Development of functional gold nanorods for bioimaging and photothermal therapy

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Abstract. Gold nanorods have strong surface plasmon band at near-infrared light region, and are used as a photothermal converter. Since the near-infrared light penetrates into tissues deeply, it has been expected as a contrast agent for near infrared light bioimaging, a photosensitizer for photothermal therapy, and functional device for drug delivery system responding to near-infrared light irradiation. In this study, the surface plasmon bands of intravenously injected gold nanorods were monitored in the mouse abdomen using a spectrophotometer equipped with an integrating sphere, then we determined pharmacokinetics parameters of the gold nanorods after intravenous injection. Next, the PEG-modified gold nanorods were directly injected into subcutaneous tumors in mice, then, near-infrared pulsed laser light was irradiated the tumors. Significant tumor damage and suppression of the tumor growth was observed. We constructed targeted delivery system of the gold nanorods by modifying with a thermo-responsive polymer and a peptide responding to a protease activity. These modified gold nanorods are expected as functional nanodevices for photothermal therapy and drug delivery system.

1. Introduction
Gold nanorods, rod-shaped gold nanoparticles, have unique optical properties [1,2]. They show two surface plasmon bands corresponding to the transverse and longitudinal surface plasmon bands in the visible and the near infrared regions, respectively (Figure 1). The longitudinal band has a substantially larger extinction coefficient than the transverse band, and show efficient photothermal effect. The near infrared region (650 – 900 nm) is ideally suited for in vivo imaging and phototherapy due to minimum light absorption by intrinsic chromophores, hemoglobin, and water. Recently, we succeeded to prepare biocompatible gold nanorods by coating PEG chain [3,4]. Long lasting circulation of the PEG-modified gold nanorods was observed after intravenous injection. In this study, we tried to apply the gold nanorods as a contrast agent for near infrared light bioimaging, a photosensitizer for photothermal therapy, and a functional device for drug delivery system responding to near-infrared light irradiation and protease activity.

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2. Experimental

2.1. Gold nanorods
Gold nanorods, prepared with a modification of our previous methods [5], were supplied by Dai Nihon Toryo Co. Ltd. and Mitsubishi Materials Co. Ltd. Average length and width of as-prepared gold nanorods were 65 ± 5 nm and 11 ± 1 nm, respectively (aspect ratio: 5.9). PEG-modified gold nanorods were prepared as follows. A solution of gold nanorods containing CTAB was centrifuged at 14,000 g for 10 min, decanted, and resuspended in water to remove excess CTAB. 200 µl 5 mM mPEG₅₀₀₀-SH were added to 1 ml 1 mM gold nanorod solution. The mixture was stirred for 24 h at room temperature, and was dialyzed for 3 d.

2.2. Measurement of absorption spectra of gold nanorods
All animal experiments were performed according to Guidelines for Animal care and Use committee, Kyushu University. The anesthetized mouse was placed on a port of the integral sphere equipped with a conventional spectrophotometer (JASCO V-670), then, monochromatic light from the spectrophotometer was introduced into the abdomen of the mouse. The absorbance spectra of the mouse abdomen were obtained by scanning the wavelength of the light from spectrophotometer.

2.3. Preparation of NIPAM-modified gold nanorods
PEG-modified gold nanorods were coated with a silica layer using the modified Stöber method, which is based on the hydrolysis of tetraethyl orthosilicate (TEOS) in ethanol/water mixture in the presence of nanorods, which act as “seeds”. Silane-coupling agent, 3-(methacryloxy) propyl triethoxysilane (MPS) was modified with the surface of the silica-coated nanorods. Next, silica-coated nanorods were modified with pNIPAM shells by precipitation polymerization. In the presence of MPS-modified silica nanorods, precipitation polymerization of NIPAM and N,N'-methylene bisacrylamide as a crosslinker was done. Finally, the silica templates were selectively etched using hydrofluoric acid.

