Surface chemistry-mediated penetration and gold nanorod thermotherapy in multicellular tumor spheroids

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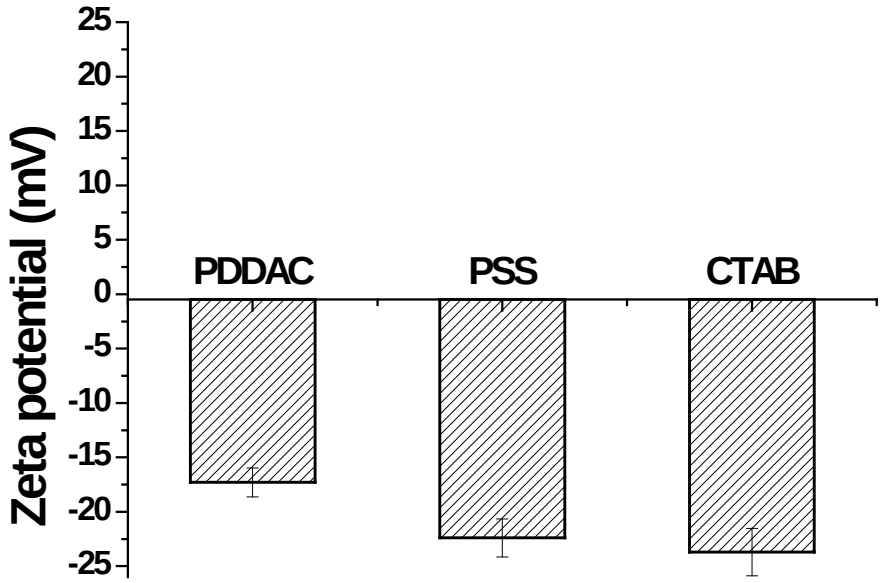

Fig. S1 Zeta potentials of Au NRs after incubation with serum containing medium for 24 hours.

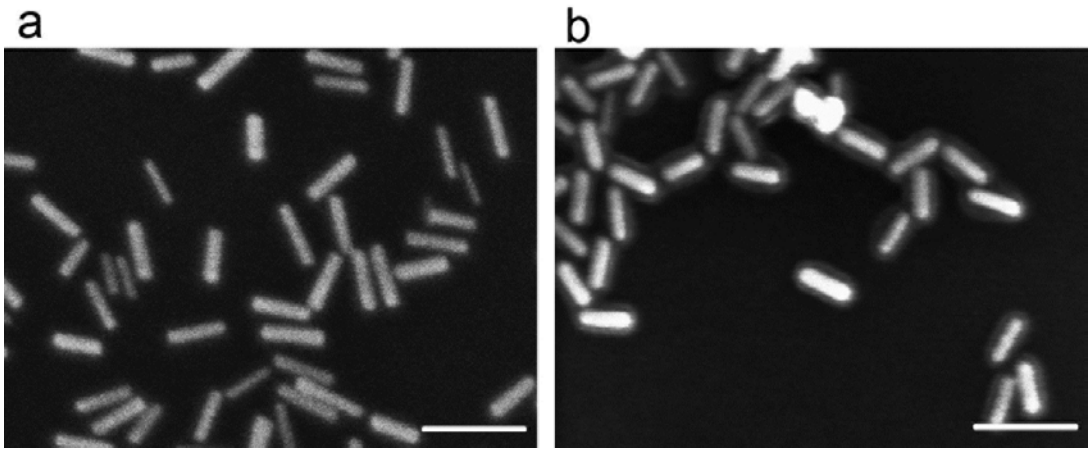

Fig. S2 Scanning electron microscopy (SEM) pictures of Au NRs after incubation with serum-containing medium for 24 hours. (a) Negatively charged Au NRs; (b) Positively charged Au NRs. The white coatings around Au NRs in Fig. S2b are protein coronas (Scale bar, 100 nm).

MATERIALS AND METHODS

MCF-7 MULTICELLULAR SPHEROID CULTURE

MCF-7 spheroids were cultured using a liquid overlay method. Briefly cells were detached from the monolayers, and single cell suspension (200 µl per well containing 600 cells quantitated with an Attune® acoustic focusing cytometer, Life Technology, Germantown, MD) was transferred into flat-bottomed 96-well plates pre-coated with 1 % agarose. The cells were incubated for approximately 7 days in DMEM (Dulbecco's Modified Eagle Medium) at 37 ºC with 5 % CO_2. The culture medium was partially (100 µl) replaced by fresh medium every other day.

ESEM OBSERVATION OF MCF-7 TUMOR SPHEROIDS

An environmental scanning electron microscopy (ESEM) was used to examine the outer morphology of the spheroid. After incubation for a week, the spheroids were removed from the wells, washed with PBS and fixed overnight in 5 % glutaraldehyde solution at room temperature. The spheroids were then progressively dehydrated in an ethanol series from 70 % to 100 %, followed by critical-point drying with isoamyl acetate. Finally, the spheroids (without gold coating) were directly observed using the ESEM (FEI Quanta 200).

TEM OBSERVATION OF SPHEROIDS

Spheroids cultured for 7 days were removed, washed with PBS and fixed overnight using 3 % glutaraldehyde solution at room temperature. This was followed by a

secondary fixation with 1 % osmium tetroxide; subsequently, a serial dehydration in graded ethanol series. Each spheroid was embedded in Epon resin and polymerized for 3 days at 60ºC. The embedded samples were sectioned, stained with uranyl acetate and examined under electron microscopy (JEM-1400, JEOL).

