New Simple Synthesis of Cu–TiO_2 Nanocomposite: Highly Enhanced Photocatalytic Killing of Epithelia Carcinoma (HeLa) Cells

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Cu–TiO_2 nanocomposites with different Cu/TiO_2 molar ratios were synthesized by a new simple chemical reduction method. The morphological structure and the surface species of Cu–TiO_2 nanocomposites were determined by using UV–vis absorption spectroscopy, TEM, XPS, XRD, and EDX techniques. The photocatalytic cytotoxicity of Cu–TiO_2 nanocomposites was investigated under UV–vis light irradiation. The cytotoxicity depends on the molar ratio of Cu to TiO_2, ranging from 0.2 to 5.

Titanium dioxide has been broadly studied for photocatalytic activity, and many functional materials on TiO_2 surfaces have been developed for maximizing the efficiency of photocatalytic reactions.[1,2] A variety of nanocomposite semiconductor materials have been synthesized in recent years to improve the selectivity and efficiency of photocatalytic processes. The functional properties of such materials can be greatly improved by capping a semiconductor or a metal nanocluster with another layer of a compatible material.[3,4] The deposition of a noble metal on semiconductor nanoparticles is an essential factor for maximizing the efficiency of photocatalytic reactions. The noble metal (e.g., Pt), acting as a sink for photoinduced charge carriers promotes interfacial charge-transfer processes.[1] Metal oxide-supported copper nanoparticles are employed as highly active heterogeneous catalysts for the photodegradation of organic pollutants.[5] However, some studies have suggested that observed enhancement of the photocatalytic degradation rates was due to electron trapping by copper ions leading to the prevention of electron–hole recombination.[6]

In the present study, we deal with the preparation and characterization of Cu–TiO_2 nanocomposites and their successful utilization for photocatalytic cancer cell killing under UV–vis light irradiation. Moreover, the advantage of Cu nanoparticles as the promoter for the TiO_2 photocatalyst is reported.

Generally Cu nanoparticles are chemically very reactive; they can be oxidized by direct contact with TiO_2 and can produce cuprous oxide (Cu_2O). For this problem to prevent oxidation we introduce a very simple new method to prepare a Cu–TiO_2 nanocomposite by the borohydride reduction of copper nitrate salt in acetonitrile–water mixed solvent under Ar-purged inert atmosphere. The synthesis method was as follows: The surface of commercially available TiO_2 (P25) (Degussa Corp., diameter ca. 20 nm, surface area 49.9 $m^2\,g^{-1}$) was modified by using hydrochloric acid. The modified TiO_2 colloidal suspension (0.01 M (1 M = 1 mol dm^{-3})) was prepared by dropwise addition of 0.001 M hydrochloric acid until the pH rose to ca. 3 in 70 vol % acetonitrile–water mixture under vigorous stirring. Then copper(II) nitrate ($Cu(NO_3)_2 \cdot 3H_2O$) salt (0.002–0.05 M) was mixed in solution under Ar atmosphere in a rubber septum-capped Pyrex conical flask of ca. 154 cm^3, maintaining the reaction mixture in suspension by magnetic stirring. Under vigorous stirring, freshly prepared sodium borohydride (0.15 M) was injected drop by drop in the reaction medium via a syringe. The injection of the borohydride solution was continued (2 to 8 mL) until the color of the reaction medium turned wine-reddish. After completion of reduction, the suspension was centrifuged and the particles were separated, followed by washing with methanol and drying in a vacuum. The application methods to the cancer-killing were as follows: HeLa cells were cultured in minimum essential medium (MEM) solution with 10% newborn calf serum (NBS) in a humidified incubator under an atmosphere of 5% CO_2 in air at 37 °C. The 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 30 mW cm^{-2} was used for the light irradiation on HeLa cells. 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.

