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Killing of cancer cell line by photoexcitation of folic acid-modified titanium dioxide nanoparticles

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ABSTRACT

Folic acid (FA) was coupled onto titanium dioxide nanoparticles to provide a specificity of cell targeting. Modification was confirmed by infrared absorption spectra and zeta potential that suggested a formation of linkage between carboxylic acid of FA and titanium dioxide. The surface attachment of folic acid did not cause a significantly change in particles size, but led to a reduction of photocatalytic activity of TiO_2. The FA-modified TiO_2 nanoparticles could be internalized by cells at a much faster rate than the unmodified TiO_2, due to the mediation of folate receptor on the cancer cells. The UV irradiation caused death of HeLa cells pretreated with FA-modified TiO_2 more effectively than that of HeLa cells treated with unmodified TiO_2. Results suggest that to modify TiO_2 with folic acid using appropriately the FA-to-TiO_2 mass ratio of 0.2 could yield nanoparticles having higher cytotoxicity under photoexcitation. Photocatalytic TiO_2 nanoparticles could not only damage to cellular components including plasma membranes leading to cell nucrosis but also induce the programming cell death. Results from flow cytometry-based analysis indicated that the mechanism of cell death was a combination of necrosis and apoptosis.

1. Introduction

The photocatalysis of titanium dioxide (TiO_2) has been investigated for decades since Fujishima and Honda published their findings in 1972 [1]. Upon UV radiation, TiO_2 particles can generate active free radicals ($OH^\bullet$ and O_2^-) that are responsible of decomposing organics on the particle surface. Because of this photocatalytic property, titanium dioxide has been widely used for decomposition of organic pollutants [2–5]. In addition to the finding by Matsunaga et al. that microbial cells can be killed by the photocatalysis of titanium dioxide [6,7], numerous papers have been published on using this semiconductor photocatalyst for disinfection and killing of bacteria, viruses, fungi, and even cancer cells [8,9]. Although the biocide activity and induced cytotoxicity by photoexcited TiO_2 particles is well reported, the mechanism underlying the cell-killing process remains largely unknown. On the bactericidal activity, Maness et al. proposed that TiO_2 promotes peroxidation of the polyunsaturated phospholipid component of the lipid membrane and induce a major disorder in the *Escherichia coli* cell membrane, which subsequently results in the loss of cell viability [10]. Since TiO_2 particles are subject to uptake by cell via phagocytosis, cells are damaged also from the inside of cytoplasm [11]. In the present paper, we attempt to reveal the mechanism and cell-killing effect of photoexcited TiO_2 nanoparticles modified with folic acid.

Folic acid (FA) has been used for drug targeting to cancer cells since the folate receptor is significantly overexpressed on the surface of human cancer cells [12,13]. The folate receptor-mediated drug delivery is based on the conjugation of drug with folic acid, which is internalized by folate receptor-mediated endocytosis. Furthermore, the attachment of drug to folic acid does not normally interferer with the binding of folate for its receptor. Folic acid has been immobilized on superparamagnetic magnetite [14], polymer nanoparticles [15], or incorporated to a dendrimer-based therapeutic nanodevice [16] for tumor cell-selective targeting. In the present study, folic acid was coupled on the surface of TiO_2 for the selective binding to cancer cells.

2. Experimental

2.1. Modification of TiO_2 with folic acid

The TiO_2 used in this study was Degussa P-25, obtained as a gift from Degussa, Taiwan Branch. A specified amount of folic acid (Sigma) was dissolved in 0.1 M sodium hydrogen carbonate ($NaHCO_3$) solution adjusted to pH 5.5 with HCl and NaOH. TiO_2 nanoparticles were dispersed in deionized water to a concentration of 0.1 g/ml by sonication for 10 min. The average particle diameter of TiO_2 prior to modification was estimated to be ca. 28 nm on the basis

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of TEM image. The hydrodynamic diameter for Degussa P-25 TiO_2, however, was subject to change by the dose concentration in the culture medium as indicated in the literature [17]. The TiO_2 dispersion then added slowly to a folic acid solution with a volume ratio of 1:9, and the resultant mixture was stirred for reaction for one day. The reaction mixture was finally dialyzed against 1 mM $NaHCO_3$ solution for one day to remove unreacted folic acid, yielding folic acid-modified TiO_2 particles. It is noted that the whole process for the preparation and preservation of modified TiO_2 should be kept in dark conditions.

