DNA Hydrogel Delivery Vehicle for Light-triggered and Synergistic Cancer Therapy

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Supplementary Information

CONTENTS

S1. Materials and General Methods

Hexadecyltrimethylammonium bromide (CTAB) was purchased from Acros organics. Minimum essential medium with Earle's balanced salts (MEM/EBSS) for cell growth was purchased from HyClone. Fetal bovine serum (FBS) and penicillin–streptomycin (PS) were obtained from GIBCO. Other reagents were obtained from Aldrich, and all chemicals were used as received without further purification. Water was triply distilled using a Millipore filtration system. Solutions of CTAB, $HAuCl_4$, $AgNO_3$, ascorbic acid, and NaBH4 were freshly prepared in distilled water, and all the reactions were carried out at room temperature. UV/Vis absorption spectra were obtained using an Agilent 8453. Hydrodynamic size and zeta potential were measured using Malvern Zetasizer Nano S and Zetasizer Nano Z, respectively. TEM images were recorded using a JEOL JEM-2100.

S2. Synthetic Procedures

Synthesis of gold nanorod (AuNR). AuNRs were prepared by following a seedless growth method described in the literature with slight modifications.[1] A 20 mM aqueous solution of $HAuCl_4$ (125 μL, 2.5 μmol) was mixed with 5 mL aqueous solution of CTAB (0.162 g, 0.44 mmol) and stirred for 10 min. Then, a 10 mM aqueous solution of $AgNO_3$ (20 μL, 0.2 μmol) was added to the mixture and stirred for 5 min, followed by addition of 100 mM ascorbic acid (30 μL, 3 μmol). Finally, a 1.6 mM aqueous solution of $NaBH_4$ (2 μL, 3.2 nmol) was added and vigorously stirred overnight. The resultant AuNR solution was centrifuged at 18,000 g to get rid of excessive free CTAB molecules. AuNR surface was dispersed in aqueous solution that contains lipoic acid derivative molecules with quaternary ammonium (LA-QA), which was reported previously, to enhance the colloidal stability.[2] AuNR solution was mixed with

excessive LA-QA under continuous sonication for 1 h, and then centrifuged at 18,000 g to remove free surface molecules followed by redispersion in distil led water.

Synthesis of Dgel. The X-shaped DNA monomers were constructed by annealing four single-stranded oligonucleotides that are partially complementary to each other. The Dgel was fabricated by ligating 10 nmol of the X-DNA monomers using 6 units of T4 DNA ligase (Promega) at 16°C for 12 hr. Dgel has been made by enzymatic ligating of X-shaped DNA building blocks composed of three sticky arms and the other arm as shown in Fig. S1. The other arm has no phosphate group at 5' end so ligation can't be performed at the end (Table S1). The Dgel made from the disabled X-DNA at our experimental condition showed nanoscale size distribution (~87 nm) while Dgel made from perfect X-DNA with four crosslinkerble arms results in one piece of bulky hydrogel.[3]

Synthesis of AuNR-Dgel and Dox-AuNR-Dgel. Freezing dried Dgel (before dry volume 50 μL) was swelled under AuNR aqueous solution (100 μL, 2.5 pmol) by overnight mixing without agitations. Then, the mixture was centrifuged at 5,000 g followed by the addition of 400 μL of distilled water to remove unassembled free Dgels and AuNRs; this process was repeated three times. For further Dox incorporation, 100 μM aqueous solution of Dox was added to AuNR-Dgel solution and reacted for 1 h. They are centrifuged at 5,000 g to wash unbound Dox and recover Dox-AuNR-Dgel in the precipitate.

S3. Experiment Procedures to Investigate Combination Effect

Fluorescence and dark-field microscopy. B16 F10 mouse melanoma cells were purchased from the Korean Cell Line Bank. B16 F10 cells were maintained in MEM/EBSS which was supplemented with 10% FBS and 1% PS. B16 F10 cells were grown onto 12 mm glass coverslips in 24-well plates at a density of 1×10^5 cells/well at 37 °C under 5% CO_2. After 1 day, the cells were treated with Dox-AuNR-Dgel. As controls, AuNR-Dgel, or Dox were used. Dox-AuNR-Dgel had been prepared by the ligation of 0.24 nmol X-DNA and subsequent co-loading of 0.06 pmol AuNR and 0.03 nmol Dox. These samples were incubated for 1, 6, 12, 24 h at 37 °C under 5% CO_2. The cells were rinsed with PBS three times and fixed with 4% formaldehyde at room temperature for 20 min. The cells were further washed with PBS three times. They were then mounted onto slide glasses using an aqueous mounting medium with an anti-fading agent (Biomeda). The fluorescence and dark-field images were recorded using a Zeiss Axioplan 2 microscope. A high numerical dark-field condenser (0.75-1.0) and a 100× / 1.3 oil iris objective were used for dark-field images. The pictures were taken using a Zeiss Axiocam HR camera.

