Electronic Supplementary Information

Immobilization of iron oxide nanoparticles within alginate nanogels for enhanced MR imaging applications†

Wenjie Suna1, Jia Yangb1, Jianzhi Zhua, Yiwei Zhoua, Jingchao Lia, Xiaoyue Zhua, Mingwushenan, c*, Guixiang Zhangb*, Xiangyang Shiana*

a College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, People’s Republic of China
b Department of Radiology, Shanghai General Hospital, School of Medicine, Shanghai Jiaotong University, Shanghai 200080, People’s Republic of China
c State Key Laboratory of Molecular Engineering of Polymers, Fudan University, Shanghai 200433, People’s Republic of China

* To whom correspondence should be addressed. E-mail addresses: mwshen@dhu.edu.cn (M. Shen), guixiangzhang@sina.com (G. Zhang), xshi@dhu.edu.cn (X. Shi).
† Authors contributed equally to this work.
Part of Experimental Details:

**Synthesis of the PEI-Fe$_3$O$_4$ NPs:** PEI-Fe$_3$O$_4$ NPs were synthesized using a facile hydrothermal method reported in our previous work.$^1$ Briefly, ammonium hydroxide (12.5 mL) was added dropwise into FeCl$_2$·4H$_2$O aqueous solution (2.5 g, 15.5 mL) under vigorous stirring in air for about 10-15 min. After the color of the mixture turned dark, the reaction mixture was transferred into an autoclave with a volume of 50 mL (Shanghai Yuying Instrument Co., Ltd., Shanghai, China). A PEI aqueous solution (1.0 g, 10 mL) was also added into the autoclave and the mixture was blended thoroughly. The reaction mixture was sealed in the autoclave with a gauge pressure of 2 bar and heated at 134 °C for 3 h. After the reaction was completed, the solution was cooled down to room temperature. The black precipitate was collected in the presence of an external magnetic field (a magnet) and purified with water for 5 times to remove excess reactants and by-products. Finally, the obtained PEI-Fe$_3$O$_4$ NPs were dispersed in 20 mL water.

**Hemolysis Assay:** Fresh human whole blood was donated by Shanghai General Hospital (Shanghai, China) after approval by the ethical committee of Shanghai General Hospital. Before hemolysis assay, healthy human red blood cells (HRBCs) were acquired according to the protocols reported in the literature.$^2$ In brief, the HRBCs were separated from the whole blood by centrifugation at 1000 rpm for 10 min to remove the plasma and purified with PBS for 5 times. After that, the suspension of HRBCs was 10-fold diluted with PBS. The diluted HRBC suspension (0.1 mL) was then added to water (0.9 mL) as a positive control, PBS (0.9 mL) as a negative control, and PBS (0.9 mL) containing AG/PEI-Fe$_3$O$_4$ NGs at different Fe concentrations (0.025-0.2 mM), respectively. After standing at room temperature for 2 h, all the mixtures were centrifuged at 10000 rpm for 1 min. The absorbance of the supernatants was recorded by a PerkinElmer Lambda 25 UV-vis spectrophotometer (Waltham, MA), and the digital pictures of the samples were taken. The
hemolysis percentages (HPs) of the samples were calculated according to the following equation:

$$HPs(\%) = \frac{D_t - D_{nc}}{D_{pc} - D_{nc}} \times 100$$

where $D_t$ is the absorbance of the test samples at 541 nm and $D_{pc}$ and $D_{nc}$ are the absorbances of the positive and negative controls at 541 nm, respectively.

**Cytotoxicity Assay:** To test the cytotoxicity of the AG/PEI-Fe$_3$O$_4$ NGs, a resazurin reduction assay was performed after the HeLa cells were treated with the NGs at different Fe concentrations. HeLa cells suspended in DMEM containing 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin were seeded into 96-well plates with a density of $1 \times 10^4$ cells/well. After cultivation for 24 h in a 37 °C incubator with 5% CO$_2$, the medium was replaced with 200 µL fresh DMEM containing 20 µL PBS or PBS solution of the AG/PEI-Fe$_3$O$_4$ NGs at different Fe concentrations (0.02, 0.04, 0.06, 0.08, or 0.1 mM, respectively). After 24 h incubation at 5% CO$_2$ and 37 °C, the medium was removed and the cells were washed with PBS for 3 times, followed by addition of 200 µL fresh DMEM containing 20 µL resazurin (with a final concentration of 0.1 mg/mL) into each well. After incubation of the cells at 37 °C for additional 4 h, 100 µL of the supernatant from each well was transferred into a black 96-well plate. Finally, a multifunctional ELISA reader (Biotek, Winooski, VT) was used to read the resoruflin fluorescence ($\lambda_{ex} = 530$ nm, $\lambda_{em} = 590$ nm). Mean and standard deviation for the quintuplicate wells of each sample were recorded.

Likewise, after treatment with PBS or AG/PEI-Fe$_3$O$_4$ NGs at different Fe concentrations (0.02, 0.04, 0.06, 0.08, or 0.1 mM, respectively) for 24 h, the cell morphology was observed by a Leica DM IL LED inverted phase contrast microscope (Wetzlar, Germany) with a magnification of 100 × for each sample to further assess the cytotoxicity of the NGs.

