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Controlled-release system of single-stranded DNA triggered by the photothermal effect of gold nanorods and its in vivo application

Shuji Yamashita [a], Hiromitsu Fukushima [a], Yasuyuki Akiyama [a], Yasuro Niidome [a], Takeshi Mori [a], Yoshiki Katayama [a,b,c], Takuro Niidome [a,b,c,d,*]

[a] Department of Applied Chemistry, Faculty of Engineering, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan
[b] Center for Future Chemistry, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan
[c] International Research Center for Molecular Systems, Kyushu University, 744 Motooka, Nishi-ku, Fukuoka 819-0395, Japan
[d] PRESTO, Japan Science and Technology Corporation, Kawaguchi 332-0012, Japan

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ABSTRACT

Gold nanorods have strong absorption bands in the near-infrared region, in which light penetrates deeply into tissues. The absorbed light energy is converted into heat by gold nanorods, the so-called 'photothermal effect'. Hence, gold nanorods are expected to act not only as on-demand thermal converters for photothermal therapy but also as controllers of a drug-release system responding to irradiation by near-infrared light. To achieve a controlled-release system that can be triggered by light irradiation, double-stranded DNA (dsDNA) was modified on gold nanorods. When the dsDNA-modified gold nanorods were irradiated by near-infrared light, the single-stranded DNA (ssDNA) was released from gold nanorods due to the photothermal effect. The amount of released ssDNA was dependent upon the power and exposure time of light irradiation. Release of ssDNA was also observed in tumors grown on mice after light irradiation. Such a controlled-release system of oligonucleotide triggered by the photothermal effect could expand the applications of gold nanorods that have unique optical characteristics in medicinal fields.

1. Introduction

Various types of nanoparticles (e.g., liposomes, polymer micelles, inorganic nanoparticles) have attracted attention as drug carriers for the delivery of drugs into tumors.[1,2] Traditional tumor-targeting strategies are classified into 'passive targeting' and 'active targeting'. The former relies on the 'enhanced permeability and retention' (EPR) effect. Tumors contain defective and permeable blood vessels that allow particles to accumulate within them.[3,4] Active targeting involves conjugating molecules that have affinity for the cell surface. Antibodies, folate, arginine–glycine–aspartic acid (RGD) peptide, transferrin and aptamers as active targeting molecules have been studied.[5–13]

Recently, as other strategies to deliver nucleic acids and drugs to tumors, a controlled-release system responding to the unique environments of tissues and external stimuli has been investigated. The extracellular pH of tumors is slightly acidic (pH 6.5–7.2),[14,15] so a pH-response reaction or polymers have been used for the controlled-release system.[16–22] The protease activity is also considered to be a unique environment of tumors.[23–30] As the external stimulations, radiofrequency,[31,32] ultrasound,[33,34] light,[22,35] heat[22,36] have been used to control the drug-release system. For example, magnetic nanoparticles have been used as a heating nanodevice responding to irradiation of the radiofrequency. The produced heat not only triggers thermal damage of the irradiated tissue but also releases drugs from the nanoparticles.[31,32] Liposomes, which release encapsulated contents in response to ultrasound, have also been developed.[33,34]

Gold nanoparticles are promising agents for drug delivery because they can be readily prepared by reduction of the Au ion in appropriate conditions and do not show significant toxicity in in vitro and in vivo systems.[35,37] Among them, gold nanorods (which are rod-shaped gold nanoparticles) have been expected to be functional nanodevices. Gold nanorods have two distinctive adsorption bands corresponding to the transverse and longitudinal surface plasmon oscillations of free electrons in the visible (~520 nm) and near-infrared (~900 nm) regions, respectively.[38,39] The near-infrared region locates in the minimum light absorption band of tissue between the absorptions of intrinsic chromophores, hemoglobin (<650 nm), and water (>900 nm), resulting in maximal

Abbreviations: CTAB, hexadecyltrimethylammonium bromide; CW, continuous wave; PEG, polyethylene glycol; ssDNA, single-stranded deoxyribonucleic acid; dsDNA, double-stranded deoxyribonucleic acid; TEM, transmission electron microscopy.

* Corresponding author. Tel./fax: +81 92 802 2851.
E-mail address: niidome.takuro.655@m.kyushu-u.ac.jp (T. Niidome).

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penetration of light into tissues.[40] Gold nanorods also possess photothermal effects that can efficiently convert the absorbed light into heat.[41] Therefore, gold nanorods are expected to act as contrast agents for in vivo bioimaging and a thermal converter for thermal therapy.[42–44]

Nucleic acids are expected to behave as drugs. Plasmid DNA gene is used for gene therapy, and various types of oligonucleotides are also used as antisense, decoy, small interfering RNA (siRNA), ribozymes, and aptamers, which control cellular function by regulating gene expression in cells.[45–49] Chen et al. reported that plasmid DNA encoding the enhanced green fluorescent protein (EGFP) gene was released from gold nanorods responding to near-infrared laser irradiation, which induced reduction of the surface area of the particles by changing the rod shape to a sphere. The gene expression of EGFP from the plasmid DNA was then observed in an in vitro system.[50] Wijaya et al. reported that two different oligonucleotides were modified with two types of gold nanorods with different sizes. They succeeded in selective release of the oligonucleotide from each gold nanorod by irradiating laser light corresponding to the absorption band of the gold nanorod.[51] We also previously reported release of plasmid DNA and polyethylene glycol (PEG) chains from gold nanorods responding to pulsed near-infrared light irradiation that induced a change in the shape of the gold nanorods.[52–54]

