Matrix Metalloproteinase Sensitive Gold Nanorod for Simultaneous Bioimaging and Photothermal Therapy of Cancer

Dong Kee Yi,[†,⊥] *In-Cheol Sun,*[‡,⊥] *Ju Hee Ryu,*[‡] *Heebeom Koo,*[‡] *Chul Wan Park,*[§] *In-Chan Youn,*[‡] *Kuiwon Choi,*[‡] *Ick Chan Kwon*[‡], *Kwangmeyung Kim,*[*,‡] *and Cheol-Hee Ahn* [*,∥]

Supporting informations

Preparation of Cy5.5-MMP substrate conjugated gold nanorod (MMP-AuNR): Cy5.5 was conjugated to MMP substrate by same method in previous report.[18] AuNR was functionalized with Cy5.5-MMP by simple mixing method. First, cetyltrimethylammonium bromide (CTAB) stabilized AuNR was centrifuged (9000 rpm, 12 min) and washed twice to remove with TCNB buffer containing 100 mM of Tris, 5 mM of calcium chloride, 200 mM of NaCl, and 0.1% of Brij. Cy5.5-MMP (0.1 ml, 100 μg/ml) solution was added in AuNR colloid and it was kept in the absence of light at 25°C under stirring for 24 h. The solution was purified by repeated centrifugation (9000 rpm, 12 min) and washing 2 times in the presence of 20 % (v/v) of DMSO.

In vitro fluorescence recovery test: UV absorbance titration of Cy5.5 (675 nm) was performed to determine the amount of Cy5.5-MMP bound to the AuNR surface. The Cy5.5-MMP bound to AuNR was detached by addition of dithiothreitol (DTT, 10 mM) and its UV-

vis absorption was detected with UV-vis spectroscopy. The concentration of free Cy5.5-MMP was calibrated with a standard UV absorbance curve of Cy5.5 in aqueous solution and the amount of Cy5.5-MMP was calculated from known concentrations of DTT-treated AuNR. The NIRF recovery of MMP-AuNR was examined in terms of MMP enzyme specificity. MMP enzymes (MMP-2, 3, 7, 9 or 13) were activated by incubation at 37 ^{o}C for 1 h with 2.5 nM of 4-Aminophenylmercuric Acetate (APMA) in the TCNB buffer. Inhibitor of MMP-2 was co-incubated with MMP-2 enzyme before reaction with MMP-AuNR. The same amount of MMP-AuNR colloid was added to MMP enzyme solution and incubated additionally at 37 ^{o}C for 1 h. After incubation, samples were centrifuged and the fluorescence intensity of supernatant was monitored with F-7000 fluorescence spectrometer (Hitachi, Tokyo, Japan). The excitation wavelength was fixed at 675 nm and emission spectra were recorded from 680 to 800 nm using a 1 ml cuvette. In addition, NIRF images of MMP-AuNR was obtained from a 12-bit CCD (charge coupled device) camera (Kodak Image Station 4000MM, New Haven, CT, USA) equipped with the special C-mount lens and the Cy5.5 bandpass emission filter (680 nm to 720 nm; Omega Optical Inc., Brattlebore, VT, USA).

In vitro imaging and photothermal therapy: HeLa cells were cultured in DMEM medium at 37 °C in a humidified atmosphere of 5 % CO_2. Cells were seeded in 96-well flatbottomed plates at a density of 0, 10^2, 10^3, 10^4 and 10^5 cells/well respectively and allowed to adhere for 24 h. The cell media was extracted from 96-well plate and mixed with MMP-AuNR colloid (final concentration : 2 mg/ml) in Eppendorf tubes at 37 °C for 1 h. The mixture was moved to a 96-well plate and visualized with a Kodak image station 4000MM. For NIR photothermal therapy, NIR Diode Pumped Solid State (DPSS) laser (671 nm, 200 mW) was used. HeLa cells in 12-well plate (10 x 10^4 cell/ml) was exposed to 0.8 mg/ml of MMP-AuNR for 1 h and then washed with DPBS buffer and then exposed to the red laser light for

10 min. After treatment, cells were stained with 0.4% trypan blue (Gibco Invitrogen, Paisley, UK) for 10 min and observed through microscope and imaged under X10 in bright field.

Annexin V assay : annexin V-FITC fluorescence microscopy kit (BD Bioscience, San Jose, CA, USA) was used according to the protocol provided by manufacturer. Briefly, after the treatment of HeLa cells with laser, they were washed once with Annexin V Binding Buffer after fixation. Then, the cells were stained with annexin V-FITC solution for 10 min. The annexin V-FITC solution was removed and washed with Binding Buffer once for the observation under the microscopy. The cells were visualized using IX81 fluorescence microscope (Olympus, Tokyo, Japan) with excitation at 488 nm and emission at 530 nm.

In vivo imaging and photothermal therapy: Squamous cell carcinoma (SCC) - 7 cells cell line (1 X 10^6 cells/mouse) was cultured in RPMI 1640 medium containing 10% fetal bovine serum and xenografted subcutaneously into the backs of athymic nude mice (Crlj-nu, 5-6 weeks, male). When the size of tumors became 5 mm in diameter, 50 μl of MMP-AuNR (2 mg/ml) probe was injected intratumorally. For MMP inhibition experiment, MMP inhibitor was intratumorally administered into the other SCC-7 tumor, 30 min prior to injection of the MMP-AuNR. After injection of MMP-AuNR, the fluorescence recovery profiles of SCC-7 tumor-bearing mice were imaged using eXplore Optix system (ART advanced Research Technologies Inc., Montreal, Canada). A 670 nm pulsed laser diode was used and the laser power and count time settings were optimized to 15 μW and 0.3 per point, respectively. NIRF tomographic images were obtained by collecting NIR fluorescence emission at 700 nm with a fast photomultiplier tube (Hamamatsu, Japan) and time-correlated single photon counting system (Becker and Hickl GmbH, Berlin, Germany). After 4 hours of post-injection, tumors were dissected and ex vivo NIR fluorescence images were taken using Kodak Image Station 4000MM. For therapy, tumor-bearing mice were exposed to NIR laser after injection of MMP-AuNR under same condition and their infrared thermal images were obtained

according to irradiation time (1, 2, 4, 8 min, respectively). The exact temperature was measured with a 33 gauge T-type hypodermic thermocouple (Omega Engineering, Inc., Stamford, CT, USA). After irradiation, tumors were dissected, fixed and stained with hematoxylin-eosin for microscopic observation.