Electronic Supplementary Information

Magnetic Polyaniline Nanohybrid for MR Imaging and Redox-sensing of Cancer Cells

Yoochan Hong[a,b,†], Seungyeon Hwang[b,c,†], Dan Heo[b,c], Bongjune Kim[d], Minhee Ku[b,e], Eugene Lee[b,c], Seungjoo Haam[d,f], Dae Sung Yoon[a], Jaemoon Yang[b,f]*, and Jin-Suck Suh[b,f,g,*]

[a]Department of Biomedical Engineering, Yonsei University, Maeji 234, Heungup, Wonju, Gangwondo, Republic of Korea 220-710.

[b]Department of Radiology, College of Medicine, Yonsei University, Shinchon, Seodaemoon, Seoul, Republic of Korea 120-752.

[c]Nanomedical National Core Research Center, Yonsei University, Shinchon, Seodaemoon, Seoul, Republic of Korea 120-749.

[d]Department of Chemical and Biomolecular Engineering, Yonsei University, Shinchon, Seodaemoon, Seoul, Republic of Korea 120-749.

[e]Brain Korea 21 Plus Project for Medical Science, Yonsei University, Shinchon, Seodaemoon, Seoul, Republic of Korea 120-752.

[f]YUHS-KRIBB Medical Convergence Research Institute, Shinchon, Seodaemoon, Seoul, Republic of Korea 120-752.

[g]Severance Biomedical Science Institute (SBSI), Shinchon, Seodaemoon, Seoul, Republic of Korea 120-752.

[†]These authors contributed equally to this work

*Corresponding author: 177hum@yuhs.ac, and jssuh@yuhs.ac

Experimental Section

Materials: Polyaniline (PAni, 5,000 Da), benzyl ether, Tween 80, dichloromethane, and Iron (III) acetylacetonate were purchased from Sigma-Aldrich (St. Louis, MO, USA). 3-maleimidopropionic acid is purchased from Tokyo Chemical Industry Ltd, and membrane type-1 matrix metalloproteinase (MT1-MMP) targetable peptide is obtained from Peptron. 1-Methyl-2-Pyrrolidone (NMP) and ethanol (EtOH) were purchased from Dae-Jung, KOREA. All other chemicals and reagents were analytical grade. Ultrapure deionized water (DW) was used for all of the synthetic processes.

Synthesis of polyaniline-coated magnetic nanohybrid (MPNH): The synthetic process of immaculate product was as follows: 1g of PAni was dissolved in 20 mL of NMP. 706.4 mg iron (III) acetylacetonate was dissolved in 20 mL of benzyl ether. After mixing of the two solutions, then the mixture was pre-heated to 200^{o}C for 1 hour, repeatedly heated to 300^{o}C for 30 minutes. The reactant was cooled in room temperature for 3 hours. Subsequently, the product was washed with excess of EtOH, and separated the precipitants by centrifuging at 3,000 rpm for 10 minutes. This centrifugation was repeated for three times, and then, the product was re-dispersed in EtOH. The end product was dispersed in NMP. After synthesis of MPNH, the morphology and structure of MPNH were evaluated with a transmission electron microscope (TEM, JEM-1011, JEOL Ltd). Fourier transform infrared spectra (FT-IR spectrum Two, Perkin Elmer) analysis was performed to confirm the characteristic bands of the synthesized MPNH. The absorbance of MPNH was analyzed UV-Vis spectrophotometer (Optizen 2120UV, MECASYS Co.).

Preparation of maleimide modification of TWEEN80: 1 g of TWEEN80 was dissolved in 50 mL of dichloromethane. 0.709 g of N,N'-dicyclohexylcarbodiimide, 0.420 g of 4-dimethylaminopyridine, 0.194 g of 3-maleimidopropionic acid, and 0.479 mL of triethylamine were added the solution, respectively. The mixed solution was stirred for 48 hours at the room temperature. The unwanted solution was rapidly removed using the rotary evaporator. The product was re-dissolved in DW, and then dialyzed excess deionized water (DW) for 24 hours. After dialysis, the mixture was filtered using filter paper to remove the unwanted precipitate. Finally, the product was freeze-dried and stored.