DNA can be used as a heat-labile linker. That is, double-stranded DNA (dsDNA) dissociates to single-stranded DNA (ssDNA) by increasing the temperature. Derfus et al. modified magnetic nanoparticles with dsDNA. They then succeeded in the controlled-release of ssDNA from the dsDNA by irradiating the nanoparticles using a radiofrequency that could heat the nanoparticles.[55] Gold nanoshells, which can be heated by near-infrared light irradiation, were also modified with dsDNA, and ssDNA was released by the light irradiation.[56] Lee et al. succeeded in the release of ssDNA from dsDNA modified on gold nanorods controlled by near-infrared light irradiation in an in vitro experimental system.[57]

In the present study, to examine if a controlled-release system responding to near-infrared light irradiation works in vivo, we constructed dsDNA-modified gold nanorods. The controlled-release of ssDNA induced by the photothermal effect of the gold nanorods was then examined in tumors inoculated in mice.

2. Results and discussion

2.1. Preparation of DNA-modified gold nanorods

dsDNA was modified on the gold surface through several steps (Fig. 1). Briefly, the PEG chain was modified with the surface of the gold nanorods via the Au–S bond.[58] PEG-modified gold nanorods were coated with a silica layer using the Stöber method.[59–62] After coating the silica layer around the gold nanorods, branched-polyethyleneimine (PEI) was coated onto the silica layer on gold nanorods to arrange amino groups on their surface.[63–66] The coating with the silica layer on the gold surface produced a red shift of the absorption of the gold nanorods that corresponded to the longitudinal oscillation mode because of a changing local refractive index around the surface of gold by the coated silica layer (see Supplementary data Fig. S1).[42,61,62,67,68] dsDNA, which has a thiol group and a fluorescent group (Cy3) at the 5′-end of each chain (see Section 4.1), was modified with the PEI-coated gold nanorods through a crosslinker (Mal–PEO_{12}–NHS). To confirm surface modification in each step, the ζ-potentials of the gold nanorods were measured. The values of ζ-potentials of each step of the gold nanorods corresponded to the surfaces in each modification step (see Supplementary data Table S1).

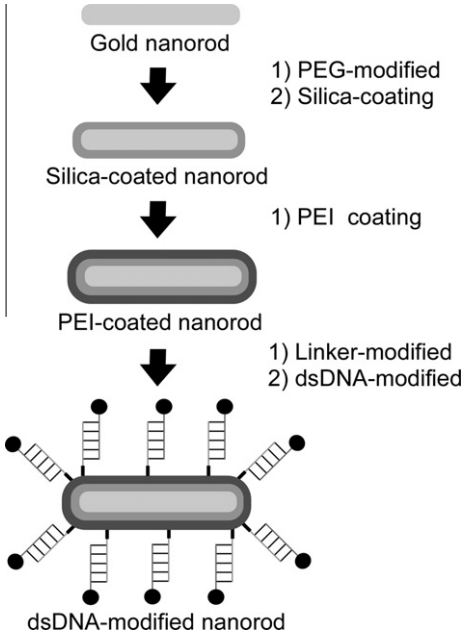

Figure 1. Preparation of dsDNA-modified gold nanorods (schematic). Actual thickness of each layer was shown in Figure S1.

Methods of direct oligonucleotide modification on the gold nanorods have been already reported.[51,57] We tried these methods first. However, aggregates of gold nanorods were formed at a step of addition of oligonucleotides. Even if the DNA-modified gold nanorods were able to be prepared, the resultant gold nanorods were unstable. It would be due to difference of chemical constitution of surfaces of gold nanorods between their and our preparations of the gold nanorods. Therefore, we employed such multi-steps modification using PEG, silica, PEI to prepare the dsDNA-modified gold nanorods in this study.

2.2. Release of ssDNA by heat treatment

Release of ssDNA induced by heat treatment of the dsDNA-modified gold nanorods was examined. To avoid released Cy3-modified ssDNA rehybridizing to the parent ssDNA fixed on the gold surface, an excess amount of complementary ssDNA was mixed in the reaction mixture (Fig. 2A). The mixture was heated at 80 °C for 10 min, then release of Cy3-modified ssDNA was measured with a fluorescence spectrometer. The fluorescence of the dye is quenched by the gold nanorods, which has been well studied as a quenching property of metallic nanoparticles,[69–72] so the release of ssDNA can be evaluated as the increase in fluorescence intensity.

After incubation at 80 °C for 10 min, the fluorescence intensity increased significantly (Fig. 2B-e). This indicated that the Cy3-modified ssDNA was released from the gold surface at 80 °C and then hybridized to the free complementary ssDNA after cooling the mixture. In the absence of the free complementary ssDNA, the fluorescence intensity increased slightly after incubation at 80 °C (Fig. 2B-c). This result indicated that most of the released Cy3-modified ssDNA re-hybridized back to the ssDNA on the gold surface. The slight increase in the fluorescence intensity would be due to the contribution of the remaining ssDNA that could not re-hybridize back to the gold surface. The fluorescence intensity in the presence of the free non-complementary ssDNA (Fig. 2B-b) was identical to that in the presence of the free complementary ssDNA (Fig. 2B-a). This indicated that simple strand exchange of Cy3-modified ssDNA with the complementary ssDNA on the gold

