Bifunctional gold nanorod-loaded polymeric microcapsules for both contrast-enhanced ultrasound imaging and photothermal therapy†

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Electronic Supplementary Information

Supplementary Figures:

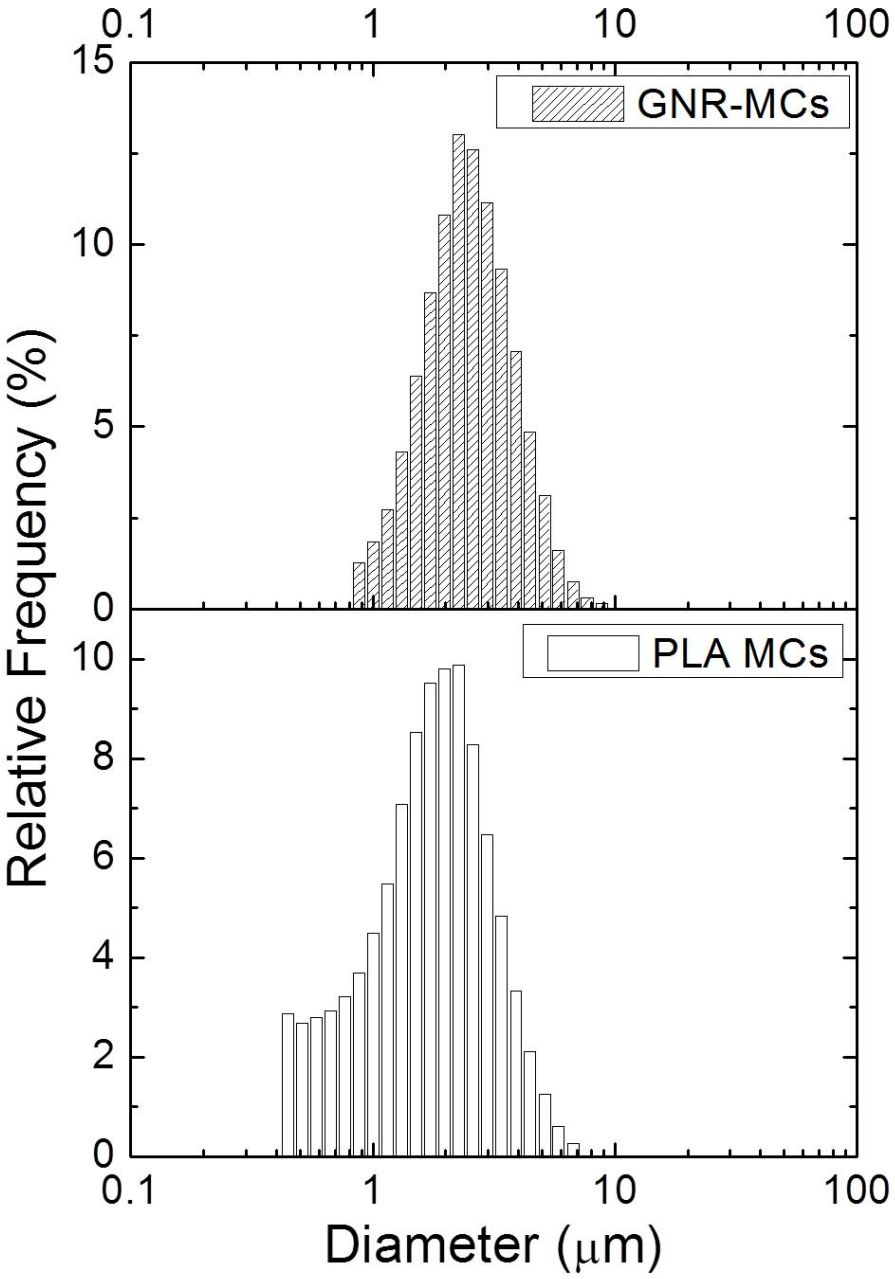

Figure S1. Size distribution of blank MCs and GNR-MCs by static light scattering

2. Experimental Section

2.1 Materials

Poly(lactic acid) (PLA, 80k MW, Shandong Medical Instrumental Institute, China), polyvinyl alcohol (PVA, 86-89% hydrolyzed, low molecular weight, Alfa Aesar) were used as received. Poly(allylamine hydrochloride) (PAH, 56k MW), poly(styrene sulfonate) (PSS, 70k MW) and camphor were purchased from Sigma-Aldrich, and hydrogen tetra-chloroaurate (III) hydrate ($HAuCl_4 \cdot 4H_2O$), cetyltrimethylammonium bromide (CATB), silver nitrate ($AgNO_3$), ascorbic acid and sodium borohydride ($NaBH_4$) were obtained from SinoReagent, China. De-ionized water (DI water, 18.2 MΩ·cm) from Milli-Q Gradient System was used in all the preparations.

2.2 Preparation of CTAB-Stabilized Gold nanorods (GNRs)

GNRs were prepared using a seedless growth method as previously reported [*J. Mater. Chem., 2010, 20, 3260-3264*]. Briefly, 120 mL $AgNO_3$ (0.1 M) was added to 100 mL aqueous solution containing 0.1 M CTAB and 0.5 mM HAuCl4. Gold reduction was achieved by adding 0.6 mL ascorbic acid solution (0.1 M) under vigorous stirring. Thirty seconds later, 25 mL $NaBH_4$ solution (2 mM) was quickly injected under vigorous stirring for another 10 s. The solution was then kept still for 40 min. The resulting nanorods were collected and washed by centrifugation (2-16PK, Sigma) at 12,000 rpm for 10 min.

2.3 Preparation of PSS-coated GNRs (PSS/GNR)

After centrifugation, a pellet of gold nanorods was form at the bottom of the tubes. 8

mL PSS (2 mg/mL in 0.5 M NaCl) was added in and mixed softly for electrostatic adsorption. Fifteen minutes later, the excess PSS were removed by centrifugation at 12,000 rpm for 10 min and the obtained PSS/GNR were washed by DI water.

2.4 Preparation of PLA microcapsules (MCs)

