Electronic Supplementary Material (ESI) for Polymer Chemistry

Fluorescent Dextran-Based Nanogels: Efficient Imaging Nanoprobes for Adipose-Derived Stem Cells

Shuyan Zhou,[a] Hongjing Dou,*[a] Zhaofeng Zhang,*[b] Kang Sun,[a] Yuqing Jin,[b] Tingting Dai,[c] Guangdong Zhou,[c] and Zunli Shen[b]

[a] *The State Key Laboratory of Metal Matrix Composites, School of Materials Science and Engineering, Shanghai Jiao Tong University, Shanghai 200240, P. R. China, Fax: +86-21-34202745; Tel: +86-21-34202956; E-mail: hjdou@sjtu.edu.cn (H. Dou);*

[b] *Department of Plastic and Reconstructive Surgery, Shanghai First People's Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200080, P. R. China; E-mail: zzf555@sina.com (Z. Zhang);*

[c] *Department of Plastic and Reconstructive Surgery, Shanghai 9th People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai Key Laboratory of Tissue Engineering, National Tissue Engineering Center of China, Shanghai 200011, China*

Supporting Information

Experimental Section

Materials

7-Amino-4-methyl coumarin (AMC) was purchased from Aladdin Reagent Co., Ltd. Dextran (also abbreviated as Dex, M_w = 40,000 Daltons), acrylic acid (AA), and ceric ammonium nitrate (CAN) were purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (EDC·HCl) and *N*-hydroxysuccinimide (NHS) were purchased from Shanghai Medpep Co., Ltd. Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Gibco and Sigma, respectively. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma. Other chemical reagents were all purchased from Shanghai Sinopharm Chemical Reagent Co., Ltd. Dialysis membrane bags with a cut-off molecular weight of 14,000 Da were purchased from Shanghai Green Bird Co., Ltd.

Functionalization of Dex-PAA NGs by loading diverse cargoes

The fluorescent agent 7-amino-4-methyl coumarin (AMC) was conjugated with NG4M2 through an amide condensation reaction using EDC/NHS as the catalyst system. NG4M2 was dispersed in water at a concentration of 1 mg/mL, then EDC and NHS (10 eq. of the acrylic acid monomer used for the synthesis of the nanogels) were added. Half an hour later, a DMSO solution containing an equivalent number of AMC molecules to the amount of the acrylic acid used for the synthesis of the nanogels was added into the reaction solution. The reaction was allowed to proceed overnight at room temperature and then the reaction mixture was dialyzed against pure water for a week to remove the catalyst and any unreacted AMC molecules. The entire preparation was performed in the dark to avoid possible fluorescent quenching. The product was denoted as FNG(AMC).

Magnetic nanogels (MNGs) was synthesized by an *in situ* co-precipitation method as reported previously.[1] 0.50 g of NG4M2 was dissolved in 75 mL of deionized water and mixed with 50 mL of a degassed aqueous solution containing 0.10 g of $FeCl_2 \cdot 4H_2O$ and 0.27 g of $FeCl_3 \cdot 6H_2O$ ($n(Fe^{2+}):n(Fe^{3+})$ = 1:2) for at least 15 min to allow the complete interaction of the negatively charged carboxyl groups of NG4M2 with the iron cations. Then the mixed solution was slowly added dropwise into an excess of aqueous ammonia under nitrogen protection and vigorous stirring (~1000 rpm) at 80 ^{o}C. The color of the solution changed from yellow to brown, and then

quickly changed to dark red and eventually turned black, which indicated the formation of Fe_3O_4. The reaction continued for 1 h and then the reaction mixture was dialyzed against distilled water for 3 days. The resultant MNGs were collected by magnetic separation and lyophilization.

Characterization of FNG(AMC) and MNG

Fluorescence spectrum of FNG(AMC) was recorded on a RF-5301PC Fluorescence Spectrophotometer (Shimadzu, Japan) with an excitation wavelength of 350 nm and an excitation and emission slit of (3,3). Magnetic measurements of the MNGs were carried out at room temperature by using a vibrating sample magnetometer (VSM 7407, Lakeshore, U.S.A.) with a maximum magnetic field of 18,000 G.