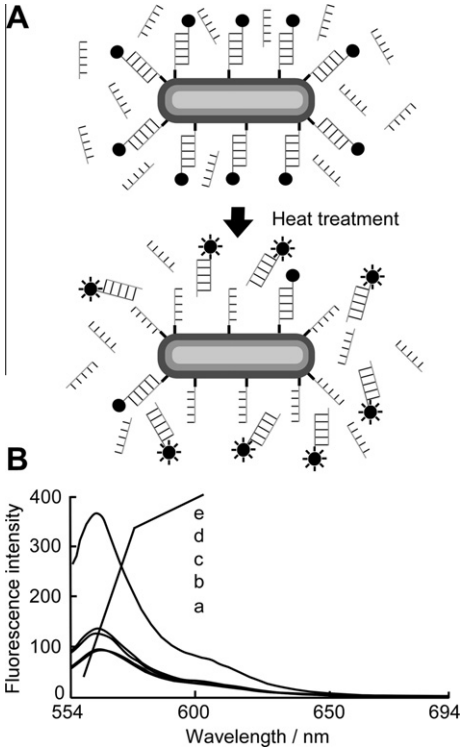

Figure 2. Release of ssDNA from gold nanorods by heat treatment. (A) Schematic diagram of the released ssDNA after heat treatment with free complementary ssDNA. (B) Evaluation of the released ssDNA by the increase in the fluorescence intensity spectrum. (a and e) The dsDNA-modified gold nanorods solution with free complementary ssDNA before and after heat treatment, respectively. (b and d) The dsDNA-modified gold nanorods solution with free non-complementary ssDNA before and after heat treatment, respectively. (c) The dsDNA-modified gold nanorods solution without free complementary ssDNA after heat treatment.

nanorods at room temperature did not occur. In the presence of non-complementary ssDNA (Fig. 2B-d), the increase was as slight as the case in the absence of the free complementary ssDNA (Fig. 2B-c). This result opposed the notion that the dsDNA was released with the PEI chains, which were modified electrostatically with the silica layer on the gold surface by the heat treatment.

2.3. Release of ssDNA by the photothermal effect

To investigate the release of the ssDNA triggered by the photothermal effect of the gold nanorods, dsDNA-modified gold nanorods were irradiated by continuous wave (CW) near-infrared laser light (wavelength 807 nm, 500 mW). After irradiation for 1 min, a significant increase of fluorescence intensity was observed (Fig. 3b), which then reached to plateau at 5 min (Fig 3c and d). However, the maximum intensity induced by the light irradiation was lower than that in the positive control, in which dsDNA-modified gold nanorods were incubated at 80 °C for 10 min (Fig. 3e). This indicated that 80% of the ssDNA could be released from the gold surface under this condition.

2.4. Effect of laser power on the release of ssDNA

The dsDNA-modified gold nanorods were irradiated by CW near-infrared laser light with various powers (100, 300, 500 mW). Fluorescence intensities were then measured (Fig. 4). An increase in fluorescent intensity was dependent upon laser power. That is, the increase in the laser power of 300 mW was lower than that of 500 mW. In the case of a laser power of 100 mW, a significant increase in fluorescent intensity was not observed.

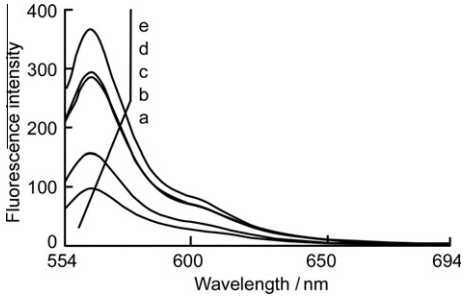

Figure 3. Release of ssDNA from gold nanorods induced by the photothermal effect. (a) Without CW near-infrared laser light laser irradiation dsDNA-modified gold nanorods solution. (b) To (d) with the light irradiation dsDNA-modified gold nanorods solution at 500 mW for 1, 5, and 10 min, respectively. (e) The dsDNA-modified gold nanorods solution after incubation (80 °C for 10 min) (positive control).

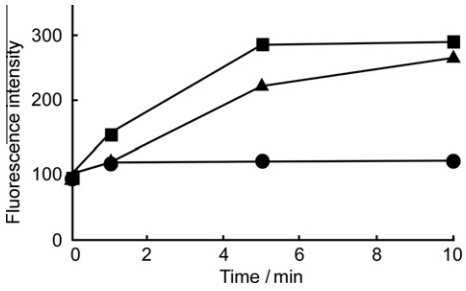

Figure 4. Relationship between laser irradiation and release of ssDNA. dsDNA-modified gold nanorods solution was irradiated with CW near-infrared laser light at various power values (●; 100, ▲; 300, ■; 500 mW) for various exposure times (1, 5, 10 min).