The photocatalytic activity was characterized by the rate constant for the degradation of methylene blue (Riedel-deHaën) [18]. The rate constant (k, s^{-1}) was evaluated from the plot of $\ln(C_{A0}/C_A)$ vs. irradiation time (t), where C_{A0} and C_A are the concentrations of methylene blue at the initial time and at time t, respectively. The concentration of methylene blue was determined by evaluating the absorbance at 664 nm.

The zeta potential of unmodified and folic acid-modified TiO_2 was determined using Malvern Zetasizer-3000 and samples were buffered with 1 mM NaCl and adjusted to different pH values with NaOH and HCl. For FT-IR analysis, the sample was mixed with KBr (1:10, w/w), pressed into a pellet and placed into the infrared spectrometer. Unmodified and folic acid-modified TiO_2 nanoparticles were also characterized by transmission electron microscopy (TEM) on Hitachi H600.

2.2. Cellular uptake of unmodified and FA-modified TiO_2

HeLa cells were cultured in the MEM medium containing non-essential amino acids solution, sodium pyruvate, and Penicillin/Fungizone/Streptomycin (all medium components were obtained from Sigma). Cells were seeded on a 35 mm dish (1×10^6/dish) for one day for cell adhesion. A dispersion of unmodified or folic acid-modified TiO_2 (200 μg/ml in MEM medium) was then applied to the HeLa cells for culture for 5 min, 30 min, 1 h, 2 h or 6 h. The uptake of TiO_2 by cells was characterized by using flow cytometry to evaluate the intensity of side angle scatter (SSC). Chinese hamster ovary (CHO) cells, as the control of non-folate receptor expression cells [19], were also cultured with TiO_2 particles for 30 min and cellular uptake of TiO_2 particles was estimated by the same method for comparison.

2.3. Photokilling study

Cells were seeded onto a 24-well multiplate (8×10^4/well) for culture for one day. After washing with PBS buffer, cells were incubated with a dispersion of TiO_2 in MEM medium for 24 h or folic acid-modified TiO_2 for 30 min for cellular uptake. After a wash, cells were mixed with 0.5 ml PBS and irradiated with UV at a distance of 1 cm from the 100 W long-wave ultraviolet lamp (Blak-Ray model B 100AP; UVP, Upland, CA) for 5 min, 15 min or 30 min. Cells were then subjected to viability assay.

The cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method, which was based on measuring the activity of living cells via mitochondrial dehydrogenases. Cells were washed with PBS buffer and then incubated with MTT (Sigma) solution (0.5 mg MTT/ml in PBS) at 37 °C for 4 h. After incubation, an aliquot of MTT solubilization solution (10% Triton X-100 plus 0.1 N HCl in anhydrous isopropanol) was added to dissolve the resulted formazan crystals. Afterward, the product was quantified by measuring absorbance at 570 nm. The MTT stock solution (0.5 mg MTT/ml PBS) was stored at 4 °C in dark not longer than 2 weeks and filtered with a 0.22 μm filter prior to use. The cell survival was defined as the ratio of the viability of treated cells and that of non-treated control (neither TiO_2 treatment nor UV illumination).

Cells after incubation with TiO_2 and UV illumination were characterized by flow cytometry using Annexin-V-FITC and propidium iodide (PI) labeling. The staining solution was prepared by mixing 20 μl Annexin-V-FITC labeling reagent and 20 μl PI into 1 ml Annexin V binding buffer (all from Strong Biotech Corp). Cells released from 35 mm dish by using trypsin-EDTA were mixed with 3× MEM medium, centrifuged, and washed with PBS buffer. The cell pallet was then suspended in 100 μl of the staining solution and the resulting suspension was allowed for incubation for 10–15 min in dark at the room temperature. Finally, the stained cell suspension was added with 0.8 ml PBS buffer and subjected to flow cytometry analysis.