**In Vitro Cellular Uptake Assay:** The cellular uptake of the AG/PEI-Fe$_3$O$_4$ NGs by HeLa cells was evaluated by ICP-OES. Fe$_3$O$_4$-PEI.SAH NPs synthesized according to our previous report were
also tested for comparison. Briefly, HeLa cells in DMEM were seeded in 24-well plates at a density of $5 \times 10^5$ cells/well. After overnight incubation to bring the cells to confluence, the medium was replaced with fresh DMEM containing the AG/PEI-Fe$_3$O$_4$ NGs or Fe$_3$O$_4$-PEI.SAH NPs ([Fe] = 0, 0.025, 0.05, or 0.1 mM, respectively) and the cells were then incubated at 37 °C and 5% CO$_2$ for 6 h. Thereafter, the cells were washed with PBS for 3 times, digested with trypsinization, centrifuged, and resuspended in PBS. A portion of the cell suspension was used to count the cell density and the remaining cells were collected by centrifugation (1000 rpm, 5 min) and lysed using 0.5 mL aqua regia solution (nitric acid/hydrochloric acid, v/v = 1:3). After diluting the samples with PBS, the cellular Fe uptake was quantified by ICP-OES.

To qualitatively confirm the Fe uptake of the AG/PEI-Fe$_3$O$_4$ NGs by HeLa cells, Prussian blue staining of cells was performed. In brief, $5 \times 10^5$ cells were seeded into each well of 24-well cell culture plates. After overnight incubation at 37 °C and 5% CO$_2$, the medium was replaced with fresh medium containing the AG/PEI-Fe$_3$O$_4$ NGs or Fe$_3$O$_4$-PEI.SAH NPs ([Fe] = 0, 0.025, 0.05, or 0.1 mM, respectively). After 6 h incubation, the cells were washed with PBS for 3 times, fixed with 2.5% glutaraldehyde solution at 4 °C for 15 min, and stained with Prussian blue reagent (1% Potassium ferrocyanide aqueous solution was mixed with 2% HCl by equal volume) at 37 °C for 30 min. Finally, the cells were imaged by phase contrast microscope with a magnification of 100 × for each sample.

**In Vitro MR Imaging of Cancer Cells:** HeLa cells were seeded into 25 cm$^2$ culture flasks at a density of $5 \times 10^5$ cells/flask with 5 mL of DMEM and incubated at 37 °C and 5% CO$_2$. After overnight culture, the medium was replaced with 5 mL fresh medium containing AG/PEI-Fe$_3$O$_4$ NGs at different Fe concentrations (0, 0.0125, 0.025, 0.05, or 0.1 mM, respectively). After incubation at 37 °C and 5% CO$_2$ for 6 h, the cells were washed with PBS for 3 times, trypsinized, centrifuged, and
resuspended in 1 mL PBS (containing 0.5% agarose) in a 2 mL Eppendorf tube before MR imaging.

A 3.0 T Signa HDxt superconductor clinical MR system (GE Medical Systems, Fairfield, CT) with a wrist receiver coil and a fast spin-echo (FSE) sequence (TR/TE = 3000/96.1 ms, matrix = 256 × 256, section thickness = 2 mm, and FOV = 6 × 6 cm) was used to obtain the $T_2$-weighted MR images.

References


Table S1. The surface potential, hydrodynamic size, and polydispersity index (PDI) of the AG/PEI-Fe₃O₄ NGs and Fe₃O₄-PEI.SAH NPs.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Surface potential (mV)</th>
<th>Hydrodynamic size (nm)</th>
<th>Polydispersity index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG/PEI-Fe₃O₄ NGs</td>
<td>-7.6 ± 0.3</td>
<td>308.8 ± 16.3</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>Fe₃O₄-PEI.SAH NPs</td>
<td>-15.6 ± 0.2</td>
<td>231.5 ± 7.6</td>
<td>0.3 ± 0.02</td>
</tr>
</tbody>
</table>

Figure S1. Photographs of the AG/PEI-Fe₃O₄ NGs dispersed in water, PBS, and cell culture medium for one week.

Figure S2. TGA curves of the PEI-Fe₃O₄ NPs (a) and the AG/PEI-Fe₃O₄ NGs (b).
Figure S3. FTIR spectra of AG (a), PEI-Fe₃O₄ NPs (b), and AG/PEI-Fe₃O₄ NGs (c).

Figure S4. Hemolytic activity of the AG/PEI-Fe₃O₄ NGs at different Fe concentrations (0.025, 0.05, 0.1, and 0.2 mM, respectively). PBS and water were used as negative and positive controls, respectively. The bottom right inset shows the photograph of HRBCs exposed to water, PBS, and PBS containing AG/PEI-Fe₃O₄ NGs with different concentrations, respectively for 2 h, followed by centrifugation. The top right inset shows the enlarged UV-vis spectra (as indicated by the arrow).
Figure S5. Phase contrast microscopic images of HeLa cells treated with PBS (a) or AG/PEI-Fe₃O₄ NGs at the Fe concentrations of 0.02 mM (b), 0.04 mM (c), 0.06 mM (d), 0.08 mM (e), and 0.1 mM (f), respectively for 24 h.
Figure S6. Phase contrast microscopic images of HeLa cells after Prussian blue staining. HeLa cells were treated with the AG/PEI-Fe$_3$O$_4$ NGs or Fe$_3$O$_4$-PEI.SAH NPs at different Fe concentrations for 6 h.
Figure S7. T₂-weighted MR images (a) and MR signal intensity (b) of mouse liver before and after intravenous injection of the AG/PEI-Fe₃O₄ NGs ([Fe] = 27.21 mM, in 0.2 mL PBS) at different time points.