FNG4M2 uptake by macrophage cells

Mouse Ana-1 macrophage cells (purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China)) were seeded onto tissue culture dishes with a diameter of 6 cm and cultured for 24 hours in an atmosphere of 5% CO_2, 37 ºC and saturated humidity. FNG4M2 were added into a DMEM medium to reach a final concentration of 100 μg/mL, and co-incubated with the Ana-1 cells for another 2 h or 24 h. Subsequently, the supernatant medium was sucked, and the cells were washed three times with PBS buffer, immersed in the DMEM culture medium again, and then observed under a fluorescence microscope (IX-70, Olympus, Japan).

TEM imaging of the cellular uptake of FNG4M2

The cellular uptake of FNG4M2 by both rat kidney NRK and liver BRL-3A cells (both cells were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, China)) was visualized by transmission electron microscopy (TEM). For TEM observation, NRK cells or BRL-3A cells were co-incubated with FNG4M2 for 24 hours and then thoroughly washed three times with PBS and then fixed in PBS with glutaraldehyde (2 wt.%) for 2 h at 4 ºC, washed with PBS (2 x 10 min), and subsequently equilibrated in a PBS solution containing osmium tetraoxide (1 wt.%) for 2 h at 4 ºC. Thereafter, the sample was washed and dehydrated in a series of graded ethanol solutions (30%, 50%, 70% containing 3 wt.% uranyl acetate, 80%, 95%, 100%, each for 10 min). Epoxypropane was used as the transition fluid between ethanol and epoxy. After infiltration series, the blocks were polymerized at 60 ºC for 48 h, trimmed and sectioned. Ultrathin sections were stained with lead citrate, and were observed with a Philips CM-120 Transmission

Electron Microscope.

Cytotoxicity of FNG4M2 with NRK and BRL-3A cells

Cytotoxicity experiments were performed on both rat kidney NRK cells and rat liver BRL-3A cells through the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. NRK or BRL-3A cells were seeded at a density of 6000 cells/well in 96-well culture plates and incubated for 24 h. An equal volume (0.15 mL) of FNG4M2 at various concentrations (0, 25, 100, 200, or 400 μg/mL) were dissolved in a culture medium and were added into each well, with at least 5 parallel experiments. The co-incubation process was performed in an incubator (5% CO_2, 37 ^oC and saturated humidity) for 24 h. Then the supernatant was removed and cells were subsequently washed with PBS. 0.15 mL of the medium containing 10 % MTT was then added into each well and incubated for 4 h at 37 ^oC. The supernatant was removed and aliquots of DMSO (0.15 mL) were added into each well to solubilize any resulting formazan crystals for 10 min. Optical density (OD) values of the samples were measured at 560 nm with a Microplate Reader (Varioskan Flash, Thermo Scientific, U.S.A.). The cell viability rate was calculated using Equation S1.

$$\text{Cell Viability Rate} = \frac{OD_{experiment}}{OD_{control}} * 100\% \qquad \text{(Equation S1)}$$

The 5-AF content in FNGs

The ratio of 5-aminofluorescein in FNG4M1, FNG4M2 and FNG4M3 was calculated from the UV-Vis absorption of FNGs at 488 nm according to the calibration curve of 5-AF monomer dispersed in water.

Results and Discussion

Characterization of FNG(AMC) and MNG

A FNG(AMC) nanogel exhibiting blue fluorescence with an maximum emission wavelength of 413 nm was fabricated and the fluorescence spectrum of this nanogel is shown in Figure S1. FNG(AMC) was also incubated with P2 ADSCs cells for 24 h and a fluorescence microscopy image of the incubated cells is shown in the inset of Figure S1a. The fluorescence microscopy observation confirmed that this nanogel was readily internalized by P2 ADSC cells.

MNG was prepared through the *in situ* co-precipitation of Fe_3O_4, and the product was super-paramagnetic, with a maximum magnetization of 6 emu/g. The MNG could be thoroughly attracted by a magnet after 24 hours' contact as could be seen in

Figure S1b, which demonstrated that the MNG exhibited a strong magnetic response.

Macrophage uptake of FNG4M2

As shown in Figure S2, FNG4M2 could be easily uptaken by macrophage cells, and the fluorescence was strong enough to be observed in 2 h, and the fluorescence grew much stronger after 24 h of incubation.