3. Results

3.1. Modification of TiO_2 with folic acid

Folic acid is a water-soluble vitamin B9 and has a pI value of 5.3. Since the pI value of TiO_2 is ca. 6, the modification of folic acid on TiO_2 was then carried out at a pH between 5.3 and 6 in order to enhance the interaction between folic acid and TiO_2. The conjugation of folic acid and TiO_2 was achieved simply by incubation at pH 5.5 in dark conditions for 1 day. As expected, the zeta potential of TiO_2 decreased with pH and its value switched from positive to negative around pH 6, as shown in Fig. 1. When an FA-to-TiO_2 mass ratio of 1 was used, the zeta potential curve for modified TiO_2 particles shifted significantly to the left. Increasing the FA-to-TiO_2 ratio to 5, the left shift of zeta potential curve was more severe. If the FA-to-TiO_2 ratio increased to 10.3, however, the curve was not very different from that for the FA-to-TiO_2 ratio of 5. These results suggest that there was a saturation of functionality on TiO_2 for complexion with folic acid.

Infrared absorption spectra of unmodified and folic acid-modified TiO_2 are shown in Fig. 2. The characteristic peak around 1637 cm^{-1} for TiO2, which is the characteristic peak for banding vibration of water molecule adsorbed on TiO_2, disappeared after the modification with folic acid. Also, the stretching vibration of OH groups of water molecules adsorbed on the unmodified TiO_2 was observed. The water molecules adsorbed on the (1 1 1) face of anatase TiO_2 containing five coordinate Ti(IV) atoms characterize an IR peak at ~3200 cm^{-1} [20]. But this wide and strong peak was smoothed out after coupling with folic acid. After the adsorption of folic acid, the appearance of bands due to asymmetric and symmetric stretching vibrations of carboxylate salt (–COOM) peaks (1512 cm^{-1} and 1440 cm^{-1}) suggested a formation of linkage between carboxylic acid of FA and titanium atom. The free car-

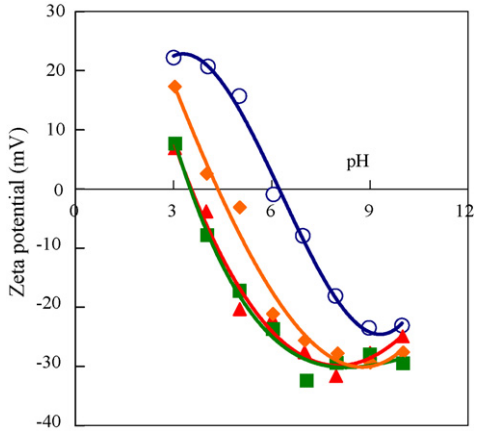

Fig. 1. Zeta potential of unmodified (○) and modified titanium dioxide with folic acid using FA-to-TiO_2 ratios of 1 (♦), 5 (■), and 10.3 (▲).

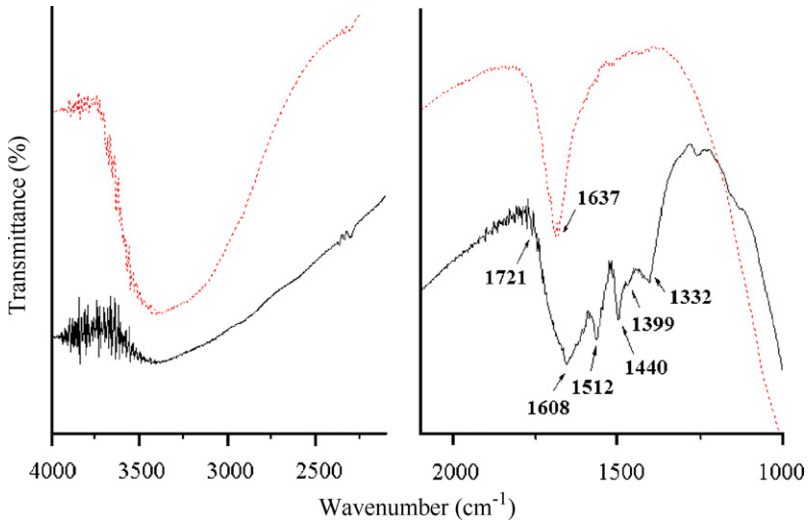

Fig. 2. FT-IR spectra of titanium dioxide (dashed curve) and FA-modified titanium dioxide (solid curve), prepared by using a FA-to-TiO_2 ratio of 1.

boxylic acid groups on FA characterized by a C=O stretch between 1680 cm^{-1} and 1725 cm^{-1} was also observed.

