Electronic Supplementary Information

Carboxylated SiO$_2$-coated $\alpha$-Fe nanoparticles: towards a versatile platform for biomedical applications

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Experimental

1. Preparation of SiO$_2$-coated Fe$_3$O$_4$ nanoparticles. The SiO$_2$-coated Fe$_3$O$_4$ nanoparticles were prepared according to the procedures described elsewhere [1]. In brief, the Fe$_3$O$_4$ nanoparticles with an average particle size of 25 nm were prepared [2] and the SiO$_2$ coating was performed through the formation of water-in-cyclohexane reverse microemulsion [3]. To a cyclohexane solution (47.74 g) containing polyoxyethylene(5)nonylphenyl ether (3.65 g) and the Fe$_3$O$_4$ nanoparticles (90 mg), ammonium hydroxide (28%, 0.38 ml) was added, and the resulting mixture was magnetically stirred for 30 min to form a transparent, brown solution of reverse microemulsion. Then, tetraethyl orthosilicate (0.4 g) was added, and the coating reaction was continued for 20 h at room temperature. The SiO$_2$-coated Fe$_3$O$_4$ nanoparticles were precipitated by adding ethanol to the reaction solution. They were collected by a magnet, washed with ethanol, and dried in vacuum.

2. Reduction with CaH$_2$. The reduction was done according to the method described elsewhere [1]. In brief, a silica-coated sample and a four-weight excess of CaH$_2$ were finely ground in an Ar-filled glove box, sealed in an evacuated Pyrex tube, and heated at 400 °C for 48 hrs. Residual CaH$_2$ and CaO produced during the reduction
were washed out with an NH$_4$Cl/methanol solution in air. The SiO$_2$-coated $\alpha$-Fe nanoparticles were collected by a magnet, washed with methanol, and dried in vacuum.

3. **Estimation of Fe content in the SiO$_2$-coated nanoparticles:** The amount of Fe in a sample was determined by means of Thermogravimetry (TG, Bruker AXS, TG-DTA2000SA). Both the SiO$_2$-coated $\alpha$-Fe and SiO$_2$-coated Fe$_3$O$_4$ nanoparticles were transformed to SiO$_2$-coated $\gamma$-Fe$_2$O$_3$ nanoparticles by oxidization at 500 $^\circ$C for 10 hrs in a stream of O$_2$, which were subsequently reduced in a stream of H$_2$ at 700 $^\circ$C to SiO$_2$-coated $\alpha$-Fe. The amount of Fe in an initial sample was calculated from the decrease in weight on the reduction by assuming that SiO$_2$ remained intact during the reduction.

4. **Modification with COOH-silane:** To a 40 wt% ethanolic aqueous solution (30 g) of the SiO$_2$-coated $\alpha$-Fe nanoparticles (40 mg) was added COOH-silane (0.3 g, N-(trimethoxysilylpropyl) ethylenediamine triacetic acid trisodium salt) and the resulting mixture was kept at 80 $^\circ$C for 3 days while stirring. Thus-obtained $\alpha$-Fe@SiO$_2$@COOH NPs were collected by centrifugation, washed with water, and
centrifuged again. The collected powder was redispersed in water and stored at room temperature.

5. **Cell Viability:** The viability of cells treated with nanoparticles was measured using a CellTiter-Glo™ Luminescent Cell Viability Assay (Promega). The cells were seeded onto a 96-well plate at a density of 1 X 10^4 cells per well. After 1 day of culture, different concentrations of the nanoparticles were added to the wells. The cells were incubated for 24 hrs and then 100 μl of the reagent was directly added to the wells. After 15 min of incubation at 37 °C, absorbance at 490 nm was measured with a standard microplate reader (Fluoroskan Asent FL, Thermo scientific). Each experiment was done in triplicate. ATP concentration in the cells was estimated based on the emission intensity-ATP concentration calibration. The relative cell viability (%) relative to that for control nanoparticle-free wells was calculated by \[ \frac{[A]_{\text{exp}}}{[A]_{\text{control}}} \times 100 \], where \([A]_{\text{exp}}\) and \([A]_{\text{control}}\) are the absorbance of the experimental and control sample, respectively.

6. **Other characterization methods.** Low magnification TEM observations were performed by using JEOL JEM-1010D and JEM-1400. High resolution TEM
observation was performed by using JEOL JEM-2200FS. TEM specimens were prepared by dropping a particle-containing solution on a carbon-coated copper grid. Cryogenic transmission electron microscopic (Cryo-TEM) images were obtained with JEOL JEM-2100F(G5) operated at an acceleration voltage of 200 kV. For the specimen preparation, a thin layer of sample solution with 50-500 nm thickness was rapidly frozen in liquid propane maintained at 100 K (Leica, Reichert KF 80 plunger) and then transferred into the cryo-TEM column. The observation was made at 4.2 K using a special sample stage. XRD measurements were performed using Bruker New D8 ADVANCE with Cu Kα radiation (λ = 0.154 nm). Magnetic properties were characterized by using a Physical Properties Measurement System (PPMS, Quantum Design PPMS-9RST) with a vibrating sample magnetometer (VSM) attachment. IR spectra were collected on JASCO FT/IR-4200. The samples were each mixed with KBr and compressed into pellets. Zeta potential measurements were performed using ZETASIZER Nano-Z (MALVERN).
References for ESI


Figure S1.
(a): high resolution TEM image of the SiO$_2$-coated $\alpha$-Fe nanoparticles.
(b): magnified image of the core $\alpha$-Fe nanoparticle.
(c): a fast Fourier transform (FFT) image of the area surrounded by dotted line in (a).
(d): an FFT image of the area surrounded by broken line in (a).

The core $\alpha$-Fe nanoparticle surface was slightly oxidized to FeO due to the washing under air (see Experimental 2 in ESI) and exposure to air for ca. 2 days before high resolution TEM measurements.
**Figure S2.** A photo of dispersion of the dextran-modified sample in phosphate buffered saline. The triangle indicates the fluid level.