TEM images of FNG4M2 up taken by NRK and BRL-3A cells

After staining, FNG4M2 were readily visible within cellular lysosome and endosome, which indicated that FNG4M2 mostly entered cells in an endocytosis way as reported elsewhere.[2, 3]

Cytotoxicity of FNG4M2 with NRK and BRL-3A cells

The cytotoxicity of FNG4M2 was also evaluated on both rat kidney NRK cells and rat liver BRL-3A cells. Both results showed that FNG4M2 had good biocompatibility and high cell viability.

5-AF content in FNGs

The calibration curve of the 5-AF at 448 nm were shown in Fig. S5(a), and the weight ratio of 5-AF in the nanogels were calculated according to the Equation S2,

$$5\text{-}\,\mathrm{AF\,content\ ratio\,(wt.\%)} = \frac{A_{\mathrm{FNGs,448nm}} - 0.0572}{0.0661 \times c_{\mathrm{FNGs}}} \times 100\% \qquad \text{(Equation S2)}$$

*Here $A_{\mathrm{FNGs,448\ nm}}$ denotes the absorbance value of FNGs at a wavelength of 448 nm, 0.0572 and 0.0661 are calculated from the 5-AF calibration curve, c_{FNGs} is the concentration of FNGs used for the UV absorbance measurement (μg/mL).

where the $A_{\mathrm{FNGs,448nm}}$ denotes the UV-Vis absorption at 448 nm of the aqueous nanogel solution due to the existence of 5-aminofluorescein (5-AF) in the nanogels. The weight ratio of 5-AF in the FNG4M1, FNG4M2 and FNG4M3 nanogels were determined as 1.55%, 1.56% and 1.59%, respectively. For example, the calibration curve of the 5-AF at 448 nm and the UV-Vis absorption spectra of NG4M2 and FNG4M2 (500 μg/mL) are as shown in Figure S5(b).

References

1 H. Dou, B. Xu, K. Tao, M. Tang and K. Sun, J. Mater. Sci.: Mater. Med., 2008, **19**, 2575-2580.

1 2 T.-G. Iversen, T. Skotland and K. Sandvig, Nano Today, 2011, **6**, 176-185.

2 3 V. Mailänder and K. Landfester, Biomacromolecules, 2009, **10**, 2379-2400.

