Gold Nanorod-sensitized Cell Death: Microscopic Observation of Single Living Cells Irradiated by Pulsed Near-infrared Laser Light in the Presence of Gold Nanorods

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Pulsed near-infrared (near-IR) laser irradiation damaged cultured cells in the presence of phosphatidylcholine- passivated gold nanorods (PC-NRs). When single cells were irradiated with a focused pulsed near-IR laser, photoreaction of the PC-NRs caused damage within a very small area around the PC-NRs, and achieved selective cell death of single cells.

Gold nanorods (NRs) are rod-shaped gold nanoparticles that show unique optical properties depending on their shapes.[1,2] One of the surface plasmon (SP) bands is located in the near-infrared (near-IR) region with intense absorption coefficiency.[2] Thus, NRs represent unusual materials with an intense absorption band in the near-IR region.[3–5] The distinct SP bands of NRs provide highly efficient photothermal conversion.[6] Photothermal conversion using near-IR light affords potential as an effective sensitizer for photoinduced treatment of tumors,[7] because near-IR light is transmitted deep into body tissues without significant damage to the tissues themselves. Thus, the optical property of NRs is expected to be useful for photothermal therapy.

Continuous laser light has normally been used for photodynamic and photothermal therapy.[7–9] On the other hand, pulsed laser light are useful for controlling irradiation conditions (repetition rate, pulse width, and so on).[10] Thus, pulsed-laser irradiation is advantageous for selectively affecting specific cells while leaving the surrounding cells intact, compared with the use of continuous laser irradiation. Previously, Hosokawa et al. reported that femtosecond pulsed-laser irradiation was able to realize nondestructive detachment of single cells.[11] This finding represents a typical example of the potential use of pulsed-laser irradiation for designing spatially selective photoreactions that could affect single living cells under microscopic observation. Moreover, pulsed near-IR light introduces local excessive heat only around the NRs for a very short time period.[12] Thus, pulsed-laser irradiation of NRs is expected to cause selective damage to single living cells, while leaving the surrounding cells intact.

Because of the presence of hexadecyltrimethylammonium bromide (CTAB), as-prepared NR solutions show high cytotoxicity.[13,14] We previously succeeded in preparing phosphatidylcholine (PC)-passivated NRs (PC-NRs), that show very low cytotoxicity.[15] In the present study, we investigated the usefulness of these PC-NRs as photosensitizers for damaging single cells using pulsed near-IR light under microscopic observation.

NRs, prepared by a modification of our previously reported method,[16] were supplied by Mitsubishi Materials Co., Ltd. The initial NR solution (≈1 mM (Au atoms)) contained 80 mM CTAB, and the zeta potential of the NRs was about +65 mV. Some of the precipitated CTAB was removed using a membrane filter (pore size, 0.8 μm). The residual CTAB in the NR solution (20 mL) was extracted into 10 mL of a chloroform solution containing PC (10 mg/mL; from egg yolk; Nacalai Tesque). After repeating the extraction procedures a further two times, the NR solution was centrifuged once, and the PC-NRs were obtained in water.

For microscopic observation of cultured cells treated with PC-NRs, HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) containing 10% (v/v) heat-inactivated fetal bovine serum and Antibiotic-Antimycotic (GIBCO) in a glass-based dish (35 mm; glass diameter, 12 mm). Following a 24-h incubation (37 °C, 5% CO_2), the culture medium was removed, and a mixed solution of 5 μL of PC-NR solution (8 mM (Au atoms)) and 25 μL of DMEM was poured into the dish. After a 30-min incubation, 10 μL of Trypan blue solution was added to investigate the incidence of cell death.[17] The cultured cells in the dishes were then observed by differential interference microscopy (TE2000; Nikon) and irradiated using the fundamental light of a Q-switched Nd:YAG laser (Polaris III; 1064 nm; pulse duration, 6–9 ns; repetition rate, 10 Hz) under microscopic observation.