Relative viability levels in dark condition. A B16 F10 cell suspension (5000 cells/well) was dispensed in a 96-well plate and incubated for 1 day at 37 °C under 5% CO_2. Cells were co-incubated in dark condition with AuNR-Dgel, Dox-AuNR-Dgel, or Dox. Dox-AuNR-Dgel had been prepared by the ligation of 0.24 nmol X-DNA and subsequent co-loading of 0.06 pmol AuNR and 0.03 nmol Dox. These samples were incubated for 1, 6, 12, 24 h at 37 °C under 5% CO_2. At the end of each incubation time, Cell Counting Kit-8 solution (CCK-8, Dojindo Laboratories) was added to the samples according to the manufacturer's instructions. After further incubation for 2 h, the absorbance at 450 nm was measured using a microplate

reader. The results of this measurement are expressed as the ratio between the absorbance of the sample and that of the negative control cells without sample incubations.

Cancer therapy at the cellular level. B16 F10 cell suspension (5000 cells/well) was dispensed into a 384-well plate and incubated at 37 °C under 5% CO_2 for 1 day. To demonstrate the synergistic effect, AuNR-Dgel, Dox-AuNR-Dgel, or Dox were co-incubated with cells for 12 h. Dox-AuNR-Dgel had been prepared by the ligation of 0.24 nmol X-DNA and subsequent co-loading of 0.06 pmol AuNR and 0.03 nmol Dox. The cells were then irradiated with a 660-nm cw laser for 0, 1, 3, 5, 10 min at a power density of 16 W/cm^2. Viability was evaluated by adding CCK-8 to each cell solution according to the manufacturer's instructions. After 2 h incubation, the absorbance at 450 nm was measured using a microplate reader. These results are expressed as the ratio of the absorbance of the sample to that of the negative control, cells exposed to no laser irradiation without sample co-incubation. To determine the threshold power densities, cells (3×10^5 cells/well) were grown onto 12-well plates. After 1 day, the cells were co-incubated with AuNR-Dgel, Dox-AuNR-Dgel, or Dox for 12 h. As a control, cells were incubated without any samples. The cells were rinsed with culture medium three times and exposed to laser irradiation for 5 min at different power densities. Trypan blue was applied to the samples at room temperature for 5 min to reveal cell mortality as blue staining.

Cancer therapy at the whole-body level. Five-week-old female C57BL/6 mice (20-25 g body weight) were obtained from Orient Co. Ltd (Korea). Mice were anesthetized by intraperitoneal injection of Avertin, and then B16 F10 cells (1×10^6) were injected

subcutaneously into the shaved area of the both flanks. The mice were randomly sorted for treatments when the tumors reached approximately 10 mm in diameter as measured with a digital caliper (within 2 weeks of subcutaneous injection of B16 F10 cells). The mice were anesthetized, and AuNR-Dgel, Dox-AuNR-Dgel, or Dox was intratumorally injected. Dox-AuNR-Dgel had been prepared by the ligation of 6 nmol X-DNA and subsequent co-loading of 1.5 pmol AuNR and 15 nmol Dox. After 10 min post-injection, the tumors were either exposed to laser light (660-nm cw diode laser, ca. 1 cm diameter, 1 W/cm^2) for 5 min under anesthesia or to anesthesia without irradiation. The sizes of the tumors were measured at regular intervals using a digital caliper, and the tumor volume was estimated by ellipsoidal calculation as $V = (\text{width})^2 \times \text{length} \times \pi/6$. Errors are reported as the standard deviation of the mean, and the significance was determined using three different mice for each sample. All experimental animal procedures were approved by the institutional animal care and use committee of the Pohang University of Science and Technology.