The temperatures of the irradiated solutions were also measured simultaneously using a radiation thermometer (Fig. 5). The temperature increase was dependent upon the exposure time and power of the laser. The temperature of the mixture at 500 mW reached ~60 °C, whereas those at 300 and 100 mW reached 50 and 37 °C, respectively. The melting temperature (T_m) of the dsDNA was ~60 °C (see Supplementary data Fig. S2). This means that, in the case of 300 mW, ssDNA was released at a lower temperature of the solution (50 °C) than the T_m of the dsDNA. This inconsistency could be because strong and rapid temperature increases of a limited area on the gold surface would be induced by the photothermal effect without increasing the ambient temperature of the solution.[56,57]

To confirm that ssDNA was released from dsDNA modified on the gold nanorods, gel electrophoresis of the treated solutions was undertaken (Fig. 6). When the sample was incubated at

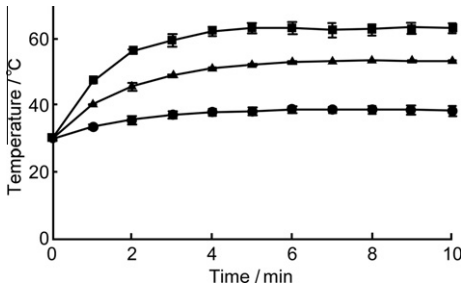

Figure 5. Temperature increase in dsDNA-modified gold nanorods solution during CW near-infrared laser light irradiation at various power values (■; 500 mW, ▲; 300 mW, ●; 100 mW) for various exposure times (1, 5, 10 min).

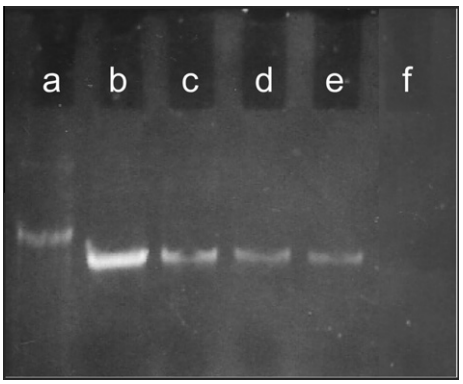

Figure 6. Gel electrophoresis of dsDNA consisting of released ssDNA from the gold nanorods and free complementary ssDNA. (a) Original dsDNA used in the preparation of dsDNA-modified gold nanorods. (b) Heat-treated sample of dsDNA-modified gold nanorods. (c–f) CW near-infrared laser light-irradiated samples of dsDNA-modified gold nanorods at 500 mW for 10 (c), 5 (d), 1 (e), and 0 min (f).

80 °C for 10 min, released ssDNA forming dsDNA with the free complementary ssDNA in the solution was clearly detected (Fig. 6, lane b). The free complementary ssDNA was shorter than the parent ssDNA (thiol-modified ssDNA) on the gold nanorods (Section 4.2), so the molecular weight of the band in lane b was lower that that in lane a (where the original dsDNA was used for the modification of the gold nanorods). By irradiating the solution with the CW near-infrared laser light at 500 mW for 10 min, release of the ssDNA was observed, and it was dependent upon the irradiation time (Fig. 6, lanes c–e). In a control condition without laser irradiation, no band was detected (Fig. 6, lane f).

2.5. Release of ssDNA in tumors by laser irradiation

A dsDNA-modified gold nanorods solution (20 μL of 1 mM (Au atoms) in 5% glucose with an excess amount of free complementary ssDNA) was directly injected into tumors grown in mice.

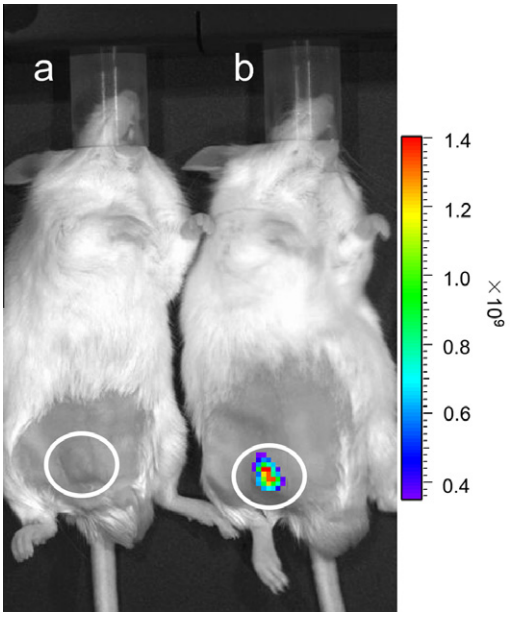

Figure 7. Release of ssDNA from dsDNA modified on gold nanorods followed by CW near-infrared laser light irradiation in vivo. Circles indicate the positions of tumors. The fluorescence of released Cy3-modified ssDNA was imaged in tumors with (a) or without (b) near-infrared laser light irradiation.

Immediately after the injection, the tumor was irradiated with CW near-infrared laser light for 3 min at 500 mW with a beam diameter of 7.0 mm. The fluorescence of the Cy3-modified ssDNA released from the gold nanorods was detected by an in vivo fluorescence imager (Fig. 7). Significant fluorescence was observed from the irradiated tumor by the laser light, whereas no fluorescence was detected from the tumor without the light irradiation. These results indicated that the photothermal release of ssDNA was triggered by the laser irradiation in the mouse tumor.

3. Conclusion

The release of ssDNA from dsDNA-modified gold nanorods was triggered by the photothermal effect induced by near-infrared laser light irradiation. The amount of released ssDNA was dependent upon the laser power and period of laser irradiation. The release of ssDNA was associated with rapid temperature increases of a limited area of the gold surface without increasing the ambient temperature of the solution. The controlled-release of ssDNA by the laser irradiation was also achieved in tumors in mice. Using this system, functional oligonucleotides, for example, siRNA, antisense oligonucleotide, decoy oligonucleotide, and aptamers could be modified, and a controlled-release system responding to light irradiation will be constructed. If a drug is modified on the released ssDNA, a controlled drug-release system can be also proposed. Also, simultaneous monitoring of the drug-release is possible by combining fluorescence drugs and/or dyes with a fluorescence bioimaging technique.

