Supporting Information

HSA Coated MnO Nanoparticles with Prominent MRI Contrast for Tumor Imaging

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S.1 Preparation of MnO nanoparticles (MONPs)

Hydrophobic MONPs were synthesized by thermal decomposition of Mn-oleate complex following a previously published protocol. In brief, 1.98 g manganese chloride tetrahydrate (MnCl₂•4H₂O, 10 mmol, Sigma-Aldrich, 98 %) and 6.09 g of sodium oleate (20 mmol, TCI, 95 %) were added to a mixture composed of 10 mL of ethanol, 40 mL of distilled water and 50 mL of n-hexane. The resulting mixture was heated to 70 °C and stirred overnight. Then the solution was transferred to a separatory funnel. The upper organic layer containing the Mn-oleate complex was washed several times with distilled water and dried at 60 °C under Ar atmosphere. 2 mmol of the Mn-oleate was dissolved in 10 mL of 1-octadecene (Aldrich Chemical Co., 90%) to yield a transparent red solution. The mixture solution was purged with Ar for 1 h to remove water and oxygen and then heated to 300 °C with vigorous stirring. The reaction system was maintained at 300 °C for 1 h and then cooled down to room temperature. 20 mL of hexane was added into the raw product, followed by the addition of 80 mL acetone to precipitate the nanoparticles. The precipitate was collected by centrifugation and washed for two more runs to remove excess surfactant and solvent.

S.2 Preparation of water soluble MONPs

~5 mg as-synthesized MONPs and 20 mg dopamine were dissolved in a mixture solution of 5 mL chloroform and 5 mL DMSO. The mixture was heated at 70 °C for 1 h and then cooled to room temperature. The MONPs were collected by centrifugation and washed twice with chloroform/DMSO 1:1 mixture. Afterwards, the product was blown dried with N₂ and redispersed in DMSO. The MONPs in DMSO were drop-wise added into HSA solution (50 mg HSA dissolved in borate buffer, 50 mM, pH 8.5) with the aid of sonication. The nanoparticles were centrifuged at 30,000 g for 20 min (3×) to remove free HSA, and the precipitate was re-dispersed in phosphate buffered saline (PBS) buffer (pH 7.4). A small portion of aggregate was removed by passing the particles through a syringe filter (0.22 µm). The 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-methoxy(polyethylene glycol)-2000 (Avanti Polar Lipids, Inc.) coated MnO (DSPE-MnO) nanoparticles were prepared following a previously reported protocols.

S.3 The labeling of HSA-MONPs
The HSA-MnO nanoparticle labeling was performed in a way similar to the labeling of HSA coated iron oxide nanoparticle.\textsuperscript{4} In brief, DOTA-NHS (Macrocyclics, Dallas, TX) in DMSO was added at 5:1 DOTA:HSA ratio to HSA-MONP solution in borate buffer (pH 8.5, 50 mM), and the reaction went on for 1 h. The solution was purified through a PD-10 column, and the buffer solution was exchanged to PBS buffer (pH 7.4). Details regarding the DOTA labeling have been reported elsewhere.\textsuperscript{5,6}

S.4 Phantom studies with MONPs

T1 relaxivities of HSA- and DSPE- MONPs were assessed on a 7.0 T small animal MRI scanner (GE Healthcare). The particles were dispersed in 1% agarose gel in 0.2 mL tubes with elevated Mn concentrations. The MR images were acquired with the following spin echo sequence: repetition time (TR) = 32, 62, 125, 250, 500, 1000, 2000 ms, echo time (TE) = 3.5 ms, matrix = 256 × 256, field of view (FOV) = 5.0 × 5.0 cm, slice thickness = 1.00 mm, number of excitation (NEX) = 1.

S.5 Animal model preparation

Human glioblastoma cancer cell line U87MG was purchased from the American Type Culture Collection (ATCC, Manassas, VA) and cultured in ATCC-formulated Eagle's Minimum Essential Medium (with 10% FBS) in a cell culture incubator. Athymic nude mice were purchased from Harlan Laboratories (Indianapolis, IN). Approximately $5 \times 10^6$ U87MG cells were inoculated subcutaneously onto the right front flank of each mouse, and the \textit{in vivo} imaging was carried out about 3 weeks later, when the tumor size reached a size of about 100 mm$^3$. All the animal work was conducted following a protocol approved by the Stanford University Administrative Panel on Laboratory Animal Care (APLAC).

S.6 Small-animal MR imaging

The HSA-MONPs were injected as a bolus through a tail vein line at a dose of 10 mg Mn (measured by ICP-AES) per kg of mouse body weight. The animals were anesthetized before imaging with 2% isoflurane and were then set into an MR-compatible cradle. To investigate the migration of the MnO nanoparticles, T1 weighted fast spin-echo images were acquired before, and 1, 4, 24 h after administration of the MnO nanoparticles in 3 animals,
with the following parameters: TE 12 ms, TR 400 ms, thickness 1 mm, FOV 5 × 5, NEX 6.0, Echo 1/1. The signal intensities in defined ROIs were analyzed by Image J (National Institutes of Health).

S.7 Small-animal PET imaging

The details of small-animal PET imaging and region-of-interest (ROI) analysis have been reported elsewhere.\(^5,6\) In brief, \(^{64}\)Cu-labeled HSA-MONPs were i.v. injected into U87MG tumor-bearing mice at a dosage of 10 mg Mn per kg of mouse body weight. Small-animal PET imaging was performed with a microPET R4 rodent scanner (Siemens Medical Solutions) at 1, 4 and 24 h post injection time points. For each small-animal PET scan, three dimensional ROIs were drawn over the tumor on decay-corrected whole-body coronal images. The average radioactivity was obtained from the mean pixel values within the ROI volume, and was converted to counts per milliliter per minute by the use of a predetermined conversion factor.\(^5,7\) Given a tissue density of 1 g/mL, the counts per milliliter per minute were then converted to counts per gram per minute, and the values were divided by the injected dose to obtain the imaging ROI derived percentage injected dose per gram (‰ID/g). At the end of the 24 h scanning, the mice were sacrificed. Tumors and the major organs were collected and subjected to \textit{ex vivo} PET imaging with the microPET R4 rodent scanner.

S.8 \textit{Ex vivo} Transmission Electron Microscopy (TEM) analysis on tissue samples

U87MG xenograft models were injected with the HSA-MONPs (10 mg/kg) and were sacrificed after the imaging at 4 h. Tumors and liver parenchyma were collected and frozen into blocks in OCT at -80 °C. Such tissue sections were then cut into 60 μm-thick slices, fixed, and stained under a standard protocol. Briefly, the tissue slices were fixed with glutaraldehyde and paraformaldehyde for 1 h; afterwards, the buffer solution was exchanged to OsO\(_4\) solution to render contrast against the background. After 1 h, the sections were washed with PBS and then dehydrated in an alcohol series, embedded in Epon, and sliced to a thickness of 50 nm. Images of the slices are taken with a JEOL 1230 transmission electron microscope.
References:


