ADVANCED MATERIALS

Supporting Information

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Designed Fabrication of Multifunctional Polymer Nanomedical Platforms for Simultaneous Cancer-Targeted Imaging and Magnetically-Guided Drug Delivery**

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Materials

Poly(D,L-Lactide-co-glycolide) with a lactide:glycolide molar ratio of 50:50 copolymer was purchased from Boehringer Ingelheim (Resomer RG502H). Pluronic F127 was purchased from BASF Co. Ethylenediamine, poly(ε-carbobenzoxy-L-lysine) (ε-CBZ-PLL, MW=1000), folate, and N-hydroxysuccinimide (NHS) were all obtained from Sigma Chemical Co. Poly(ethylene glycol) (NH_2-PEG-COOH, MW=3400) was purchased from Nektar. 1,3-dicyclohexylcarbodiimide(DCC)was supplied from Fluka Chemie. Fetal bovine serum (FBS), folate-free Roswell Park Memorial Institute 1640 medium (RPMI 1640), and Dulbecco's Phosphate Buffered Saline (PBS) were obtained from Gibco BRL. All other chemicals and reagents were of analytical grade.

Characterization

The transmission electron microscopy (TEM) images were collected on a JEOL JEM-2010 electron microscope operated at an accelerating voltage of 200 kV equipped with ultra scan CCD camera unit of Gatan USC 100. The field emission scanning electron microscopy (FE-SEM) images were recorded with a Jeol JSM-6700F microscope. The UV-Vis absorption spectra were taken with a UV-Visible spectrometer (Jasco V-550). The photoluminescence (PL) spectra were obtained using a fluorescence spectrometer (Jasco FP-6500). The confocal laser scanning microscopy data were obtained using Confocal Laser Scanning Microscope (LSM 510, Carl-Zeiss Inc., USA). The extents of intracellular uptake of PLGA nanoparticles by the cells were monitored by flow cytometry (FACScan, Becton Dickinson) using a CELLQUEST software (PharMingen).

Experimental

Synthesis of PLGA(MNP/DOXO) and PLGA(QD/DOXO) nanoparticles

PLGA nanoparticles containing 15 nm Fe_3O_4 (or 3 nm CdSe/Zn) nanocrystals and doxorubicin were prepared using the conventional oil-in-water (O/W) single emulsion-evaporation technique. Methylene chloride solution of doxorubicin was prepared by extracting DOXO from aqueous solution (1 mg/mL) of DOXO·HCl after adding three equivalents of triethylamine. Initially, as-synthesized nanocrystals were dispersed in methylene chloride, and the resulting dispersion was mixed with the doxorubicin solution. 10 mg of PLGA was added to 1 mL nanocrystals/DOXO solution of various nanocrystal concentrations. This mixture was emulsified with 50 mL of the F127 aqueous solution (7 wt %) using a probe type sonicator (Sonics & materials inc. VCX600) at 600 watt for 10 min in ice bath. The resulting suspension was stirred for 5 h at room temperature to evaporate organic solvent and was subsequently centrifuged at 10000 rpm for 20 min. The supernatant was removed and the precipitate was redispersed in water. The centrifugation followed by dispersion in water was repeated twice to remove the remaining F127 and free PLGA. Finally, the resulting solution was filtered with 800 nm pore size cellulose acetate filter and was stored at 4 °C.

The Calculation of the Doxorubicin-Loading Content

The PLGA(MNP/DOXO) nanoparticle solution was lyophilized to yield the solid samples. The dried samples were weighed and re-dissolved in a mixture of chloroform and DMSO (1:1, v/v). The Fe_3O_4 nanoparticles were removed by centrifugation and the supernatant was used for the UV-Vis measurement. The doxorubicin concentration was calculated based on the absorbance intensity at 480 nm compared to standard absorption curve of the doxorubicin.

In Vitro Release of Doxorubicin from PLGA(MNP/DOXO) nanoparticles

For release experiments, 1 mL of PLGA(MNP/DOXO) nanoparticles (4.5 mg/mL) was placed into a dialysis tube with an MW cutoff of 5000 Da. The tube was placed into 25 ml phosphate buffered (pH 7.4) or acetate buffered (pH 5.0) solutions. Release study was performed at 37 ^{o}C under stirring of buffered solution. At selected time intervals, buffered solution outside the dialysis tube was removed for UV-Vis analysis and replaced with fresh buffer solution. The doxorubicin concentration was calculated based on the absorbance intensity at 480 nm using the collected buffered solution.

Preparation of PLL-PEG-FOL conjugates

PLL-PEG-FOL was synthesized by coupling ε-CBZ-PLL-NH_2 to COOH-PEG-FOL according to the previous report (S. H. Kim, J. H. Jeong, K. W. Chun, T. G. Park, *Langmuir* **2005**, *21*, 8852).

Preparation of PLGA-FOL nanoparticles

PLGA nanoparticles with negative-charged surface charges due to carboxyl groups exposed on the surfaces were coated with PLL-PEG-FOL conjugate via ionic interactions in an aqueous phase. PLGA nanoparticles in 50 μL of phosphate buffered saline (PBS, pH 7.4) was mixed with a desired amount of PLL-PEG-FOL (0-50 μg) at room temperature for 15 min.

Cell culture

A human epidermal carcinoma cell line (folate receptor overexpressing cell line), KB cells were purchased from the Korean Cell Line Bank (Seoul, Korea). KB cells were maintained in RPMI 1640 medium without folate at 37 °C in a humidified atmosphere of 5 % of CO_2. The cell culture media were supplemented with 100 units/mL penicillin, and 100 μg/mL streptomycin.

In Vitro Cytotoxicity against KB cells

KB cells were seeded in a 96-well plate at a density of 1×10^4 cells per well and were grown in RPMI medium (without folate) supplemented with 10% (v/v) fetal bovine serum for 24 h at 37 ^oC. After washing (1 × PBS), the culture medium was replaced with serum free RPMI medium (without folate) containing the free DOXO, naked PLGA(MNP/DOXO), and PLGA(MNP/DOXO)-FOL nanoparticles and the cells were incubated for 4 h at 37 ^oC. Another PLGA(MNP/DOXO)-FOL nanoparticles were incubated under the same condition except applying an external magnetic field onto the bottom of the plate. The concentration of doxorubicin was 0.4 μM. Then cells were replaced with fresh serum containing medium (without folate) and further incubated for 5 days. Finally, the cell viability was determined by the CCK-8 cell viability assay.

Confocal Laser Scanning Microscopy

KB Cells were plated in chamber slides (Labtek chamber slide 4-well, Nunc) at a density of 2×10^4 cells per well 24 h before cellular uptake. The naked PLGA(MNP/DOXO), PLGA(MNP/DOXO)-FOL nanoparticles, and PLGA(QD/DOXO)-FOL nanoparticles were incubated in KB cell culture for 2 h at 37 ^oC in serum free medium. Another PLGA(MNP/DOXO)- FOL nanoparticles were incubated under the same condition except

applying an external magnetic field onto the bottom of the slide. The concentration of doxorubicin was 0.07 μM. Then the cells were washed with cold PBS three times and fixed in 1% paraformaldehyde solution. The slides were covered with a mounting medium (SuperMount, InnoGenex, San Ramon, CA). In the case of DOXO, the cells were imaged by a confocal laser scanning microscope (Carl Zeiss LSM5100, Germany) using a helium-neon laser with an excitation wavelength of 543 nm and 560 - 615 nm emission band-pass filter. An argon laser with an excitation wavelength of 488 nm was used for excitation of green QD and the image was detected using 501 nm emission band-pass filter.

Flow Cytometry Analysis and In vitro MR imaging of cancer cells

KB cells were seeded in a petri-dishes (90 mm in diameter) at a density of 5×10^6 cells per dish and were grown in RPMI medium (without folate) supplemented with 10% (v/v) fetal bovine serum for 24 h at 37 ^{o}C. After washing (1 × PBS), the culture medium was replaced with serum free RPMI medium (without folate) containing naked PLGA(MNP/DOXO) and PLGA(MNP/DOXO)-FOL nanoparticles and the cells were incubated for 30 min at 37 ^{o}C. Another PLGA(MNP/DOXO)-FOL nanoparticles were incubated under the same condition except applying an external magnetic field onto the bottom of the slide. Then cells were replaced with fresh serum containing medium and further incubated for 6 h at 37 ^{o}C. The cells were rinsed with PBS buffer solution and were cleaved by trypsin. After fixing in 1% paraformaldehyde solution, followed by incubation at 4 ^{o}C for 2 h, a portion of the cells were resuspended in PBS solution for flow cytometry. The remaining cells were centrifuged, and the resulting cell pellets were mixed with 1% agarose solution. The mixture was transferred into Eppendorf-tube for MR measurement.