Polymeric microcapsules were prepared by an adapted W/O/W double emulsion solvent evaporation process as previously described [*Ref. 5-7 of main article*]. Briefly, PLA (500 mg) and camphor (50 mg) were dissolved in methylene chloride (20 mL). The first W/O emulsion was generated by adding DI water (1.0 mL) to the polymer solution followed by continuous probe sonication for 30 s with a 1.27 cm (1/2 inch) diameter titanium alloy horn (Sonicator 4000, Misonix) under 80% output amplitude setting [*Ultrasonics, 2006, 44, 360-367*]. Then, the first W/O emulsion was poured into a PVA solution (5% w/v) and homogenized (FJ-200, Jintan Jingbo Experimental Instrument, China) for 5 min at about 9500 rpm. Afterwards, the double W/O/W emulsion was poured into isopropanol (2% v/v in DI water) with magnetic stirring at room temperature for 1 h to make almost all the methylene chloride evaporate off and harden the capsules. Then, they were collected by centrifugation (5000 g at 15 °C for 5 min, Avanti J-25, Beckman Coulter), washed with DI water, centrifuged again and washed three times with hexane to further extract the methylene chloride from the polymer. After another washing step by DI water, some of the microcapsules were ready for the deposition of gold nanorod, and the other capsules were lyophilized (−54 °C, 36 h) by using a freeze dryer (TFD5505, Ilshin Lab, Korea) to obtain blank PLA microcapsules.

2.5 Preparation of gold nanorod-loaded microcapsules (GNR-MCs) using electrostactic layer-by-layer self-assembly technique

Gold nanorod-loaded microcapsules were fabricated by electrostatic deposition with the help of positively charged PAH as an interlayer due to negative charging of both microcapsules and PSS/GNR. The microcapsule suspension (100 μL) was added in PAH solution (20 mL, 2 mg/mL in 0.5 M NaCl aqueous solution) in a 50 mL vial, shaken and mixed for about 15 min, then centrifuged at 5000 g for 5 min. The supernatant was discarded and excessive non-adsorbed PAH molecules were washed by DI water for 3 times. Then, PSS/GNR solution was added in and the adsorption/centrifuge/wash step was repeated to obtain the GNR-MCs.