Fig. 3 displays TEM images of unmodified and folic acid-modified TiO_2. These pictures indicate that all the TiO_2 particles were in the nano-sized range. When the FA-to-TiO_2 mass ratio was 0.5, particle size was not changed by the folic acid modification. The average particle size remained at ca. 30 nm. When the FA-to-TiO_2 ratio increased to 5, however, the particle size increased very slightly and most importantly particles became slightly foggy due to the presence of folic acid.

The photocatalytic activity was significantly changed by the modification of folic acid. Methylene blue solution was used for the determination of TiO_2 activities before and after the coupling of folic acid. Based on first 300 s data, the rate constants were calculated to be 0.0073 s^{-1}, 0.0044 s^{-1} and 0.002 s^{-1} for folic acid-modified TiO_2 with FA-to-TiO_2 ratios of 0.2, 0.5 and 1, respectively. In comparison with unmodified TiO_2 having a rate constant (k) of 0.0104 s^{-1}, the conjugation of folic acid led to the decrease of photocatalytic activity of TiO_2. The binding of folic acid apparently obstructed the access of methylene blue to TiO_2.

3.2. Uptake of TiO_2 by HeLa and CHO cells

A recent report suggested that the uptake of TiO_2 nanoparticles by cells could be quantitatively measured by using flow cytometric light scatter [21]. The intensity of the side-scattered light (SSC) increased with the amount of particles taken up by the cells. Fig. 4 shows side angle scatter histograms for cells incubated with unmodified and FA-modified TiO_2. The SSC intensity was minimum when cells were not treated with any nanoparticles as the control. The change of SSC became significant after the cells were treated with TiO_2 nanoparticles for 30 min. The longer the period for treatment, the larger the SSC intensity due to the increasing uptake of TiO_2 nanoparticles by cells. Like the unmodified TiO_2, folic acid-modified TiO_2 nanoparticles could also be internalized by cells but at a much higher uptake rate. A 30 min treatment of FA–TiO_2 nanoparticles could lead to a saturation of cellular uptake. A longer treatment time (1 or 2 h) could not further increase the cellular uptake of FA–TiO_2 nanoparticles, as shown in Fig. 4. However, when CHO cells were treated with unmodified and FA-modified TiO_2 nanoparticles for 30 min, there was no big differences between the amounts of these two kinds of nanoparticles internalized into cells.

3.3. Treating HeLa cells with photoexcited TiO_2 modified with folic acid

Unmodified TiO_2 particles were first tested for cytotoxicity on HeLa cells. The cytotoxicity of unmodified TiO_2 particles (without UVA irradiation) was determined by exposing cells to various concentrations of TiO_2 in MEM medium for 24 h. Results indicate that there was no significant loss of cell survival if the incubated concentration of TiO_2 was 150 μg/ml or below. As the TiO_2 concentration increased to 200 μg/ml, the survival fraction of the cells remained at ca. 90% (n = 4). These results confirmed that nonirradiated TiO_2 nanoparticles were not toxic to HeLa cells.

Fig. 5 shows the cell death due to the TiO_2-induced damage by exposure to UVA radiation. When cells were exposed to UVA in the absence of TiO_2, 5 min illumination led to a decrease in cell survival to 78%. The cell viability decreased further to 70% and 37% when the UVA radiation time was extended to 15 min and 30 min, respectively. If the HeLa cells were pre-incubated with TiO_2 (200 μg/ml in MEM medium) for 30 min and then exposed to UVA illumination, cell survival decreased severely with UV-exposure time for every cases. As shown in Fig. 5, modified TiO_2 particles prepared using an FA-to-TiO_2 ratio of 0.2 could cause worse damage to cells than other modified and unmodified TiO_2 particles. However, the higher the ratio of FA-to-TiO_2 the lower the photokilling effect when the modified particles were prepared with an FA-to-TiO_2 ratio greater than 0.2. Folic acid-modified TiO_2 with the FA-to-TiO_2 ratio less than one could cause cell death more effectively than the unmodified TiO2. For a 30 min exposure to UVA, those modified TiO_2 nanoparticles led cell survivals below 15%. When the modified particles prepared with an FA-to-TiO_2 ratio of 5, the cell killing effect was lower than the use of unmodified TiO_2.