An absorption spectrum of the as-prepared PC-NR solution (1 mM (Au atoms)) is shown in Figure 1a. The spectrum showed the typical two SP peaks corresponding to the longitudinal (≈900 nm) and transverse (≈520 nm) oscillation modes.[1,2] The zeta potential of the PC-NRs was +15 ± 1 mV. The cytotoxicity of the PC-NRs is discussed in another paper.[15] An absorption spectrum of the PC-NRs (1 mM) in DMEM showed the same two absorption bands at the identical wavelengths (Figure 1b), indicating that most of the PC-NRs had dispersed in the solution without forming aggregates. It was clear that PC-NRs could absorb near-IR light (1064 nm) in DMEM. From a TEM image (inset of Figure 1), the average length and width of the PC-

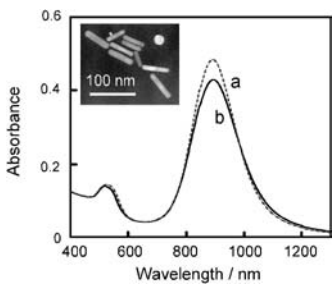

Figure 1. Absorption spectra of PC-NRs in water (a) and DMEM (b). The inset shows a TEM image of PC-NRs.

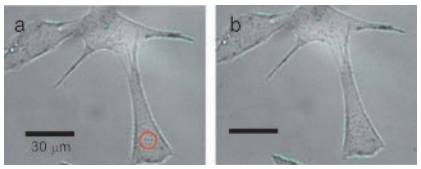

Figure 2. Microscopic images of HeLa cells without PC-NRs before (a) and after (b) laser irradiation. The medium in the dish consisted of a mixture of 30 μL of DMEM and 10 μL of Trypan blue. Fundamental light of a pulsed Nd:YAG laser (1064 nm, 2 mJ/pulse, 2 min) was passed through a 40× objective lens. The laser-irradiated spot is shown by the red circle.

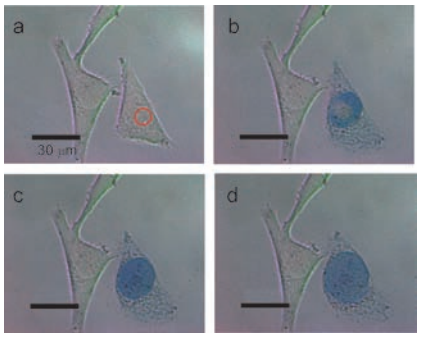

Figure 3. Microscopic images of HeLa cells treated with PC-NRs before (a) and after (b–d) laser irradiation (several seconds (b), 1 min (c) and 2 min (d) after the laser irradiation). The medium in the dish consisted of a mixture of 25 μL of DMEM, 5 μL of PC-NR solution (8 mM) and 10 μL of Trypan blue solution. The final PC-NR concentration was about 1 mM. Laser irradiation conditions were the same as those of Figure 2.

NRs were calculated to be 65 ± 5 nm and 11 ± 1 nm, respectively (aspect ratio: 5.9).

Figure 2 shows microscopic images of HeLa cells in the absence of PC-NRs. The pulsed laser (2 mJ/pulse) was focused at the area shown by the red circle in Figure 2a. The medium contained a dye (Trypan blue) that stained dead cells blue. After 2 min of laser irradiation, the laser-irradiated cell was not stained with Trypan blue, confirming that focused laser irradiation does not induce cell death in the absence of PC-NRs in the medium.

