Cytotoxic Evaluation of Ag@TiO_2 Core–Shell Composite Nanoclusters against Cancer Cells

Md. Abdulla-Al-Mamun, Yoshihumi Kusumoto,* and Md. Shariful Islam
Department of Chemistry and Bioscience, Graduate School of Science and Engineering, Kagoshima University,
1-21-35 Korimoto, Kagoshima 890-0065

(Received July 24, 2009; CL-090693; E-mail: kusumoto@sci.kagoshima-u.ac.jp)

A Ag metal core–TiO_2 shell (Ag@TiO_2) composite nanocluster photocatalyst with uniform size and shape was successfully synthesized by a new simple citrate reduction method. The cytotoxicity of colloidal Ag@TiO_2 composite nanoclusters was evaluated against cancer (HeLa) cells under UV–vis irradiation. Ag@TiO_2 composite nanoclusters were characterized by using UV–vis absorption spectroscopy, TEM, SEM, XPS, XRD, and EDX techniques. It was found that Ag@TiO_2 nanoclusters at low concentrations kill HeLa cells by 100%, whereas TiO_2 nanoparticles alone kill only ca. 30% at the same concentrations.

The photocatalytic oxidation and reduction of semiconductor TiO_2 are strongly enhanced by contact with noble metal nanoparticles. Metal nanoparticles deposited on TiO_2 nanostructures undergo Fermi level equilibration following UV-excitation and enhance efficiency of the charge-transfer process.[1,2] Charge recombination or the grain boundary in heterogeneous semiconductor nanoparticles often limits the efficiency of light energy conversion. A semiconductor–metal composite system is able to suppress the charge recombination to enhance energy conversion efficiency.[3] A variety of nanocomposite semiconductor materials have been synthesized in recent years to improve the selectivity and efficiency of photocatalytic processes.[4,5]

Ag@TiO_2 has been used to enhance the photocatalytic decomposition of organic compounds and photokilling of bacteria.[6–8] However, enhanced photoactivity against cancer cells due to both plasmon-excited metal nanoparticles and photogenerated electron–hole pairs in TiO_2 has never been reported to the best of our knowledge. Here we report the photocatalytic and photothermal effect of Ag@TiO_2 on cancer (HeLa) cells at several TiO_2 concentrations and also at different molar ratios of Ag to TiO_2. We found that the photocatalytic cytotoxicity of Ag@TiO_2 required only one-fourth the irradiation time required by TiO_2 alone and that the killing efficiency of Ag@TiO_2 was more than three times higher than that of TiO_2 alone at the same concentration.

Generally Ag nanoparticles are chemically very reactive; they can be oxidized by direct contact with TiO_2 and can produce silver oxide (Ag_2O). For this problem to prevent oxidation we introduce a very simple new method to prepare a Ag@TiO_2 nanocomposite by citrate reduction. The Ag@TiO_2 nanoclusters were prepared by adding 1 mM ($1\,M = 1\,mol\,dm^{-3}$) of $AgNO_3$ to distilled water with vigorous stirring at constant temperature (60 °C). Titanium(IV) (triethanolaminato)isopropoxide ($N((CH_2)_2O)_3TiOCH(CH_3)_2$) of different molar concentrations and 2 mL of 1% sodium citrate were added to $AgNO_3$ solution. The solution was stirred for an additional 15 min to allow complete core–shell formation. The solution temperature was increased to the boiling temperature with vigorous stirring. With continued heating of the solution, it slowly became light brown from colorless. After 90 min, the color of the suspension turned from light to dark brown. After that, the sample suspension was cooled on standing at room temperature.

Next the solution was transferred to a conical flask with a rubber septum and the solution was purged with N_2 gas. Then the solution was irradiated with ultraviolet light ($15\,mW\,cm^{-2}$) for at least 30 min to reduce unreacted Ag^+ in solution to Ag^0. Then the solution was centrifuged at 5000 rpm for 20 min. The precipitate nanoclusters were washed two times with water and one time with ethanol to remove the remaining citrate solution and minimize the water content. After the supernatant was removed, clusters were collected and sterilized in an autoclave. Then the composite nanoclusters were dispersed in 5 mL of MEM (minimum essential medium) solution and stored at 4 °C.

Cytotoxicity towards a human carcinoma cell-line was evaluated as follows. HeLa cells were cultured in MEM solution with 10% newborn calf serum (NBS) in a humidified incubator under an atmosphere of 5% CO_2 in air at 37 °C. HeLa cells were plated at a concentration of about 3×10^5 in 60-mm Petri dishes and allowed to grow for 3 days. A xenon lamp with heat cut-off and band-pass filters (350–600 nm) with an average intensity of $35\,mW\,cm^{-2}$ was used for light irradiation. The light power was measured by a spectroradiometer (Model LS-100, EKO Instrument Co., Ltd.). The images were taken using an Olympus inverted CKX41 microscope.