3.4. Mechanism of cell killing

HeLa cells after pre-incubation with modified TiO_2 particles (FA-to-TiO_2 ratio = 0.2) and exposure to UVA for 5 min were cultured in the CO_2 incubator for 6 h, 12 h, or 24 h, and then examined for cell-death mechanisms using flow cytometric method. Cells that were considered viable were both FITC Annexin V and PI negative while cells that were in early apoptosis were FITC Annexin V positive and PI negative. Cells that stained positive for both FITC Annexin V and PI were considered necrotic. Results as shown in Fig. 6 revealed that about 60% of dead cells were attributed to apoptosis and 40%

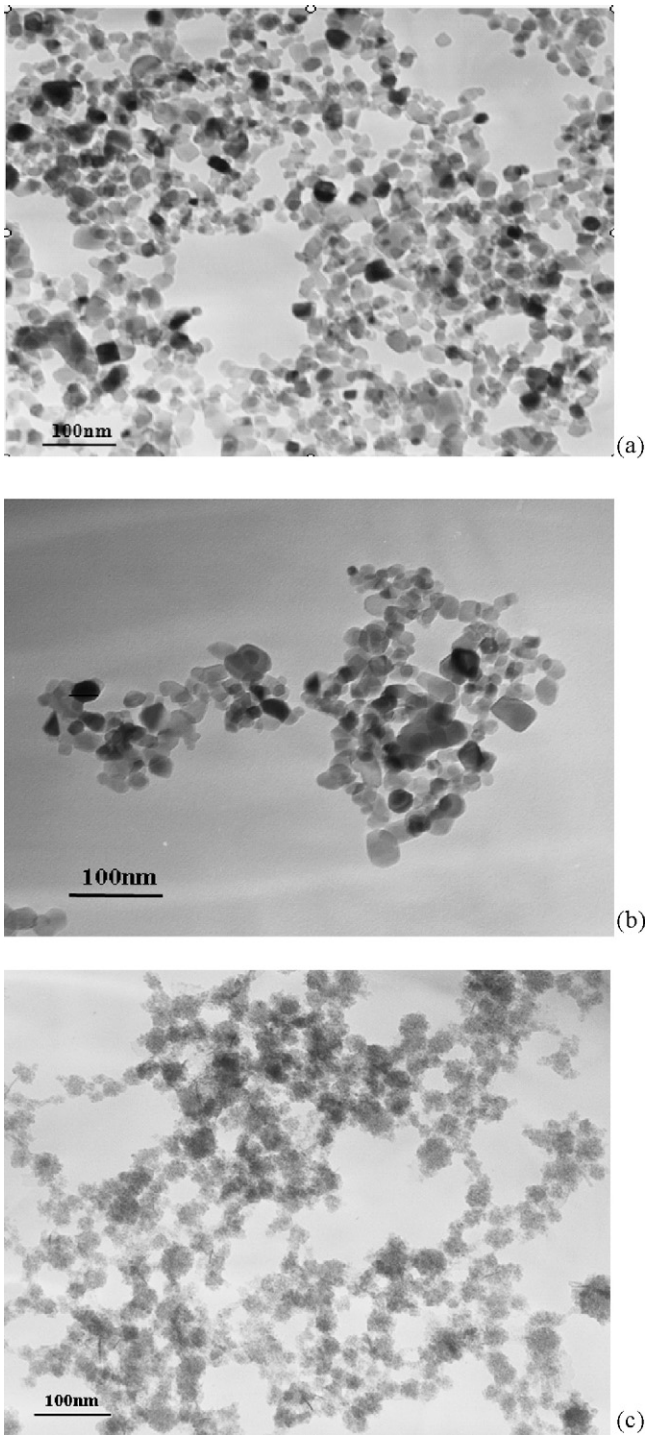

Fig. 3. TEM images of TiO_2 particles before (a) and after modification with FA using FA-to-TiO_2 ratios of 0.5 (b) and 5 (c).

of dead cells were caused by necrosis. The post-photokilling cultivation could reduce the numbers of both apoptotic and necrotic cells due to the recovery of some damaged cells. The rate of cell death caused by apoptosis decreased from 30% at 6 h to 20% at 24 h after photokilling; while the death rate by necrosis was 18% and 16%, respectively, for 6 h and 24 h of post-photokilling cultivation. The recovery of damaged cells from apoptosis was found to be more conspicuous. Among these dead cells, the faction of apoptotic cells decreased from 0.61 to 0.54 when the post-photokilling cultivation time increased from 6 h to 24 h.