3

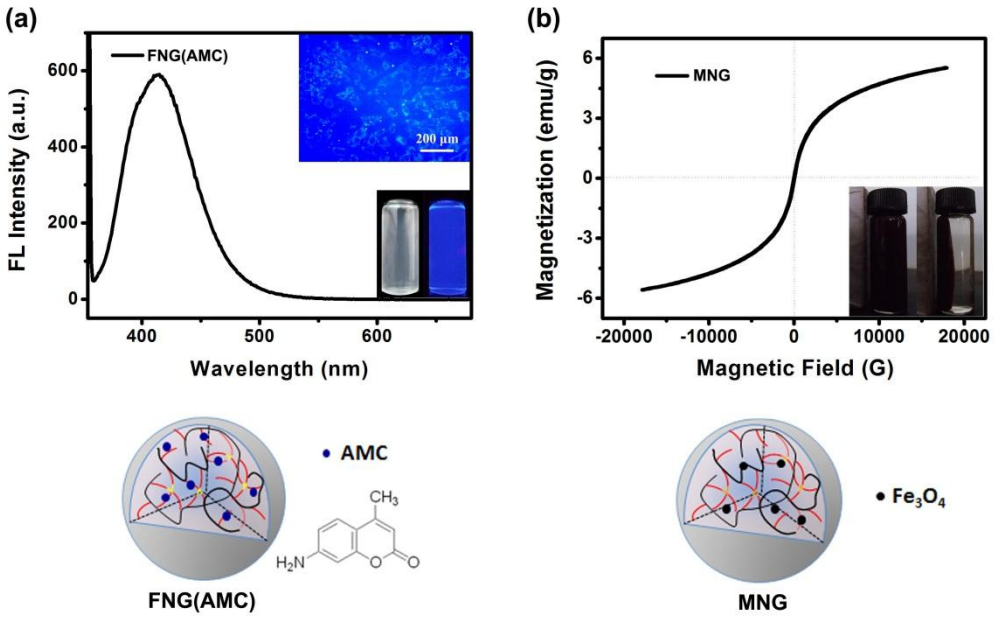

Figure S1. Characterization of FNG(AMC) and of MNG. A fluorescence spectrum and a schematic structure of FNG(AMC) are shown in (a), while the upper inset shows a fluorescence microscopy image of P2 ADSCs cells after they were co-incubated with FNG(AMC) for 24 h. The lower inset shows images of aqueous solutions of FNG(AMC) under sunlight (left) and UV light (right). A vibrating sample magnetometer (VSM) spectrum and a schematic structure of MNG are shown in (b). The inset shows images of an aqueous MNG solution before (left) and after (right) it was exposed to a magnet for 24 h.

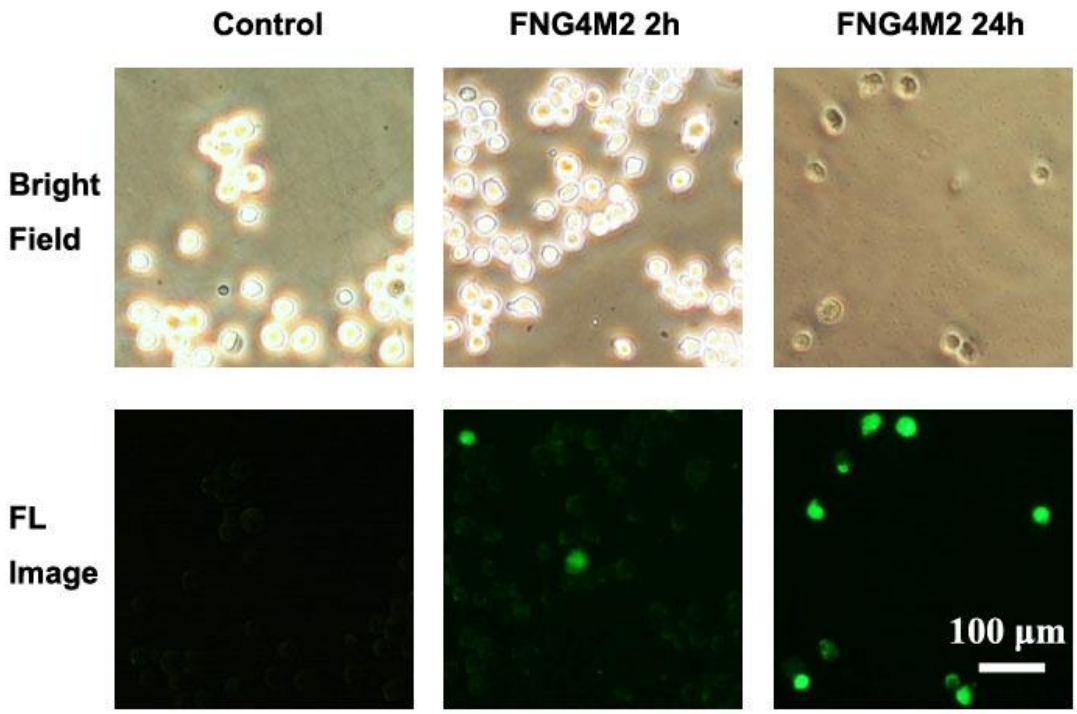

Figure S2. Optical and fluorescence microscopy images of macrophage cells after co-incubation with FNG4M2 for 2 h and 24 h (exposure time = 1 s). Images of non-incubated control macrophage cells are also shown.