4. Experimental

4.1. Materials

Gold nanorods were provided by a joint research project between Mitsubishi Materials Corporation and Dai Nippon Toryo Company Limited. The mean size of the gold nanorods was 46 ± 6 and 10 ± 1 nm in longitudinal and transverse directions, respectively. m-$PEG_{20,000}$-SH (MW ca. 20,000 Da) was purchased from NOF Company Limited (Tokyo, Japan). Tetraethylorthosilicate (TEOS), branched-polyethyleneimine (PEI) and ethanol were purchased from Wako Pure Chemical Industries, Limited (Osaka, Japan). Sodium dihydrogenphosphate and disodium hydrogenphosphate were purchased from Nacalai Tesque, Incorporated (Kyoto, Japan). Crosslinkers were purchased from Thermo Fisher Scientific, Incorporated (Waltham, MA, USA). Free ssDNA, fluorescence (Cy3)-modified ssDNA and thiol group-modified ssDNA were purchased from Nippon EGT Company Limited (Toyama, Japan).

4.2. DNA hybridization

DNA hybridization was undertaken by mixing two complementary ssDNA (thiol-modified ssDNA: 5′-thiol-GTGTGTGTGT-CCTTGAAGGGATTTCCCTCC-3′, Cy3-modified ssDNA: 5′-Cy3-GTGTGTGTGT-GGAGGGAAATCCCTTCAAGG-3′) in a 1:1 molar ratio in 0.2 mM sodium phosphate buffer at pH 7.4 containing 50 mM NaCl, heating the solution to 80 °C, then allowing it to cool slowly to room temperature in a block heater. The T_m of the dsDNA was determined from the melting curves of the dsDNA using a UV spectrometer (JASCO V-570, Tokyo, Japan).

4.3. Preparation of DNA-modified gold nanorods

PEG-modified gold nanorods were prepared as follows. A solution of gold nanorods containing hexadecyltrimethylammonium bromide (CTAB) was centrifuged at 14,000*g* for 10 min at 25 °C,

decanted, and re-suspended in water to remove excess CTAB. Thiol-terminated PEG (m-$PEG_{20,000}$-SH) solution and water were added to the suspension of gold nanorods at a PEG:Au molar ratio of 0.05. The suspension was mixed for 24 h at room temperature and dialyzed. The solution of PEG-coated gold nanorods was centrifuged at 14,000 *g* for 10 min at 25 °C, decanted, and re-suspended in water to concentrate and remove excess free PEG chains. Ethanol (final concentration, 90%), 5% ammonia solution (final concentration, 0.05%), and 1% tetraethyl orthosilicate (TEOS; final concentration, 0.1%) were added to the concentrated solution of PEG-modified gold nanorods. The reaction was allowed to proceed for 24 h at 35 °C. The reaction mixture was centrifuged at 5000*g* for 5 min at 35 °C, decanted, and re-suspended in 90% ethanol in water to remove excess TEOS. Branched-polyethyleneimine (PEI) (MW ca. 1600 Da) solution was added to the silica-coated gold nanorods solution to graft amino groups on the gold surface at a PEI:Au molar ratio of 1.4. The suspension was mixed for 24 h at 35 °C. The PEI-modified gold nanorods solution was centrifuged twice at 10,000 *g* for 5 min at 35 °C. It was then decanted, and re-suspended in 0.2 M sodium phosphate buffer (pH 8.0) to exchange the solution and remove excess free PEI. The crosslinker (Mal–PEO_{12}–NHS) solution was added to the PEI-coated gold nanorods solution at a crosslinker:Au molar ratio of 2.3. The reaction was allowed to proceed for 1.5 h at room temperature. The reaction mixture solution was centrifuged twice at 10,000 *g* for 5 min at 35 °C. It was then decanted, and re-suspended in 0.2 M sodium phosphate buffer (pH 7.4) to exchange the solution and remove excess free crosslinker. dsDNA was added to the linker-modified gold nanorods solution at a dsDNA:Au molar ratio of 0.005. The reaction was allowed to proceed for 24 h at room temperature. The reaction mixture solution was then centrifuged twice at 10,000 *g* for 5 min at 25 °C. It was then decanted, and re-suspended in 0.2 M sodium phosphate buffer (pH 7.4) to remove excess free dsDNA.

4.4. Characterization of DNA-modified gold nanorods

The surface modification in each step was confirmed by ζ-potential measurement (Malvern Zetasizer Nano ZS, Worcestershire, UK) in 0.2 M sodium phosphate buffer (pH 7.4). Dispersal of gold nanorods in solution was confirmed by the absorbance spectrum.

4.5. Release of ssDNA from gold nanorods by heat treatment

The prepared dsDNA-modified gold nanorods solutions (100 μL of 0.5 mM (Au atoms) in 50 mM NaCl in sodium phosphate buffer (pH 7.4)) were divided into three samples (with complementary ssDNA and non-complementary ssDNA, without complementary ssDNA). The sequence of ssDNA was as follows: complementary ssDNA: 5′-CCTTGAAGGGATTTCCCTCC-3′, non-complementary ssDNA: 5′-TTGGACCAAACGGGTTTGTT-3′. Each mixture of the gold nanorods solution was heated at 80 °C for 10 min. Then, the release of Cy3-modified ssDNA was measured using a fluorescence spectrometer (Shimadzu RF-5300PC, Tokyo, Japan). Fluorescence spectra were measured at an excitation wavelength of 545 nm.