ACID PHOSPHATASE ASSAY

An acid phosphatase (APH) assay was employed to evaluate the viability of the spheroids. Ten spheroids were collected and washed with PBS until the solution was totally transparent. Then, 300 µl APH buffer (0.1 M sodium acetate and 0.1 % (v: v) triton-X-100) and 250 µl PBS were mixed to incubate the spheroids. Later, 4-nitrophenyl phosphate disodium salt hexahydrate was added to a final concentration of 2 mg/ml. After further incubation at 37 ºC for 3 h, 200 µl supernatant was pipetted and placed in the 96-well plate with 10 µl 1N NaOH. Immediately, the color became yellow. The absorbance at 405 nm was measured by a plate reader (Infinite M200, Tecan Group Ltd, Switzerland), and the value was positively correlated with the viability of the spheroids.

PREPARATION AND CHARACTERIZATION OF Au NRs

The cetyltrimethylammonium bromide (CTAB), hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), silver nitrate ($AgNO_3$), L-ascorbic acid, glutaraldehyde (50 % aqueous solution), and sodium borohydride ($NaBH_4$) solutions were purchased from Alfa Aesar (Ward Hill, MA, USA). The poly(sodium-p-styrenesulfate) (PSS,

molecular weight: 70,000) and the poly (diallyldimethylammonium chloride) (PDDAC, 20 %) were obtained from Aldrich. All chemicals were used as received. In all the experiments, the deionized water (18.2 $_{M}\Omega$ cm^{-1}) was produced by Milli-Q Synthesis (Millipore Co., USA). A stock solution of sodium borohydride was freshly prepared for each experiment.

CTAB-COATED Au NRs

The CTAB-coated Au NRs were synthesized by seed-mediated growth. First, the CTAB-capped Au seeds were obtained by chemical reduction of $HAuCl_4$ with $NaBH_4$: 7.5 ml 0.1 M CTAB was mixed with 100 μl 24 mM $HAuCl_4$ and diluted with water to 9.4 ml. Then, 0.6 ml ice-cold $NaBH_4$ (0.01 M) was added while magnetically stirring. After 2 min of vigorous stirring, the seed solution was kept at room temperature (25 ℃) and used within 2-5 h. Second, the growth solution of the Au NRs, consisting of 100 ml 0.1 M CTAB, 2 ml 0.024 M $HAuCl_4$, 2 ml 0.5 M H_2SO_4, $AgNO_3$ (10 mM) and 800 μl 0.1 M ascorbic acid, was prepared. The amount of Ag ions added was used to control the aspect ratio of the Au NRs. Afterwards, 240 μl seed solution was added to the above mentioned growth solution to initiate the growth of the Au NRs. After 12 h, the reaction was stopped using centrifugation (8000 rpm for 10 min). The precipitates were collected and re-suspended in deionized water. A suspension of Au NRs to aspect ratio of 4 was obtained.

POLYELECTROLYTE-COATED Au NRs

The preparation of multilayer polyelectrolyte-coated Au NRs was obtained via a layer-by-layer approach[1, 2]. The multilayer polyelectrolyte-coated Au NRs were synthesized by sequentially coating negatively charged PSS and positively charged PDDAC onto the as-synthesized CTAB-coated Au NRs. For PSS coating, 12 ml Au NRs were centrifuged at 12000 rpm for 10 min, and the precipitate was dispersed in 12 ml of 2 mg/ml PSS aqueous solution (containing 6 mM NaCl). The solution was stirred magnetically for 3 h. Finally, the solution was centrifuged at 12,000 rpm for 10 min, and the precipitate was redispersed in water. For further coating with PDDAC, a similar procedure was applied to the PSS-coated Au NRs.

ICP-MS

For the quantitative determination of Au content, inductively coupled plasma mass spectrometry (ICP-MS, Thermo Elemental X7, Thermo Fisher Scientific Inc., USA) was used. After incubation in medium containing 150 pM Au NRs for 24 h, MCTSs were trypsinized and then soaked in aqua regia overnight. This solution was heated to approximately 140 ℃ to eliminate hydrogen chloride and nitrogen oxide vapors until the solution was colorless and clear. After making a 3-ml solution using water with 2 % nitric acid and 1% hydrogen chloride, the gold content was analyzed using ICP-MS. A bismuth solution at 10 ng/ml was used as internal standard. The concentration of the elemental gold in solution was then obtained. The concentration of gold atoms was converted to the concentration of particles using the calculated number of atoms in each nanoparticle. This was estimated from the nanoparticle

volume calculated from the TEM images using the assumption that the gold unit cell edge was 4.0786 Å. The measurement for each treatment was repeated in triplicate.

THERMAL THERAPY

After treatment with Au NRs for 24 h, the MCTSs were collected and washed with the PBS to clear the Au NRs in the culture medium. Ten MCTSs samples were placed in a 2 ml tube, and they received continuous laser radiation. Each tube contained 250 μl of medium. The MCTSs without Au NRs treatment were used as the control group. The thermal therapy was conducted with an 808 nm stabilized near-infrared diode fiber laser system purchased from China Daheng Group, Inc. The output power was set at 3140 mW to achieve a radiation intensity of 100 W/cm^2. All the samples received radiation for 4 min and were cultured in the incubator for another 48 h before the APH analysis and the hematoxylin-eosin staining (HE staining).

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