2.4. Preparation of PEG-peptide-modified gold nanorods
Peptides were synthesized by Fmoc-chemistry manually. After deprotection and cleavage from resin, the peptides containing a cysteine residue were purified by RP-HPLC. N-hydroxy succinimide-terminated PEG (NOF Co., Ltd, MW ca. 5,000 Da) was reacted with amine of α-amino group of N-terminal amino acid in the peptide, then, resulting PEG-modified peptides were dialyzed for 3 d. Original gold nanorods solution containing CTAB was centrifuged at 14,000 g for 10 min, decanted, and re-suspended in water to remove excess CTAB. The PEG-modified peptides solution was added to gold nanorods suspension at various PEG:Au molar ratios. The mixtures were stirred for over night at room temperature and centrifuged at 14,000 g for 10 min, decanted, and resuspended in water to remove remaining CTAB and excess PEG-modified peptides. Zeta potential of PEG-peptide-modified gold nanorods were around neutral.

2.5. Photothermal tumor damage
For evaluation of tumor growth suppression, B-16 melanoma cells were injected subcutaneously into abdomen of mouse (BALB/c, male, 3 weeks, 10 - 13 g) and allowed to grow for 5 days, where the tumors reached a size of ~ 5 mm in diameter. The gold nanorods (50 µl in 5% glucose, 2 mM) were injected directly into the tumor, and then the tumor was irradiated with pulsed laser light (Nd:YAG 1,064 nm, 750 mW, 20 Hz, 3 min, ~ 5 mm beam diameter) within 10 min after the injection. In the case of intravenous injection, the gold nanorods (300 µl in 5% glucose, 2 mM) were injected. At 24 h after the injection, the tumor was irradiated with laser light.

3. Results and Discussion

3.1. Measurement of absorption spectra of gold nanorods
As shown in Figure 2, spectra of the gold nanorods could be observed at abdomen immediately after injection (spectra a) and 30 min after injection (spectra b). Baseline data were recorded just before the injection. Following the injection of PEG-modified gold nanorods (Figure 2A), characteristic peaks at around 900 nm were observed. Furthermore, because aggregation of the gold nanorods dramatically changes the longitudinal surface plasmon bands in the near infrared region, the peak intensities could be assigned to the amount of the gold nanorods dispersing in the abdomen without forming aggregates. Thus, it indicates that the PEG-modified gold nanorods circulated at least 30 min without forming aggregates. In the case of injection of phosphatidylcholine (PC)-modified gold nanorods [6], a surface plasmon band from the gold nanorods was observed at immediately after the injection (Figure 2B). However, at 30 min after injection of the PC-modified gold nanorods, no absorption peak was observed in the spectrum, indicating that circulation of gold nanorods in mice was not as good as that of the PEG-modified gold nanorods.

Next, the absorbance at 900 nm was continuously monitored after the intravenous injection. The injection of the PEG-modified gold nanorods resulted in an increase of absorption in the abdomen, which then reached a plateau. On the other hand, the injections of the PC-modified nanorods resulted in an increase of absorption, as in the case of PEG-modified gold nanorods; however, the absorption intensities immediately decreased. By single-exponential fitting of the decrease, half-lives of the PEG- and PC-modified gold nanorods were estimated to be 231 and 1.3 min, respectively. The PC-modified gold nanorods have slight positive surface charge. Positively charged nanoparticles tends to be recognized by reticuloendothelial system in liver and spleen, therefore, their half-life was shorter than the PEG-modified gold nanorods that have neutral surface. Such a surface chemical structure of the gold nanorods was important factor for stability in the circulation and biodistribution.

![Figure 2](image)

**Figure 2.** Absorption spectra from the abdomen of mice after intravenous injection of gold nanorods (300 µl of 2 mM Au atoms). Spectra immediately after injection (a) and 30 min after injection (b) of PEG- (A) and PC-modified (B) gold nanorods.

3.2. Photothermal tumor damage
We tried to perform photothermal cancer therapy of tumor bearing mice. To confirm the therapeutic effect of the photothermal system, we measured the tumor growth after the treatment (Fig. 3). Only in the case where both direct injection of gold nanorods and the irradiation of laser light resulted in a significant suppression of the tumor (Figure 3, closed circles), while the tumor growth in the control mice, that is, only those mice injected with gold nanorods or those mice treated with laser irradiation (Fig. 3, open squares and closed squares, respectively) showed no difference from the case of non-treated mice (Fig. 3, closed triangles) [7]. In the case of intravenous injection of gold nanorods, the suppression of the tumor growth was lower than the case of the direct injection (data not shown), indicating that the targeted delivery of the gold nanorods to the tumor tissue is an important key to improve the therapeutic effect.