UV–vis absorption spectra of Ag@TiO_2 composite nanocluster suspensions show strong absorption in the visible region with plasmonic peaks as shown in Figure 1A. The plasmonic peaks indicate that the Ag core is not oxidized.[9] Lower ratios of the TiO_2 shell to the Ag core resulted in dampening and broadening of the surface plasmon band (Figures 1Aa and 1Ab), and at a higher ratio of the TiO_2 shell the plasmon band

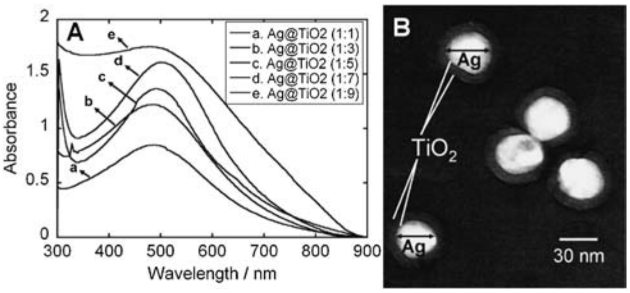

Figure 1. (A) Absorption spectra of Ag@TiO_2 various colloidal composite nanocluster suspensions. The Ag:TiO_2 molar ratios were maintained at (a) 1:1, (b) 1:3, (c) 1:5, (d) 1:7, and (e) 1:9. (B) The transmission electron microscope picture. Ag:TiO_2 = 1:7.

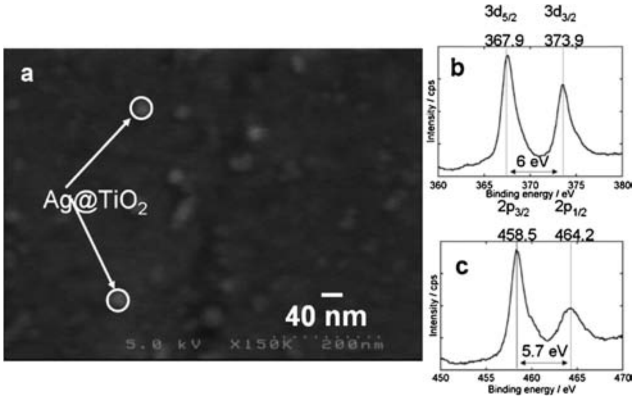

Figure 2. (a) FE-SEM image of Ag@TiO$_2$ composite nanocluster morphology. X-ray photoelectron spectra in the region of (b) Ag 3d and (c) Ti 2p of as-prepared Ag@TiO$_2$.

of Ag core becomes obscure (Figure 1Ae). The TEM image (Figure 1B) clearly shows round dark structure of the TiO$_2$ shell around the Ag core. The core is uniformly covered by the shell with particles of overall diameters of 20–40 nm and a shell thickness of about 3–5 nm.

The FE-SEM image (Figure 2a) of the Ag@TiO$_2$ composite nanocluster shows that particle diameter is about 30 nm. The presence of a metallic Ag core was confirmed by X-ray photoelectron spectroscopy (XPS). The peaks (Ag 3d$_{3/2}$ and Ag 3d$_{5/2}$) observed at 373.9 and 367.9 eV were ascribed to metallic silver (Figure 2b). An XPS analysis result of TiO$_2$ is shown in Figure 2c. The peaks located at 464.2 and 458.5 eV are assigned to Ti 2p$_{1/2}$ and Ti 2p$_{3/2}$, respectively. The splitting width between Ti 2p$_{1/2}$ and Ti 2p$_{3/2}$ is 5.7 eV, indicating a normal state of Ti^{4+} in the as-prepared mesoporous TiO$_2$.

The energy-dispersive X-ray (EDX) spectrum of the Ag@TiO$_2$ composite nanocluster (Figure 3a) indicates the presence of Ag, Ti, and O. The wide-angle X-ray diffraction (XRD) patterns of Ag@TiO$_2$ and bare TiO$_2$ (P25) particles are shown in Figure 3b. The presence of peaks (planes) at 38.09° (111), 44.28° (200), 64.42° (220), and 77.36° (311) and the absence of silver oxide peaks clearly confirm that Ag is in metallic form. It is very difficult to identify TiO$_2$ in the XRD analysis. This may be due to the low amount of TiO$_2$ (shell thickness: about 3–5 nm) in the Ag@TiO$_2$ nanocluster. However, a typical anatase peak of TiO$_2$ observed at 38.59° may overlap with the Ag (111) plane (Figure 3b).

