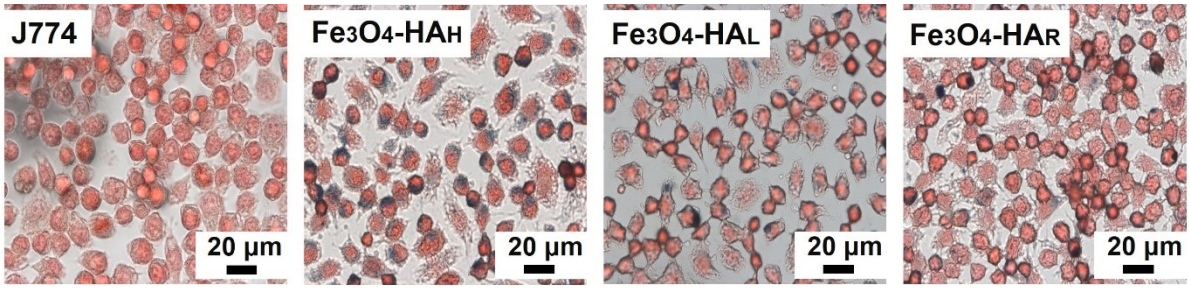

Fig. S5. Prussia blue stain images indicating iron in J774 cells treated with various NPs (20 mg Fe/L) for 12 h.

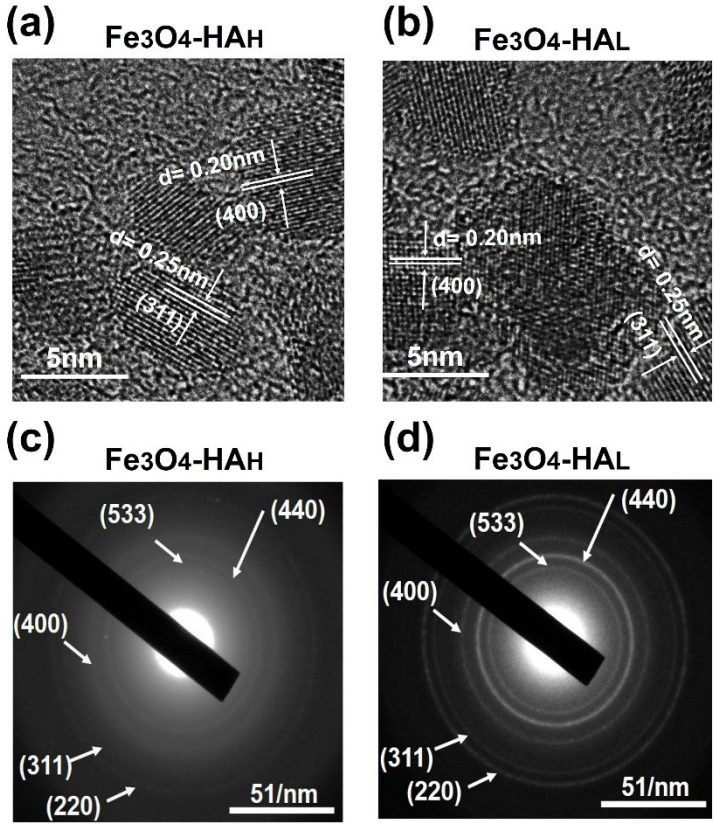

Fig. S6. HRTEM images showing lattice fringes of (a) Fe_3O_4-HA_H and (b) Fe_3O_4-$HA_{L;}$ SAED patterns of (c) Fe_3O_4-HA_H and (d) Fe_3O_4-HA_L.