The UV–vis absorption spectra of Cu–TiO_2 nanocomposite suspensions show a strong absorption in the visible region with plasmonic peaks in Figure 1A. The plasmonic peaks indicate that the Cu shell is not oxidized. The lower ratios of Cu:TiO_2 resulted in the obscurity of the surface plasmon band due to increase in excessive oxygen on the Cu nanoparticles surface (Figure 1A, spectrum a). At higher ratios of Cu:TiO_2 the plasmon bands of Cu nanoparticles result in increase in the absorbance and broadening due to increase in the particle size (Figures 1Ad and 1Ae). The results (Figure 1A) indicate that by increasing the Cu content, the plasmon absorption of the Cu–TiO_2 nanocomposite increases with increasing Cu molar ratios. The TEM image (Figure 1B) shows that the Cu nanoparticles (white spot) are fair-

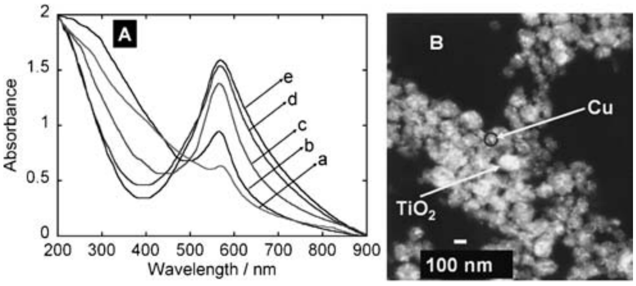

Figure 1. (A) Absorption spectra of Cu–TiO_2 various colloidal suspensions in water–acetonitrile solution. The Cu:TiO_2 molar ratios were maintained at (a) 0.2:1, (b) 0.5:1, (c) 1:1, (d) 2:1, and (e) 5:1. (B) The transmission electron microscope picture of Cu–TiO_2 nanocomposites. Cu nanoparticles (white spot) composited with TiO_2 particles are shown in the TEM image (Cu:TiO_2 = 1:1).

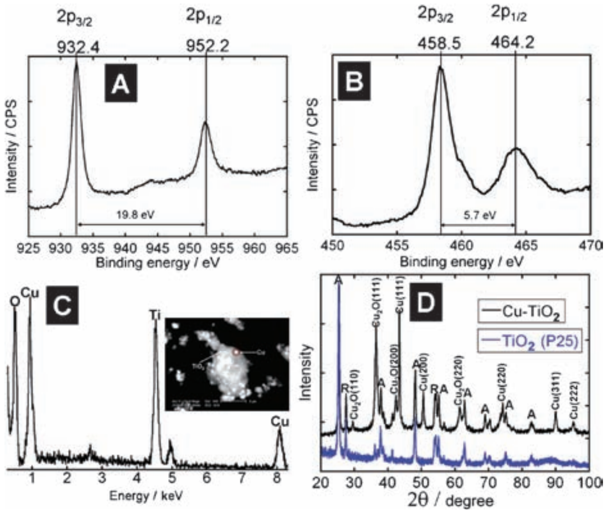

Figure 2. The X-ray photoelectron spectroscopic scan survey in the region of (A) Cu 2p (B) Ti 2p of the as-prepared Cu–TiO_2 nanocomposite. (C) The EDX spectrum of Cu–TiO_2. The strong Cu and Ti signals in the EDX spectrum indicate that Cu is metallic and Ti consists of mixed oxides. (Inset) The cold-field SEM image shows Cu on the TiO_2 surface. (D) XRD pattern of Cu–TiO_2 nanocomposite, confirming the formation of pure metallic copper–TiO_2 semiconductor nanocomposite with some partial Cu_2O. The Cu:TiO_2 molar ratio is 1:1.

ly well dispersed on the TiO_2 surface with particles of overall diameters of 10–100 nm (see also the inset in Figure 2C).

The peaks observed $2p_{3/2}$ and $2p_{1/2}$ at 932.4 and 952.2 eV were ascribed to the metallic copper (Figure 2A). The peak located at 464.2 eV corresponds to the Ti $2p_{1/2}$, and another one located at 458.5 eV is assigned to Ti $2p_{3/2}$. The splitting between Ti $2p_{1/2}$ and Ti $2p_{3/2}$ is 5.7 eV, indicating a normal state of Ti^{4+} in the mesoporous TiO_2 (Figure 2B). The EDX spectrum of Cu–TiO_2 nanocomposites (Figure 2C) indicates the presence of Cu, Ti, and O. The wide-angle X-ray diffraction (XRD) patterns of Cu–TiO_2 and bare TiO_2 (P25) particles are shown in Figure 2D. Compared with the bare TiO_2, the Cu–TiO_2 nanocomposite exhibits new peaks at $2\theta = 43.473$, 50.375, 73.997, 89.934, and 95.578 which can be attributed to the diffraction peaks of (111), (200), (220), (311), and (222) planes of polycrystalline Cu (JCPDS, PDF, File No. 00-001-1241), respectively, demonstrating the formation of the metallic Cu.