4.6. Release of ssDNA from gold nanorods by the photothermal effect

The prepared dsDNA-modified gold nanorods solution (100 μL of 0.5 mM (Au atoms)), which contained excess free complementary ssDNA in 50 mM NaCl in sodium phosphate buffer (pH 7.4), was irradiated by a CW near-infrared diode laser (wavelength, 807 nm; beam diameter, 5.5 mm; Alfright Corporation, Madison, WI, USA) at 500 mW for 10 min. The release of Cy3-modified ssDNA was measured with a fluorescence spectrometer. Fluorescence spectra were measured at an excitation wavelength of 545 nm. The temperature of the solution was monitored by a thermocouple (Horiba IT500S, Kyoto, Japan). The dsDNA-modified gold nanorods solution without light irradiation was examined as light irradiation for 0 min. The dsDNA-modified gold nanorods solution after incubation at 80 °C for 10 min was examined as a positive control. The percentage of ssDNA release was calculated with the following equation: % release = $100 \times (F - F_0/F_{100} - F_0)$, where F is the fluorescence intensity observed in the laser irradiated sample, F_0 is the intensity observed without laser irradiation, and F_{100} is the intensity observed in the positive control.

4.7. Gel electrophoresis

Each dsDNA-modified gold nanorods solution after laser irradiation or incubation (80 °C for 10 min) was centrifuged at 20,000 *g* for 30 min at 25 °C to remove gold nanorods. Obtained supernatants were dialyzed and freeze-dried (Eyela FD-5N, Tokyo, Japan) to desalt and concentrate the contents. The dried contents were added to water. This was followed by polyacrylamide gel electrophoresis (Bio-Rad Laboratories, Hercules, CA, USA). The dsDNA used in the preparation of dsDNA-modified gold nanorods was used as a control sample to compare with dsDNA comprising the released ssDNA from the gold nanorods and the free complementary ssDNA. The dsDNA-modified gold nanorods solution after incubation (80 °C for 10 min) was used as a positive control.

4.8. Animals

Male BALB/c mice (Kyudo Company Limited, Saga, Japan) were used in all experiments. Five-week old mice were maintained in a temperature-controlled environment at 24 °C with a 12-h light/dark cycle and provided with drinking water and feed ad libitum. Animal experiments were performed according to the Guidelines of the Animal Care and Use Committee of Kyushu University (Kyushu, Japan).

4.9. Implantation of tumor cells into mice

Mouse rectum carcinoma Colon-26 cells were cultured in RPMI-1640 containing 10% fetal bovine serum and 60 μg/mL kanamycin sulfate. The dishes containing cells were maintained at 37 °C in an incubator under an atmosphere of 5% CO_2. Tumor-bearing mice were prepared as follows: Colon-26 cells were injected subcutaneously into the abdomen at 2×10^6 cells in 100 μL Hank's balanced salt solution per mouse (ddY, male, 3 weeks, 11–13 g) and allowed to grow for 5 day, after which tumors reached 5–10 mm in diameter.

4.10. Evaluation of released ssDNA by laser irradiation in tumors

The tumor-bearing mice were subjected to in vivo imaging studies. Mice were anesthetized with pentobarbital sodium salt. The prepared dsDNA-modified gold nanorods solution (20 μL of 1 mM (Au atoms) in 5% glucose with excess free complementary ssDNA) was directly injected into tumors grown in mice. The tumor was irradiated with CW near-infrared laser light for 3 min at 500 mW (wavelength, 807 nm; diameter, 7.0 mm). The fluorescence images were taken by the IVIS Imaging System (Xenogen, Alameda, CA, USA) and analyzed with Living Image software. The excitation and emission wavelengths were 535 nm and 575–650 nm, respectively. The fluorescence images consisting of Cy3-modified ssDNA-released fluorescence and autofluorescence spectra were then unmixed.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.02.042.