S4. Supplementary Figures

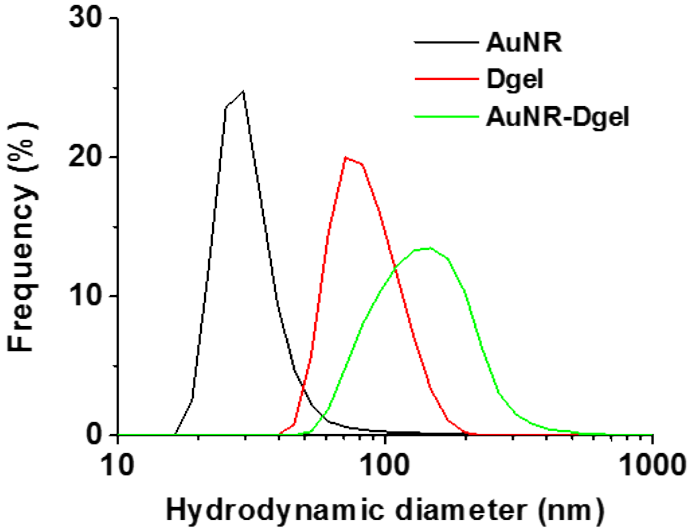

Figure S1. Hydrodynamic diameter distributions of AuNR, Dgel, and AuNR-Dgel determined by dynamic light scattering.

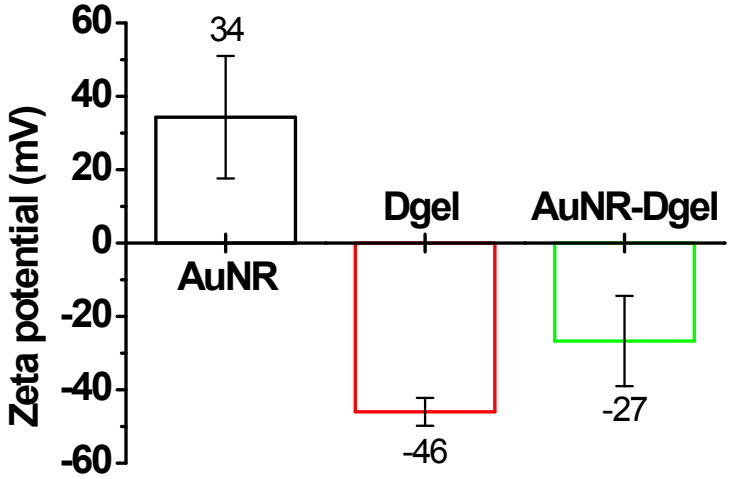

Figure S2. Zeta potential of AuNR, Dgel, and AuNR-Dgel.

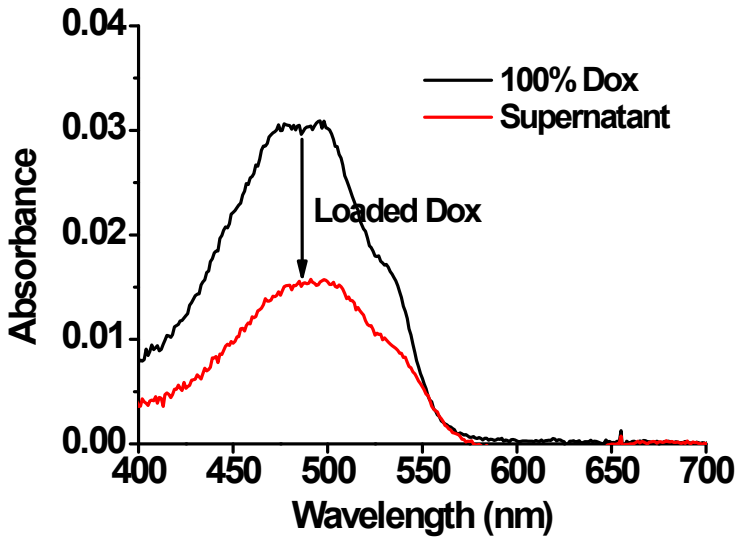

Figure S3. Absorption spectra of Dox before loading to the AuNR-Dgel (100% Dox) and the remained Dox supernatant measured after centrifugation of Dox-AuNR-Dgel at 5,000 g. The amount of loaded Dox was calculated using the absorbance decrease at 480 nm.