Figure 3 shows microscopic images of HeLa cells before (a) and after (b–d) laser irradiation in the presence of PC-NRs (1 mM). The PC-NRs themselves were too small to be observed in these microscopic images; however, they must be taken up or in contact with the cells.[15] The cells were not stained with Trypan blue before laser irradiation (a), indicating that the presence of the PC-NRs alone did not induce cell death. Next, the pulsed laser was focused at the area indicated by the red circle in Figure 3a. Several seconds after the laser irradiation (2 mJ/pulse, 10 Hz, 10 s), the laser-irradiated cell became stained with Trypan blue (Figure 3b), indicating that it had died. It could be seen that the nucleus of the cell was selectively stained. Moreover, images obtained at 1 (c) and 2 (d) min after the laser irradiation clearly indicated that the neighboring cells were not damaged by the laser irradiation. These observations indicate that the heat around the target cell caused by the pulsed-laser irradiation was insufficient to damage the neighboring cells. In the case of confluent cells, the photoreaction of PC-NRs induced cell death without damaging the neighboring cells as well as the case of Figure 3.

Therefore, the combination of PC-NRs and pulsed near-IR light resulted in selective cell death. Moreover, although the cell death was induced by photothermal conversion of the PC-NRs, the heat provided by the PC-NRs was insufficient to damage the neighboring cells. Thus, PC-NRs are effective photosensitizers driven by near-IR light to introduce excessive heat in a small localized region. When target cells are stained specifically with PC-NRs, treatment of tumors without damage to the normal tissues will be achieved. This technique provides a novel system for tumor therapy and the treatment of infectious diseases.

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References

1 Y.-Y. Yu, S.-S. Chang, C.-L. Lee, C. R. C. Wang, *J. Phys. Chem. B* **1997**, *101*, 6661.
2 K.-S. Lee, M. A. El-Sayed, *J. Phys. Chem. B* **2005**, *109*, 20331.
3 Y. Niidome, H. Takahashi, S. Urakawa, K. Nishioka, S. Yamada, *Chem. Lett.* **2004**, *33*, 454.
4 I. Gorelikov, L. M. Field, E. Kumacheva, *J. Am. Chem. Soc.* **2004**, *126*, 15938.
5 H. Takahashi, Y. Niidome, S. Yamada, *Chem. Commun.* **2005**, 2247.
6 C.-H. Chou, C.-D. Chen, C. R. C. Wang, *J. Phys. Chem. B* **2005**, *109*, 11135.
7 L. R. Hirch, R. J. Stafford, J. A. Bankson, S. R. Sershen, B. Rivera, R. E. Price, J. D. Hazle, N. J. Halas, J. L. West, *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 13549.
8 W. M. Sharman, C. M. Allen, J. E. van Lier, *Drug Discovery Today* **1999**, *4*, 507.
9 X. Huang, I. H. El-Sayed, W. Qian, M. A. El-Sayed, *J. Am. Chem. Soc.* **2006**, *128*, 2115.
10 a) Y. Miyamoto, Y. Umebayashi, T. Nishisaka, *J. Photochem. Photobiol., B* **1999**, *53*, 53. b) Y. Miyamoto, Y. Suzuki, T. Meguro, M. Iwaki, *Chem. Lett.* **2004**, *33*, 240.
11 Y. Hosokawa, H. Takabayashi, S. Miura, C. Shukunami, Y. Hiraki, H. Masuhara, *Appl. Phys. A* **2004**, *79*, 795.
12 K. Imura, T. Nagahara, H. Okamoto, *J. Phys. Chem. B* **2004**, *108*, 16344.
13 E. E. Connor, J. Mwamuka, A. Gole, C. J. Murphy, M. D. Wyatt, *Small* **2005**, *1*, 325.
14 H. Liao, J. H. Hafner, *Chem. Mater.* **2005**, *17*, 4636.
15 H. Takahashi, Y. Niidome, T. Niidome, K. Kaneko, H. Kawasaki, S. Yamada, *Langmuir* **2006**, *22*, 2.
16 Y. Niidome, K. Nishioka, H. Kawasaki, S. Yamada, *Chem. Commun.* **2003**, 2376.
17 M. Carvalho, F. Remião, N. Milhazes, F. Borges, E. Fernandes, M. Monteiro, M. J. Gonçalves, V. Seabra, F. Amado, F. Carvalho, M. L. Bastos, *Chem. Res. Toxicol.* **2004**, *17*, 623.

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