2.6 Characterization

The zeta potential of microcapsules during the alternate deposition of PAH and PSS/GNR was calculated from the electrophoretic mobility measured with a Brookhaven ZetaPALS instrument. The morphology of MCs and GNR-MCs was observed by FEI Quanta 200 scanning electron microscope. The corresponding energy dispersive X-ray spectroscopy of the samples (without gold sputtering process) was performed with a liquid nitrogen-cooled Si(Li) detector that was attached to SEM to verify the gold element on microcapsules. Transmission electron microscopic images of blank MCs and GNR-MCs were acquired by FEI Tecnai G2 Sphera Microscope with a CCD camera operated at 100 kV to confirm the deposition of gold nanorods on the microcapsules. The UV-visible extinction spectra of GNR, PSS/GNR, MCs and

GNR-MCs were analyzed by a UV- visible spectrophotometer (Varian Cary 4000) with a quartz cuvette of 1 cm optical path length to monitor the fabrication progress. Size distributions of MCs and GNR-MCs were analyzed through static light scattering using a particle size distribution analyzer (Horiba LA-920).

2.7 In vitro and in vivo Ultrasound contrast imaging

In vitro ultrasonography of GNR-MCs was carried out in the latex tube (with the inner diameter of ~5 mm) using a broadband linear array L9-3 transducer (9 to 3 MHz extended) of IU22 ultrasound system (Philips Medical Systems). The same self-made setup was used for *in vitro* study as described in our previous work [*Ref. 15 of main article*]. The GNR-MCs were dispersed in 0.9% saline at the concentration of 40 mg/mL and injected to the latex tube stimulating the blood vessel and circulated in the tube by a constant flow pump in a permanent flow rate. Ultrasonography was performed using the L9-3 transducer in both pulse inverted harmonic imaging (PIHI) mode (MI=0.42) and conventional B-mode at the same time from the longitudinal cross section of the tube.

For *in vivo* study, three rabbits (average weight of 2.4 kg) were anesthetized with pentobarbital sodium (2.0 mL per kg weight, 2% w/v in 0.9% saline) administration through ear vein, and subsequently, heparin sodium (4.0 mL, 0.2% w/v in 0.9% saline) was injected to avoid coagulation. The animals were placed on a warm blanket to keep body temperature within normal range during the experiment. The GNR-MC suspension (40 mg/mL in 0.9% saline) was intravenously injected at a concentration of 0.1 mL per kg weight through a catheter, flushed with saline (1.0 mL) thereafter. The kidney was

imaged transabdominally using a broadband L9-3 transducer in PIHI mode with MI of 0.42. All the digital clips and images were stored for off-line review. All the animal experiments were approved by institutional animal use committee and carried out ethically and humanely.

2.8 Photothermal effect of GNR-MCs

DI water, blank MCs (1 mg/mL in water), GNR-MC agents (1 mg/mL in water) were suspended in quartz cuvettes (total volume of 4.0 mL), irradiated by continuous-wave diode NIR laser (T808D2W, Xi'an Minghui Optoelectronic Technology, China) with a center wavelength of 808±10 nm and output power of 2 W for 10 min. The temperature of the solutions was measured by a digital thermometer with a thermocouple probe every 10 s.

Photothermal cell toxicity of GNR-MCs was evaluated on HeLa cells (human cervical carcinoma cell line). For qualitative analysis, HeLa cells (5×10^5 cells per well) were incubated in 6-well plates at 37°C in a humidified atmosphere containing 5% CO_2 for 24 h. After incubated with GNR-MC suspensions (2.0 mL per well, 1 mg/mL) for another 1 h, cells were then exposed to NIR laser (10 W/cm^2) for 10 min and stained with calcein AM (calcein acetoxymethyl ester) to verify the photohyperthermic effect on cancer cells.