Measurement of the specific relaxivity, r_2, of PLGA(MNP/DOXO) nanoparticles dissolved in distilled water and the cells mixed with agarose were performed with a 3.0 T whole body MRI system (Philips, Achieva ver. 1.2, Philips Medical Systems, Best, The Netherlands) equipped with 80 mT/m gradient amplitude and 200 ms/m slew rate. For r_2 measurements, spin-spin relaxation times (T_2) measurements were performed by using 10 different echo times in a multislice turbo spin echo sequence (TR/TE = 5000/20, 40, 60, 80, 100, 120, 140, 160, 180, 200 ms, in-plane resolution = 200×200 μm^2, slice thickness = 500 μm). The images were fitted into Levenberg-Margardt method to calculate T_2 values using Matlap program. The signal intensities of each ROIs (200 - 300 pixels) in the T_2 map were measured for each concentration and used for specific relaxivity calculations.

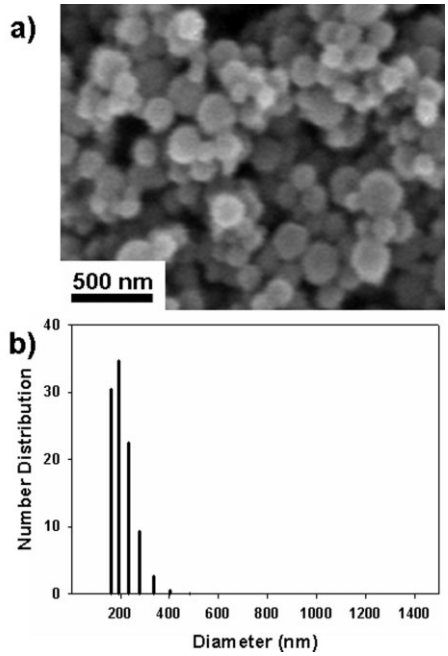

Figure S1. a) FE-SEM images of PLGA(MNP/DOXO) nanoparticles embedding 15 nm Fe_3O_4 nanoparticles. b) Size distribution of PLGA(MNP/DOXO) nanoparticles obtained by dynamic light scattering (DLS).

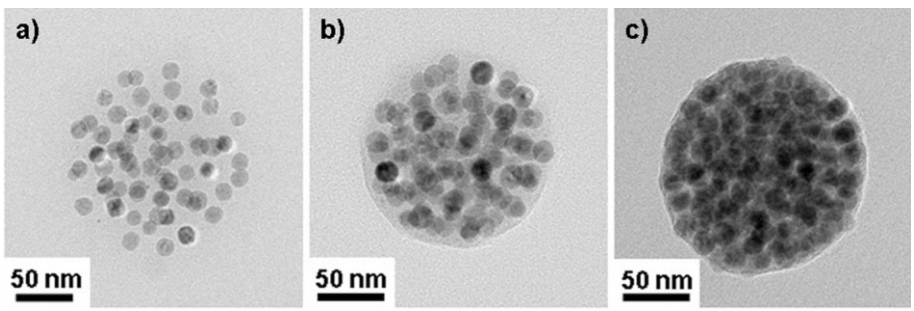

Figure S2. TEM images of PLGA(MNP/DOXO) nanoparticles synthesized with different amount of 15 nm Fe_3O_4 nanocrystals of a) 0.605 mg/mL, b) 2.42 mg/mL, and c) 6.46 mg/mL, respectively.

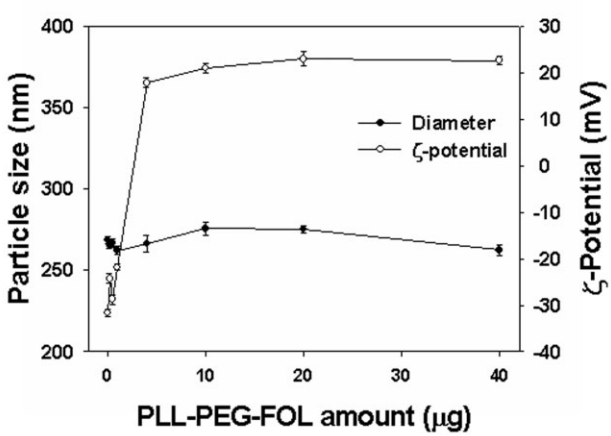

Figure S3. The particle size and the ξ-potentials of PLGA(MNP/DOXO) nanoparticles incubated with different amounts of PLL-PEG-FOL conjugate. (Actually no PLL-PEG-FOL but PLL-PEG even though there will be no differences between PLL-PEG and PLL-PEG-FOL conjugate.)

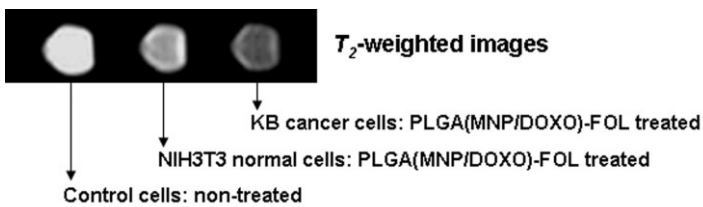

Figure S4. In vitro T_2-weighted images of KB cancer cells and NIH3T3 normal cells incubated with PLGA(MNP/DOXO)-FOL nanoparticles.