Figure 3. Tumor growth after photothermal treatment. Closed circles indicate the case of both direct injection of the gold nanorods and laser irradiation. Open squares indicate the case of direct injection of gold nanorods alone. Closed squares indicate the case of direct injection of 5% glucose followed by laser irradiation. Closed triangles indicate the case of non-treated mice. Data represent mean values for n= 6 and bars are standard deviations for the means.

3.3. Construction of targeting system of gold nanorods
To achieve targeted delivery of the nanorods to specific site, firstly we focused on thermoresponsive polymer (pNIPAM), that is, we expected that generated heat by photothermal effect of the gold nanorods induces phase transition of the pNIPAM layer to hydrophobic (Figure 4). The hydrophobic surface could interact with cellular membrane or extracellular matrix, therefore, the nanorods will accumulate to the irradiated site. We prepared hybrid nanogels (a single gold nanorod encapsulated in a PNIpAM nanogel) by colloid-template polymerization and silica etching [8]. pNIPAM-coated gold nanorods shrank considerably and rapidly when near infrared laser was irradiated (~4 W/cm²). A cycle of shrinkage and swelling could be induced by switching the laser on and off. Next, we tried a feasible study on in vivo application. The gel modified gold nanorods were intravenously injected into mice, then, the right kidney was immediately irradiated using the NIR laser. Organs were subsequently collected, and the amounts of the gold nanorods in organs were evaluated by ICP-MS. When a solution of pNIPAM-coated gold nanorods was injected, the golds were distributed primarily in the blood (47%) and lungs (46%) after 10 min. The lower critical solution temperature of the pNIPAM layer was 34°C lower than body temperature, but they circulated in the blood within 10 min because of a slow phase transition response to a jump in environmental temperature. After irradiation of the right kidney for 10 min immediately after injection of nanogels, significant accumulation of the gold in the
irradiated right kidney was observed. In that time, no gold was observed in the left kidney and the blood. pNIPAM-coated gold nanorods, which circulated in the blood after injection, could accumulate at the irradiated site due to induced rapid photothermal phase transition.

Second, we tried another strategy that focused on protease specifically expressing in tumor tissue, that is, a substrate peptide (LGGSGRSANAILE-Cys) for urokinase-type plasminogen activator (uPA) was conjugated with PEG chain, then, the PEG-modified peptide was bound on the surface of the gold nanorods (Figure 5). When purified uPA was added to the PEG-peptide-modified gold nanorods, aggregation of the nanorods was confirmed by the absorption of the near infrared light. Decrease of the absorption depended on the amount of uPA and density of PEG-peptide on the nanorods. Since uPA is specifically expressed in tumor tissue, the PEG-peptide-gold nanorods would accumulate in the tumor due to the aggregation triggered by the site-specific enzyme activity.

4. Conclusion
We succeeded in direct monitoring of gold nanorods in vivo with an integrating sphere, then, half-lives of the gold nanorods in the circulation could be calculated. Second, we demonstrated photothermal cancer therapy by combining the injection of PEG-modified gold nanorods and near infrared laser irradiation. Finally, we constructed delivery systems responding to near infrared laser irradiation and protease activity. These functional systems will allow us to diagnose the tumors using the near infrared light and improve the therapeutic effect after intravenous injection of the gold nanorods.

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References

[1] Yu Y–Y, Chang S–S, Lee C–L and Wang C R C 1997 J. Phys. Chem. B 101 6661

[2] Link S, Mohamed M B and El-Sayed M A 1999 J. Phys. Chem. B 103 3073

[3] Niidome T, Yamagata M, Okamoto Y, Akiyama Y, Takahashi H, Kawano T, Katayama Y and Niidome Y 2006 J. Control. Release 114 343

[4] Shiotani A, Mori T, Niidome T, Niidome Y and Katayama Y 2007 Langmuir 23 4012

[5] Niidome Y, Nishioka K, Kawasaki H and Yamada S 2003 Chem. Commun. 18 2376

[6] Takahashi H, Niidome Y, Niidome T, Kaneko K, Kawasaki W and Yamada S 2006 Langmuir 22 2

[7] Niidome T, Akiyama Y, Yamagata M, Kawano T, Mori T, Niidome Y and Katayama Y 2009 J. Biomater. Sci.-Polym. Ed. 20 1203

[8] Kawano T, Niidome Y, Mori T, Katayama Y and Niidome T 2009 Bioconjugate Chem. 20 209