References and notes

1. Gullotti, E.; Yeo, Y. *Mol. Pharm.* **2009**, *6*, 1041.
2. Sokolova, V.; Epple, M. *Angew. Chem., Int. Ed.* **2008**, *47*, 1382.
3. Matsumura, Y.; Maeda, H. *Cancer Res.* **1986**, *46*, 6387.
4. Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K. *J. Controlled Release* **2000**, *65*, 271.
5. Hirsch, L. R.; Stafford, R. J.; Bankson, J. A.; Sershen, S. R.; Rivera, B.; Price, R. E.; Hazle, J. D.; Halas, N. J.; West, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *23*, 13549.
6. Kirpotin, D. B.; Drummond, D. C.; Shao, Y.; Shalaby, M. R.; Hong, K.; Nielsen, U. B.; Marks, J. D.; Benz, C. C.; Park, J. W. *Cancer Res.* **2006**, *66*, 6732.
7. Huang, X.; El-Sayed, I. H.; Qian, W.; El-Sayed, M. A. *J. Am. Chem. Soc.* **2006**, *128*, 2115.
8. Wang, C.; Chen, J.; Talavage, T.; Irudayaraj, J. *Angew. Chem., Int. Ed.* **2009**, *48*, 2759.
9. Gabizon, A.; Horowitz, A. T.; Goren, D.; Tzemach, D.; Shmeeda, H.; Zalipsky, S. *Clin. Cancer Res.* **2003**, *9*, 6551.
10. Bartlett, D. W.; Su, H.; Hildebrandt, I. J.; Weber, W. A.; Davis, M. E. *Proc. Natl. Acad. Sci.* **2007**, *104*, 15549.
11. Xiong, X. B.; Huang, Y.; Lu, W. L.; Zhang, H.; Zhang, X.; Zhang, Q. *Pharm. Res.* **2005**, *22*, 933.
12. Li, Z.; Huang, P.; Zhang, X.; Lin, J.; Yang, S.; Liu, B.; Gao, F.; Xi, P.; Ren, Q.; Cui, D. *Mol. Pharm.* **2010**, *7*, 94.
13. Farokhzad, O. C.; Jon, S.; Khademhosseini, A.; Tran, T. N.; Lavan, D. A.; Langer, R. *Cancer Res.* **2004**, *64*, 7668.
14. Gerweck, L. E.; Seetharaman, K. *Cancer Res.* **1996**, *56*, 1194.
15. Tannock, I. F.; Rotin, D. *Cancer Res.* **1989**, *49*, 4373.
16. Guo, X.; Szoka, F. C., Jr. *Acc. Chem. Res.* **2003**, *36*, 335.
17. Walker, G. F.; Fella, C.; Pelisek, J.; Fahrmeir, J.; Boeckle, S.; Ogris, M.; Wagner, E. *Mol. Ther.* **2005**, *11*, 418.
18. Freiberg, S.; Zhu, X. X. *Int. J. Pharm.* **2004**, *282*, 1.
19. Torchilin, V. P. *J. Controlled Release* **2001**, *73*, 137.
20. Bae, Y.; Fukushima, S.; Harada, A.; Kataoka, K. *Angew. Chem., Int. Ed.* **2003**, *42*, 4640.
21. Lee, E. S.; Oh, K. T.; Kim, D.; Youn, Y. S.; Bae, Y. H. *J. Controlled Release* **2007**, *123*, 19.
22. Meng, F.; Zhong, Z.; Feijen, J. *Biomacromolecules* **2009**, *10*, 197.
23. Hatakeyama, H.; Akita, H.; Kogure, K.; Oishi, M.; Nagasaki, Y.; Kihira, Y.; Ueno, M.; Kobayashi, H.; Kikuchi, H.; Harashima, H. *Gene Ther.* **2007**, *14*, 68.
24. Harris, T. J.; von Maltzahn, G.; Lord, M. E.; Park, J. H.; Agrawal, A.; Min, D. H.; Sailor, M. J.; Bhatia, S. N. *Small* **2008**, *4*, 1307.
25. Lee, S.; Cha, E. J.; Park, K.; Lee, S. Y.; Hong, J. K.; Sun, I. C.; Kim, S. Y.; Choi, K.; Kwon, I. C.; Kim, K.; Ahn, C. H. *Angew. Chem., Int. Ed.* **2008**, *47*, 2804.
26. Mok, H.; Bae, K. H.; Ahn, C. H.; Park, T. G. *Langmuir* **2009**, *25*, 1645.
27. Andreasen, P. A.; Egelund, R.; Petersen, H. H. *Cell. Mol. Life Sci.* **2000**, *57*, 25.
28. Kinoh, H.; Inoue, M. *Front. Biosci.* **2008**, *1*, 2327.
29. Kinoh, H.; Inoue, M.; Komaru, A.; Ueda, Y.; Hasegawa, M.; Yonemitsu, Y. *Gene Ther.* **2009**, *16*, 392.
30. Niidome, T.; Ohga, A.; Akiyama, Y.; Watanabe, K.; Niidome, Y.; Mori, T.; Katayama, Y. *Bioorg. Med. Chem.* **2010**, *18*, 4453.
31. Brazel, C. S. *Pharm. Res.* **2009**, *10*, 643.
32. Liu, T.-Y.; Hu, S.-H.; Liu, D.-M.; Chen, S.-Y.; Chen, I.-W. *Nanotoday* **2009**, *4*, 52.
33. Hernot, S.; Klibanov, A. L. *Adv. Drug Delivery Rev.* **2008**, *60*, 1153.
34. Huang, S.-L. *Adv. Drug Delivery Rev.* **2008**, *60*, 1167.
35. Pissuwan, D.; Niidome, T.; Cortie, M. B. *J. Controlled Release* **2011**, *149*, 65.