The cell dishes were incubated for 24 h with different amounts of MEM containing Ag@TiO$_2$ composite nanocluster solutions (4, 8, 12, 16, and 20 µL), and another dish without composite nanocluster solution (control dish) was also incubated for 24 h (Figure 4). The living cells were counted and calculated as the percentage of the number of the living cells against the control dish living cells under a 10× bright field microscope.

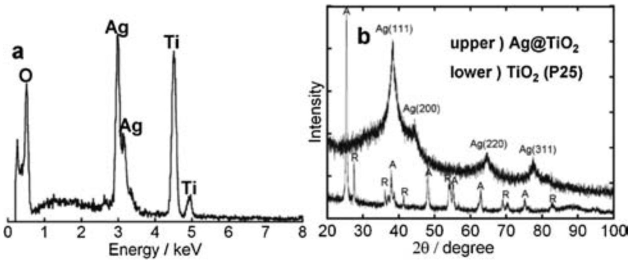

Figure 3. (a) The EDX spectrum of Ag@TiO$_2$. (b) XRD patterns of Ag@TiO$_2$ and TiO$_2$ (P25). A: anatase, R: rutile. The Ag:TiO$_2$ molar ratio is 1:7 in (a) and (b).

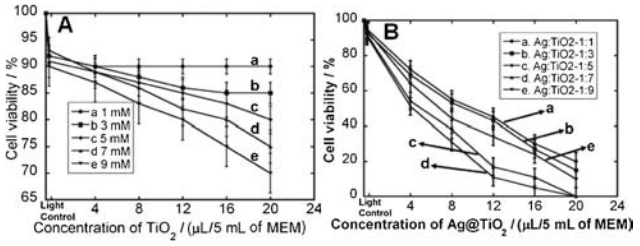

Figure 4. Surviving fractions of HeLa cells in the presence of (A) TiO$_2$ (P25) and (B) Ag@TiO$_2$ composite nanocluster colloid.

Figure 4A shows the TiO$_2$ concentration (1–9 mM) dependence of the cell viability of MEM solution treated HeLa cells under incubated conditions for 24 h. The viability under 5-min light irradiation without TiO$_2$ (light control) is 90–95%. A maximum of 30% of the cells were killed after 5-min irradiation with 20 µL of solution at 9 mM TiO$_2$ concentration in 5 mL of MEM (Figure 4Ae).

The cytotoxicity of Ag@TiO$_2$ nanoclusters depends on the Ag:TiO$_2$ molar ratio as shown in Figure 4B. Under 5-min light irradiation, almost 100% of the cells were killed in the presence of 20 µL of nanocluster Ag@TiO$_2$ (1:5 and 1:7) composite colloids (Figures 4Bc and 4Bd).

The Ag@TiO$_2$ composite nanoclusters greatly enhanced the cytotoxicity over TiO$_2$ nanoparticles. Therefore, it is reasonable to strongly suggest that the photothermal toxicity due to the plasmonic absorption of Ag nanoparticles also strongly contributes to cytotoxicity because Au nanoparticles, a noble metal, are known to show strong photothermal cytotoxicity.[10]

This research was partly supported by Grant-in-Aid for Scientific Research (B) (No. 19360367) from Japan Society for the Promotion of Science (JSPS).

References

1 P. V. Kamat, *Chem. Rev.* **1993**, *93*, 267.
2 A. L. Linsebigler, G. Lu, J. T. Yates, Jr., *Chem. Rev.* **1995**, *95*, 735.
3 P. V. Kamat, *J. Phys. Chem. B* **2002**, *106*, 7729.
4 T. Hirakawa, P. V. Kamat, *J. Am. Chem. Soc.* **2005**, *127*, 3928.
5 I. Pastoriza-Santos, D. S. Koktysh, A. A. Mamedov, M. Giersig, N. A. Kotov, L. M. Liz-Marzán, *Langmuir* **2000**, *16*, 2731.
6 M. R. Elahifard, S. Rahimnejad, S. Haghighi, M. R. Gholami, *J. Am. Chem. Soc.* **2007**, *129*, 9552.
7 C. Hu, Y. Lan, J. Qu, X. Hu, A. Wang, *J. Phys. Chem. B* **2006**, *110*, 4066.
8 M. Sökmen, F. Candan, Z. Sümer, *J. Photochem. Photobiol., A* **2001**, *143*, 241.
9 K. Awazu, M. Fujimaki, C. Rockstuhl, J. Tominaga, H. Murakami, Y. Ohki, N. Yoshida, T. Watanabe, *J. Am. Chem. Soc.* **2008**, *130*, 1676.
10 M. Abdulla-Al-Mamun, Y. Kusumoto, A. Mihata, M. S. Islam, B. Ahmmad, *Photochem. Photobiol. Sci.* **2009**, *8*, 1125.

Published on the web (Advance View) September 12, 2009; doi:10.1246/cl.2009.980