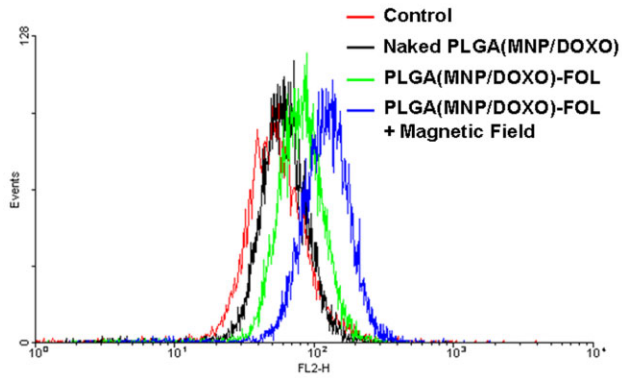

Figure S5. Flow cytometric analysis of control KB cells, and KB cells treated with naked PLGA(MNP/DOXO), PLGA(MNP/DOXO)-FOL, and PLGA(MNP/DOXO)-FOL nanoparticles under an external magnetic field.

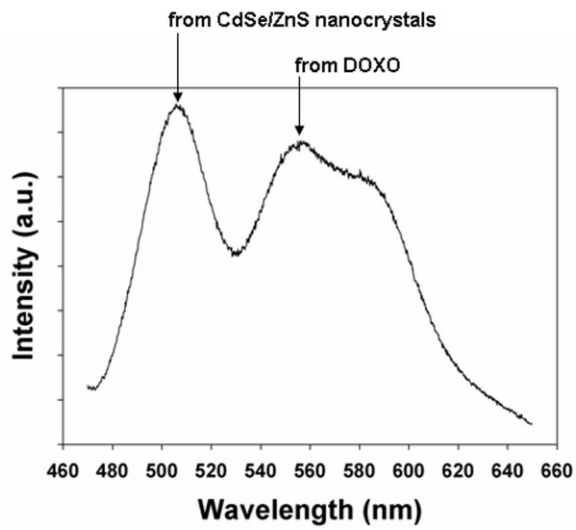

Figure S6. Photoluminescence spectrum of PLGA(QD/DOXO) nanoparticles in water (excitation at 450 nm).

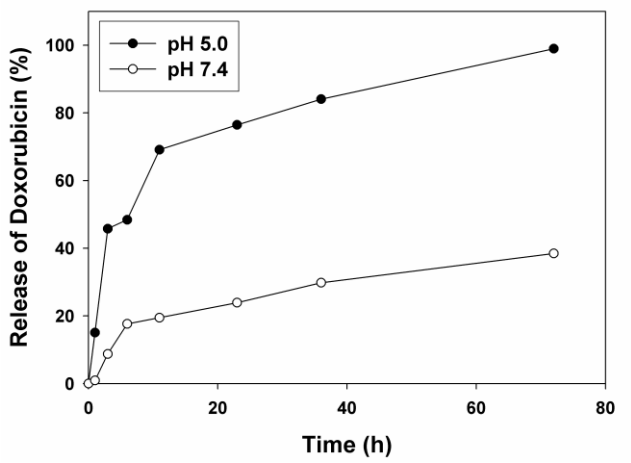

Figure S7. In vitro DOXO release from PLGA(MNP/DOXO) nanoparticles at neutral (pH 7.4) and acidic conditions (pH 5.0) at 37 ^{o}C.

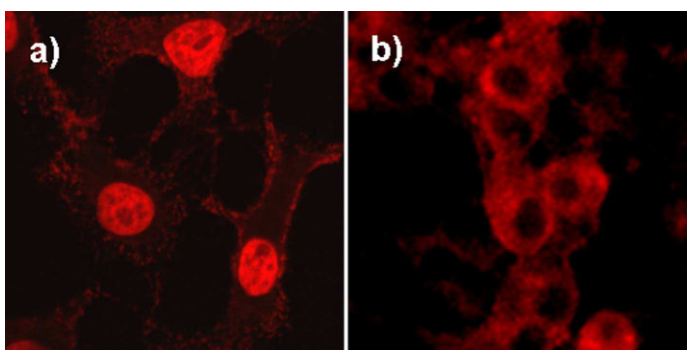

Figure S8. CLSM images of KB cells incubated with (a) the free DOXO and (b) the PLGA(MNP/DOX)-FOL nanoparticles for 2 h.