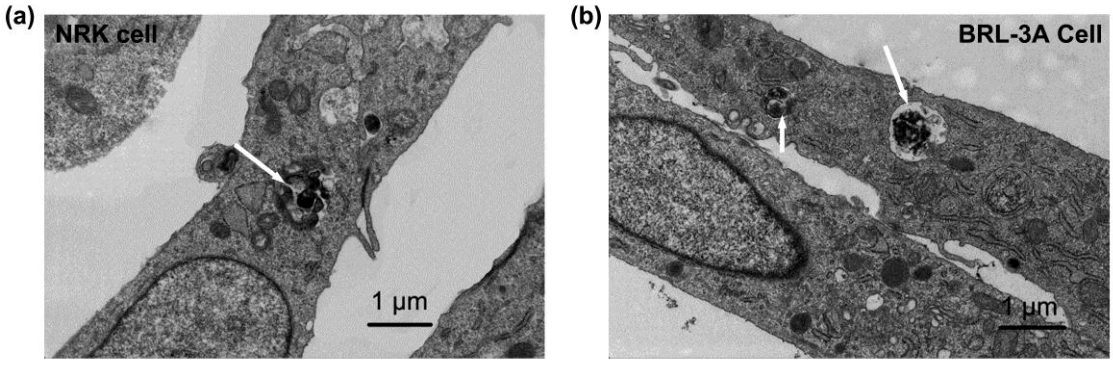

Figure S3. TEM images of NRK (a) and BRL-3A (b) cells that had internalized FNG4M2. The white arrows denote FNG4M2 nanogels inside the lysosomes.

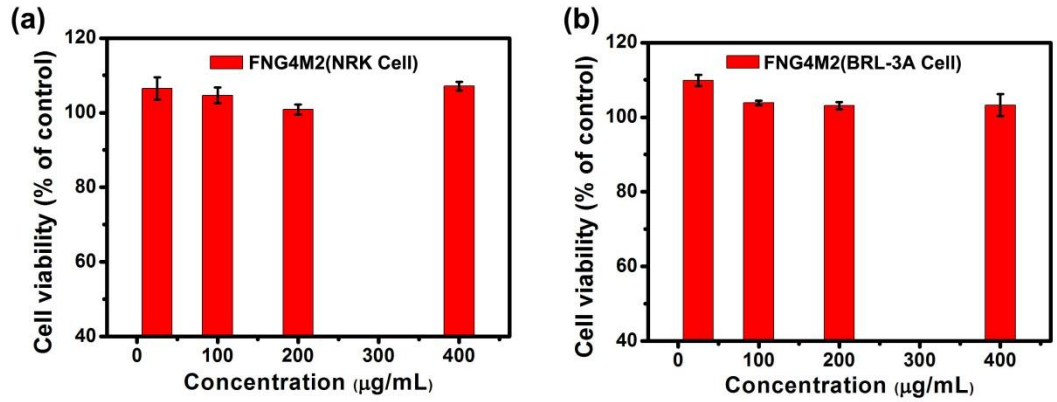

Figure S4. Cell viability data obtained by the MTT method for NRK (a) and BRL-3A (b) cells that had been co-incubated with FNG4M2 for 24 h. The data represents the mean ± SD, $n = 5$.

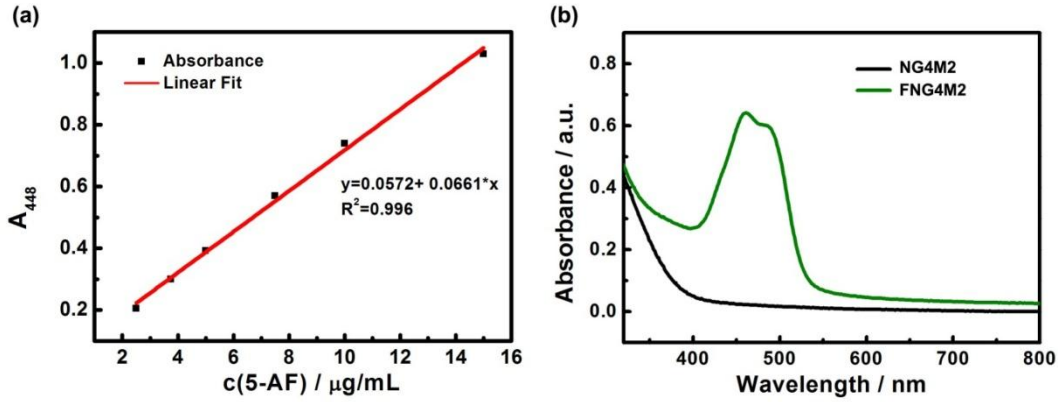

Fig.S5 The calibration curve of the 5-aminofluorescein in water at 448 nm (a) and UV-Vis absorption spectra of NG4M2 and FNG4M2 (500 μg/mL)