The cell dishes were incubated for 24 h with different amounts of MEM containing Cu–TiO_2 nanocomposite solutions (4, 8, 12, 16, and 20 µL), and another dish without nanocomposite solution (controll dish) was also incubated for 24 h (Figure 3). Under 5-min light irradiation, almost 100% of the cells were killed in the presence of 20 µL Cu–TiO_2 (1:1) nanocomposite colloid (Figure 3).

In the lower molar ratio region (ratio $<$ 1:1), some Cu_2O particles may be formed and may cover the surface of TiO_2, which may lead to decrease in the photocatalytic cytotoxicity. At the optimum ratio (1:1), the photothermal killing due to the plasmonic absorption (Figure 1A) of Cu nanoparticles may also contribute to the cytotoxicity. However, in the higher molar ratio region (ratio $>$ 1:1), excessive Cu nanoparticles obstruct the light absorption by TiO_2, which may also lead to decrease in the photocatalytic cell killing.

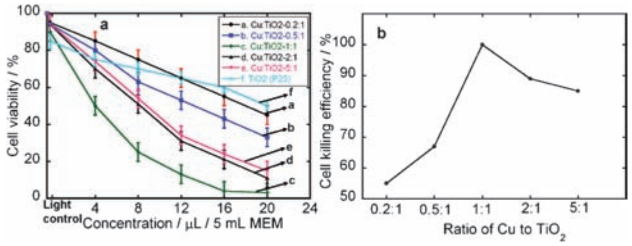

Figure 3. Surviving fractions of HeLa cells in the presence of Cu–TiO_2 nanocomposite colloid. (a) Cell viability in different molar ratios of Cu:TiO_2 and only TiO_2. (b) 100% cells were killed at a 1:1 ratio of Cu:TiO_2 in the presence of 20 µL Cu–TiO_2 colloid solution.

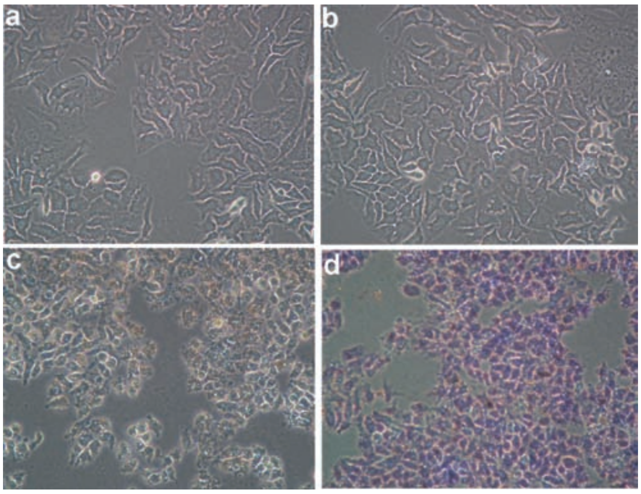

Figure 4. Microscopic images of HeLa cells (Magnification 200). (a) With neither Cu–TiO_2 nanocomposites nor light. (b) After 5-min light irradiation without the nanocomposites. (c) After 5-min light irradiation with the nanocomposite. (d) Dead cells stained with trypan blue.

Figure 4 shows microscopic images of HeLa cells before (a) and after (b)–(d) light irradiation. Figures 4a and 4b show that the cell morphology is largely unchanged after 5-min irradiation. Figure 4c shows that the HeLa cells suffer severe photochemical injury under 5-min irradiation with the Cu–TiO_2 nanocomposite. After light irradiation, cells were stained with trypan blue; cells stained blue (Figure 4d) indicated cell death.

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