Electronic Supplementary Information

Active Colorimetric Lipid-coated Polyaniline Nanoparticles for Redox State Sensing of Cancer Cells

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Materials and Methods

1. Materials

Polyaniline (Pani) Mw ~5,000, 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), and N-Methyl-2-Pyrrolidone (NMP) were purchased from Sigma-Aldrich (S. Louis, Mo, USA). Dulbecco's phosphate-buffered saline (DPBS, pH 7.4) was purchased from Welgene, Korea. All other chemicals and reagents were of analytical grade. Ultrapure deionized water (DW) was used for all of the synthesis processes.

2. Preparation of LiPani nanoparticles

Water-soluble LiPani nanoparticles were prepared by a nanoprecipitation method.[21] First, 20 mg of Pani was dissolved in 2 mL of NMP and added to 6 mL of DW containing 20 mg of DPPC as a surfactant. The mixture was vigorously stirred at room temperature for 24 hours. After the reaction, LiPani nanoparticles were dialyzed (molecular weight cut-off: 1k Da) for 24 hours to remove excess DPPC and impurities, and filtered by centrifugation using a Centricon filter (molecular weight cut-off: 3k Da) for 2 hours at 3,000 rpm.

3. Characterization of LiPani nanoparticles

The size of LiPani nanoparticles was confirmed by dynamic light scattering (DLS) method (ELS-Z, Otsuka Electronics, Japan), and atomic force microscopic (AFM) imaging (NX10, Park Systems, Korea). Fourier transform infrared spectra (FT-IR Spectrum Two, Perkin Elmer, Waltham, MA, USA) analysis was performed to confirm the characteristic bands of the synthesized LiPani nanoparticles, and the absorbance of prepared LiPani nanoparticles was measured using a UV-vis spectrophotometer (LAMBDA 45, Perkin Elmer, USA).

4. Cell viability test of LiPani nanoparticles

The cytotoxic effects of LiPani nanoparticles in HCC-1954 and HCC-1143 cells were evaluated by 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (Cell Proliferation Kit I, Roche Molecular Biochemicals, Mannhein, Germany). HCC-1954 and HCC-1143 cells were maintained in RPMI-1640 containing 10% FBS and 1% antibiotics at 37℃ in a humidified atmosphere with 5% CO_2. HCC-1954 and HCC-1143 cells (5.0 × 10 4 cells/well) were seeded into a 96-well plate at 37℃ for overnight and the cells were incubated with various concentrations of Pani for 48 hours. The cells were washed with 100 μL DPBS (pH 7.4, 1 mM), and 100 μL phenol red free RPMI-1640 was added. Subsequently, the cells were treated with MTT assay solution according to the manufacturer's instructions. Cell viability was evaluated using a microplate reader (Synergy H4 hybrid reader, BioTek) at an absorbance wavelength of 575 nm (reference wavelength of 650 nm). Cell viability was represented by normalization against of LiPani non- treated cells which were considered as having 100% cell viability.

5. Absorbance measurements of cancer cells treated with LiPani nanoparticles

HCC-1954 and HCC-1143 (1.0 x 10^6 cells/well) cells were seeded in 6-well plates and incubated for 24 hours at 37℃. Prepared various concentrations of LiPani nanoparticles were added to phenol red-free RPMI-1640 (10% FBS and 1% antibiotics). After incubation for 48 hours at 37℃ with 5% of CO_2. Transfer supernatant and spin at 15,000 rpm for 30 minutes to remove any remaining cell debris. It was measured absorbance wavelength of 600 and 900 nm using microplate reader (Synergy H4 Hydrid reader, BioTek) and used analyze software Gene5 (no 3.08, BioTek).

6. Dark field microscopic imaging of cell lines

HCC-1954 and HCC-1143 (1.0×10^6 cells/well) were seeded onto cover glass in 6-well plates

and incubated at for 4 hours at 37℃. Prepared various concentrations of LiPani were added to RPMI. After incubation for 48 hours at 37℃. To observe LiPani in the cancer cells, the dark field images were recorded using an uplight microscope (Olympus BX51, Japan) with a highly numerical dark field condenser (U-DCW, Olympus), which delivers a very narrow beam of white light from a tungsten lamp to the surface of the sample. Immersion oil (nd: 1.516, Olympus) was used to narrow the gap between the condenser and the glass slide, and to balance the refractive index.

7. Scattering spectrum measurements of Single LiPani nanoparticle

All single LiPani nanoparticle light scattering spectroscopic measurements and dark imaging were conducted using an inverted microscope (Axio Observer A1, Carl Zeiss Korea, Korea) equipped with an imaging spectrograph (Acton SP2500, Prince-ton Instruments, New Jersey) and a charge-coupled device (CCD) detector (PIXIS400B, Princeton Instruments, New Jersey). A color CCD camera (DCU224C, Thorlabs, New Jersey) was also attached to the front port of the microscope to use identification and alignment of a single LiPani nanoparticle. A dark field condenser (numerical aperture (NA) = 1.2–1.4) was used to image the single LiPani nanoparticle and a variable aperture 40× objective lens (NA = 0.6–1.5) was used to collect the light scattering signal from a single LiPani nanoparticle.

The method for scattering spectroscopic measurements are as follows. The spectrograph grating was placed in zero order and the spectrograph entrance slit was opened to the maximum setting to project a wide-field image onto the CCD detector. Next, single LiPani nanoparticle was placed in the center of the imaging field and the entrance slit was closed to about 20 μm. Next, the spectrograph grating was rotated to disperse the first-order diffracted light onto the CCD detector. To ensure that only the scattered light from a single LiPani nanoparticle was analyzed, the region of interest was selected using the CCD control software. An adjacent

empty region of the CCD detector with the same dimensions was also collected to perform background subtraction. Integration times varied, depending on lamp intensity and the scattering strength of the LiPani nanoparticle, but a typical acquisition was comprised of five exposures, each lasting 1 second. Finally, the raw scattered spectrum was normalized to correct for the lamp spectral profile, spectrograph throughput, and efficiency of the CCD detector. This process was accomplished by dividing the raw spectra by the lamp spectra, which were obtained by increasing the numerical aperture of the objective above 1.4.