Preparation of MPNHm and MPNHm-P: 0.1 g of maleimido-modified TWEEN80 was dissolved in 30 mL of DW. 5 mL of MPNH solution was added and magnetic stirred for 4 hours at the room temperature. The product was re-dissolved in DW, and then dialyzed excess DW for 2 days.and then dialyzed excess DW for 2 days. 450 μg of MT1-MMP targetable peptide was dissolved into the 5 mL of MPNHm solution. Then, the solution mixed using a vortex for 30 minutes. After synthesis of MPNHm, the morphology and structure of MPNHm were analyzed by Transmission electron microscopy. The hydrodynamic size and zeta potential were measured using dynamic light scattering method and the presence and crystallinity of magnetic substances in the MPNHm was verified using X-ray diffraction analysis. The sensitivity toward a magnetic field was investigated using vibrating sample magnetometer. MR imaging of MPNHm experiments were performed with a 1.5-Tesla clinical MRI instrument with a Micro-47 surface coil (Intera, Philips Medical Systems). R2 relaxivities of MPNHm solutions were measured at room temperature with the Carr-Purcell-Meiboom-Gill sequence (TR = 400 ms, 32 echoes with 12-ms even echo space, number of acquisitions = 1, point resolution = 0.234 × 0.234 mm, section thickness = 0.6 mm). R2 was defined as 1/T2 with units of s^{-1}. The ratio of R2 to MPNHm concentration is equal to the relaxivity coefficient (mM^{-1} s^{-1})

Cell culture conditions and viability study: Human breast cancer, MCF7, and fibrosarcoma, HT1080 cell lines were cultured in DMEM with 10% fetal bovine serum in a humidified incubator containing 5% CO_2 at 37^{o}C. The cell viability of MPNHm-P for HT1080 and MCF7 cells were quantified by a colorimetric assay based on the mitochondrial oxidation of 3-(4, 5-dimethylthiazoly-2)-2, 5-diphenyltetrazolium bromide (MTT) (Cell Proliferation Kit I, Roche, Germany). In a typical cell viability experiment, HT1080 cells and MCF7 cells (1×10^4 cells/well, respectively) were seeded into 96-well plate and incubated at 37^{o}C in a 5% CO_2 atmosphere. The cells were incubated for 4 hours with MPNHm-P, and after 24 hours, the yellow MTT solution was treated, and the formed formazan crystals were solubilized with 10% sodium dodecyl sulfate in 0.01 M HCl. Then the absorbance of the resulting colored solution was measured at 584 nm and at 650 nm as a reference using a microplate spectrophotometer (EpochTM, BioTek, USA). Cell viability was determined from the intensity ratio of treated to non-treated control cells and shown as an average ± standard deviation (n = 4).

In vitro redox-sensing: To measure the absorbance of MPNHm-P in cells, HT1080 cells and MCF7 cells (1×10^4 cells) incubated with 3.17 mM of MPNHm-P, that is, non-toxic concentration (based on iron concentration) were moved into cuvette. The cuvette was mounted on our home built absorbance measurement system between a quartz-tungsten-halogen light source (Ocean Optics, HL2000) with focusing lens and a portable spectrometer (Ocean Optics, USB4000) with collimating lens. For dark field imaging of cells with MPNHm-P, scattering imaging system was used. The imaging system was composed of an inverted microscope (Axio Observer A1, Carl Zeiss) and a color CCD camera (DCU224C, Thorlabs).

In vivo model and MR imaging: All animal experiments were conducted with the approval of the Association for Assessment and Accreditation of Laboratory Animal Care International. To establish the orthotopic mouse model of fibrosarcoma, HT1080 cells (5.0×10^6 cells) were implanted into the proximal thigh of female mice (6-week-old BALB/c-nude mice). When tumor size reached approximately 500 mm^3 (tumor volume in mm3 is calculated by following formula, tumor volume = $4/3 \times \pi \times (\text{width}/2)^2 \times (\text{length}/2)^2$), MPNHm-P was injected intravenously into the tail vein. *In vivo* MR imaging experiments were performed with a 3.0 T clinical MRI instrument (SIMENS). For T2-weighted MR imaging, the following parameters were adopted: TR = 3,000 ms even echo space, number of acquisitions = 4, point resolution of 192 × 180 mm, section thickness of 0.1 mm and TE = 79 ms.

Tissue staining and imaging: After *in vivo* MR imaging, hematoxylin and eosin (H&E) and Prussian blue staining were conducted to confirmation of MPNHm-P targeting for MT1-MMP-expressing on HT1080 cancer cells. Tissues were embedded in paraffin after being dehydrated in increasing alcohol concentrations and cleared in xylene. Slices (thickness = 10 μm) were mounted onto glass slides and were twice placed in a container filled with hematoxylin for 10 minutes, to stain the nuclei. Tissues were rinsed in water for 10 minutes to remove hematoxylin, and the cytoplasm was stained with eosin and dehydrated in the same method as described above. After washing three times for 30 minutes, we added 2 ~ 3 drops of the mount medium onto the slide and then covered the slide with a cover slip. To visualize the existence of MPNHm-P on tumor, an additional slide was stained using the Prussian blue staining kit. All of the stained tissue sections were analyzed using a virtual microscope (Olympus BX51, Japan) and Olyvia software.