36. Soga, O.; Van Nostrum, C. F.; Fens, M.; Rijcken, C. J. F.; Schiffelers, R. M.; Storm, G.; Hennink, W. E. *J. Controlled Release* **2005**, *103*, 341.
37. Timko, B. P.; Dvir, T.; Kohane, D. S. *Adv. Mater.* **2010**, *22*, 4925.
38. Yu, Y. Y.; Chang, S. S.; Lee, C. L.; Wang, C. R. C. *J. Phys. Chem. B* **1997**, *101*, 6661.
39. Link, S.; Mohamed, M. B.; El-Sayed, M. A. *J. Phys. Chem. B* **1999**, *103*, 3073.
40. Weissleder, R. *Nat. Biotechnol.* **2001**, *19*, 316.
41. Chou, C. H.; Chen, C. D.; Wang, C. R. C. *J. Phys. Chem. B* **2005**, *109*, 11135.
42. Huang, X.; Neretina, S.; El-sayed, M. A. *Adv. Mater.* **2009**, *21*, 1.
43. Yang, D.-P.; Cui, D.-X. *Chem. Asian J.* **2008**, *3*, 2010.
44. Tong, L.; Wei, Q.; Wei, A.; Cheng, J.-X. *Photochem. Photobiol.* **2009**, *85*, 21.
45. Suzuki, J.-I.; Isobe, M.; Morishita, R.; Nagai, R. *J. Biomed. Biotechnol.* **2009**, *2009*, 916514.
46. Kurreck, J. *Eur. J. Biochem.* **2003**, *270*, 1628.
47. Bhindi, R.; Fahmy, R. G.; Lowe, H. C.; Chesterman, C. N.; Dass, C. R.; Cairns, M. J.; Saravolac, E. G.; Sun, L.-Q.; Khachigian, L. M. *Am. J. Pathol.* **2007**, *171*, 1079.
48. Nimjee, S. M.; Rusconi, C. P.; Sullenger, B. A. *Annu. Rev. Med.* **2005**, *56*, 555.
49. Bunnell, B. A.; Morgan, R. A. *Clin. Microbiol. Rev.* **1998**, *11*, 42.
50. Chen, C.-C.; Lin, Y.-P.; Wang, C.-W.; Tzeng, H.-C.; Wu, C.-H.; Chen, Y.-C.; Chen, C.-P.; Chen, L.-C.; Wu, Y.-C. *J. Am. Chem. Soc.* **2006**, *128*, 3709.
51. Wijaya, A.; Schaffer, S. B.; Pallares, I. G.; Hamad-Schifferli, K. *ACS Nano.* **2009**, *3*, 80.
52. Takahashi, H.; Niidome, Y.; Yamada, S. *Chem. Commun.* **2005**, 2247.
53. Horiguti, Y.; Yamashita, S.; Niidome, T.; Nakashima, N.; Niidome, Y. *Chem. Lett.* **2008**, *37*, 718.
54. Yamashita, S.; Niidome, Y.; Katayama, Y.; Niidome, T. *Chem. Lett.* **2009**, *38*, 226.
55. Derfus, A. M.; Von Maltzahn, G.; Harris, T. J.; Duza, T.; Vecchio, K. S.; Ruoslahti, E.; Bhatia, S. N. *Adv. Mater.* **2007**, *19*, 3932.
56. Barhoumi, A.; Huschka, R.; Bardhan, R.; Knight, M. W.; Halas, N. J. *Chem. Phys. Lett.* **2009**, *482*, 171.
57. Lee, S. E.; Liu, G. L.; Kim, F.; Lee, L. P. *Nano Lett.* **2009**, *9*, 562.
58. Niidome, T.; Yamagata, M.; Okamoto, Y.; Akiyama, Y.; Takahashi, H.; Kawano, T.; Katayama, Y.; Niidome, Y. *J. Controlled Release* **2006**, *114*, 343.
59. Pastoriza-Santos, I.; Pèrez-Juste, J.; Liz-Marzàn, L. M. *Chem. Mater.* **2006**, *18*, 2465.
60. Stöber, W.; Fink, A.; Bohn, E. *J. Colloid Interface Sci.* **1968**, *26*, 62.
61. Kawano, T.; Niidome, Y.; Mori, T.; Katayama, Y.; Niidome, T. *Bioconjugate Chem.* **2009**, *20*, 209.
62. Shiotani, A.; Akiyama, Y.; Kawano, T.; Niidome, Y.; Mori, T.; Katayama, Y.; Niidome, T. *Bioconjugate Chem.* **2010**, *21*, 2049.
63. Lindquist, G. M.; Stratton, R. A. *J. Colloid Interface sci.* **1976**, *55*, 45.
64. Mèszaros, R.; Varga, I.; Gilànyi, T. *Langmuir* **2004**, *20*, 5026.
65. Mèszàros, R.; Thompson, L.; Bos, M.; De Groot, P. *Langmuir* **2002**, *18*, 6164.
66. Thierry, B.; Zimmer, L.; McNiven, S.; Finnie, K.; Barbe, C.; Griesser, H. J. *Langmuir* **2008**, *24*, 8143.
67. Link, S.; El-Sayed, M. A. *J. Phys. Chem. B.* **1999**, *103*, 8410.
68. Liz-Marzàn, L. M.; Giersig, M.; Mulvaney, P. *Langmuir* **1996**, *12*, 4329.
69. Jayagopal, A.; Halfpenny, K. C.; Perez, J. W.; Wright, D. W. *J. Am. Chem. Soc.* **2010**, *132*, 9789.
70. Jennings, T. L.; Singh, M. P.; Strouse, G. F. *J. Am. Chem. Soc.* **2006**, *128*, 5462.
71. Singh, M. P.; Strouse, G. F. *J. Am. Chem. Soc.* **2010**, *132*, 9383.
72. Yun, C. S.; Javier, A.; Jennings, T.; Fisher, M.; Hira, S.; Peterson, S.; Hopkins, B.; Reich, N. O.; Strouse, G. F. *J. Am. Chem. Soc.* **2005**, *127*, 3115.