Supporting information

Experimental

Materials

$Mg(NO_3)_2 \cdot 6H_2O$, $Al(NO_3)_3 \cdot 9H_2O$, $NaHCO_3$ and human serum (from human male AB blood type plasma, Cat No. H4522) were purchased from Sigma-Aldrich Co. LLC. (St. Louis., USA); NaOH pellets were obtained from Daejung Chemicals & Metals Co., Ltd (Siheung, Korea). Radioisotope $^{57}CoCl_2$ was purchased from Eckert & Ziegler Isotope Products Inc. (Valencia, USA). For cellular uptake, Hyclone™ RPMI (Roswell Park Memorial Institute)-1640 was purchased from GE healthcare (South Logan, USA); fetal bovine serum (FBS), phosphate buffered saline (PBS) and 0.05% trypsin-Ethylenediaminetetraacetic (EDTA) were obtained from Thermo Fisher Scientific Inc. (Gibco®, Waltham, USA). All chemicals were used without further purification.

Methods

1. Synthesis of layered double hydroxide nanoparticles with uniform particle size

For the preparation of LDH with uniform size, hydrothermal treatment after coprecipitation was utilized. Typically, a mixed metal solution (0.45 M of $Mg(NO_3)_2 \cdot 6H_2O$ and 0.225 M of $Al(NO_3)_3 \cdot 9H_2O$) was titrated by alkaline solution (1.35 M of NaOH and 1.0 M of $NaHCO_3$) until pH reached ~ 9.5, and the white suspension was sealed in autoclave, which was placed at 150 °C for 24 h. Final white precipitates were thoroughly washed with deionized water and stored as slurry state after centrifugation.

2. Characterization

The powder XRD patterns of LDHs were obtained with a Bruker AXS D2 phaser (Bruker AXS GmbH, Karlsruhe, Germany) by utilizing Ni-filtered Cu-Kα radiation (λ = 1.5406 Å) with a 1 mm air-scattering slit and a 0.1 mm equatorial slit. Data were collected from 5° to 70° (2θ) with time step increments of 0.02° and 0.5 s per step, respectively. The particle size and shape of LDH was verified by scanning electron microscopy (SEM: Quanta 250 FEG (FEI Company, Hillsboro, OR, USA) and atomic force microscopy (AFM: Park NX10 (Park Systems, Suwon, Korea)). For SEM measurement, LDH slurry was diluted with deionized water to approximate concentration ~0.4 mg/mL. Then a drop of suspension was placed on the Si wafer and dried in vacuum. The surface of specimen was sputtered with Pt/Pd plasma for 50 s and images were collected by 30 kV of accelerated electron beam. For AFM measurement LDH samples were diluted ~0.1 mg/mL and sonicated for thorough dispersion. One drop of suspension was loaded on Si wafer and dried in vacuum before measurement.

The zeta potential and hydrodynamic size of LDH were measured by light scattered electrophoresis and dynamic light scattering of ELS-Z1000 (Otsuka electronics, Osaka, Japan). For measurement, LDH was dispersed (~0.1 mg/mL concentration) in deionized water and cell culture media (RPMI-1640 with 10% FBS), respectively. The pH values of prepared colloid suspensions were adjusted ~ 7.0 with 0.01 M of HCl and NaOH. Zeta-potential and hydrodynamic size were calculated by Smoluchowski equation and Marquardt analysis, respectively, which were provided by the manufacturer.

3. Radioisotope Co-57 incorporation into LDH and its stability in human serum

All the radioisotope experiments were carried out at R&D advanced radiation

laboratory, Korea Atomic Energy Research Institute, under strict regulation.

In order to incorporate radioisotope Co-57 into LDH framework, isomorphous substitution method was utilized by modifying our previous work.[1] Typically, 1 mL of LDH suspension (5 mg/mL) was mixed with 880 μCi of Co-57 at room temperature and the mixture was immediately placed in 5 mL reaction vial for hydrothermal treatment at 150°C for 2 hours. Incorporated amount of Co-57 was evaluated by measuring radioactivity of supernatant obtained by centrifugation of reaction suspension (CRC-15R; Capintec, Inc., Ramsey, USA). The human serum stability of Co-57 incorporated LDH (Co-57/LDH) was evaluated with radio-thin layered chromatography (TLC; AR-2000, Bioscan, Poway, USA). The Co-57/LDH having 100 μCi of radioactivity was dispersed in 1 mL of human serum at 37 ºC and then vortexed. The radioactivity of free Co-57 and Co-57/LDH were determined by radio TLC (mobile phase: 0.01 M citric acid) at designed time point (15, 30, 60, 120 min and 24 hour).

4. Cellular uptake experiment

All cell lines were cultured in 6 cm Petri dishes at initial density of 1×10^7 cells/mL in 10mL Hyclone™ RPMI-1640 and 10% FBS, then were incubated at 37°C in a humidified 5% CO_2 incubator. After 3 days of culture, cells grown in a monolayer were harvested by trypsinization using a 0.05% trypsin-EDTA solution and culture medium was removed by centrifugation (1000 rpm, 3 min) and 10 mL fresh medium was added to the cell pellet for re-culture in the plate. Cellular uptake of Co-57/LDH was evaluated in both CT-26 (Mouse colon carcinoma cell) and HepG2 (Human hepatocellular carcinoma cell). Cell lines were seeded at 1×10^5 in 24 well plates at 1 mL per well and incubated at 37 ºC in a 5% CO_2 for 24 hours for an adherence and growth and then Co-57/LDH (5 μCi) was added to each well. At designated

time point (15, 30, 60 and 120 min), cells were washed twice in cold PBS to remove Co-57/LDH which did not enter cells. Then cells were detached with 0.05% trypsin-EDTA and counted by gamma counter (1470 Wizard 2, Perkin-Elmer, Waltham, USA). Cellular uptake was presented as a percentage of the injected radioactivity dose (ID%).

5. Biodistribution of radioisotope Co-57/LDH in tumor xenografted mice model

The 4-weeks-old BALB/c female mice were purchased from Orient Bio Inc. (Sungnam, Korea). The CT-26 cells (1×10^7 cell/100μL) were cultured as described in cellular uptake experiment and 100μL of cells were injected subcutaneously into right leg of mice (N=12). The mice were acclimatized until tumor diameter reached 10~15 mm during 2~3 weeks. Then, 100μL (100μCi) of Co-57/LDH suspension was injected intravenously through tail vein. At designed time point (15, 30, 60 and 120 min), the major organs (liver, spleen, kidney, and tumor) of mice were collected and the radioactivity was checked by gamma counter. The injected dose per gram% (ID/g%) was calculated by equation 1.

$$Injected\ dose/g\%\ (ID/g\%) = \frac{\left(\frac{CPM\ in\ tissue}{CPM\ of\ post\ injection}\right) \times 100}{Tissue\ weight} \quad \text{(equation 1)}$$

(CPM: Counts per minute)

Reference

1. T.-H. Kim, W.-J. Lee, J.-Y. Lee, S.-M. Paek and J.-M. Oh, *Dalton Trans.*, 2014, **43**, 10430-10437.