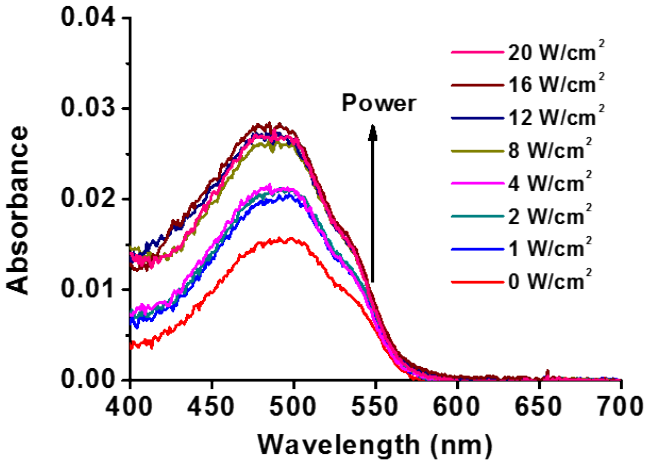

Figure S4. Absorption spectra of the Dox-AuNR-Dgel supernatant measured after laser irradiations at different power densities followed by centrifugation at 5,000 g. The percentage of released Dox was calculated using the absorbance increase at 480 nm.

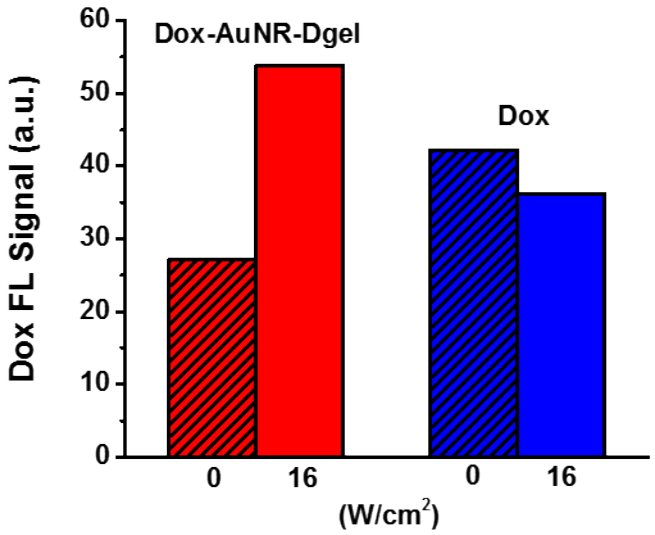

Figure S5. Dox fluorescence intensity measurements of the cells co-incubated with Dox-AuNR-Dgel (red) or with Dox only (blue). Fluorescence of the two samples were measured before the laser irradiation (bars with slanted lines) and after irradiation with 660 nm laser at 16 W/cm^2 (solid bars).

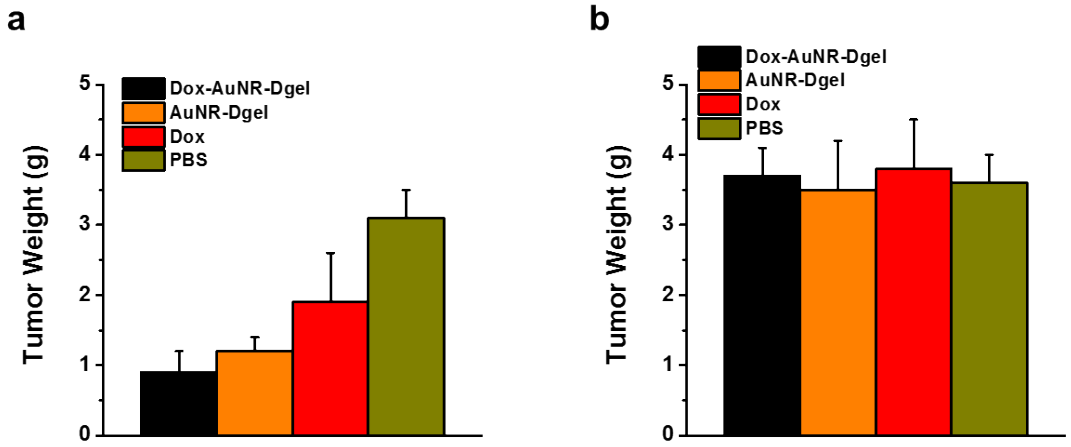

Figure. S6. Dissected tumor weights after the laser irradiation (a) or without laser irradiation (b).

Reference

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