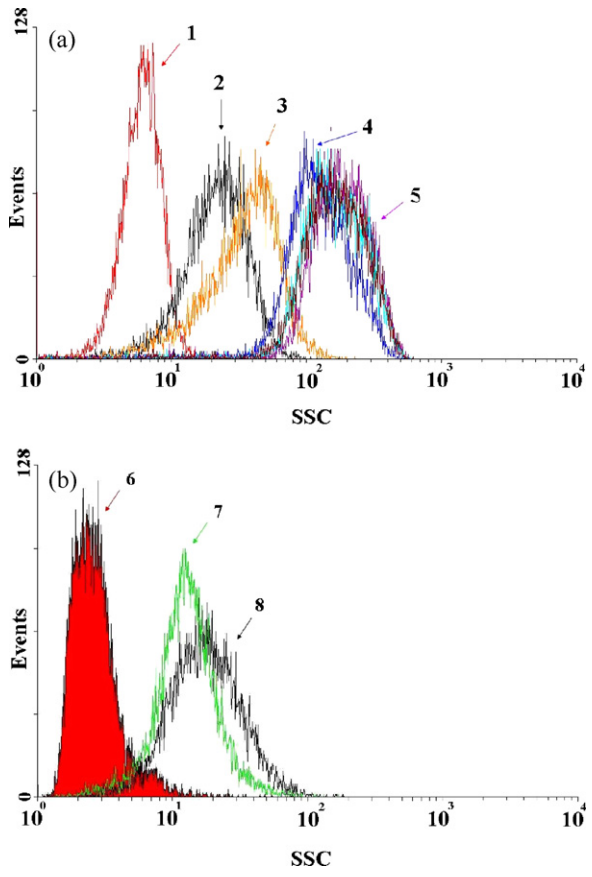

Fig. 4. Uptake of unmodified and folic acid-modified TiO_2 by HeLa (a) and CHO cells (b). HeLa cells were untreated (1), treated with unmodified TiO_2 for 30 min (2) or 6 h (4), or treated with FA–TiO_2 for 5 min (3) or 30 min and longer (1 h and 2 h) (5); CHO cells were untreated (6), treated with FA-TiO_2 (7) or unmodified TiO_2 (8) for 30 min. The FA–TiO_2 particles were prepared by using an FA-to-TiO_2 ratio of one.

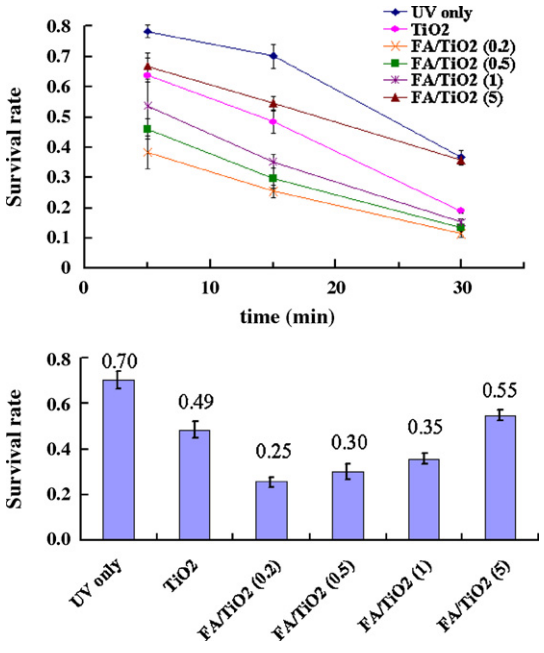

Fig. 5. Influence of folic acid modification on the photocatalytic killing of TiO_2 nanoparticles. HeLa cells were untreated, or treated with FA–TiO_2 or unmodified TiO_2 for 30 min before illumination with UVA radiation for different time periods (upper panel) and specifically for 15 min (lower panel). Data shown are the mean value ± SEM of at least three experiments.

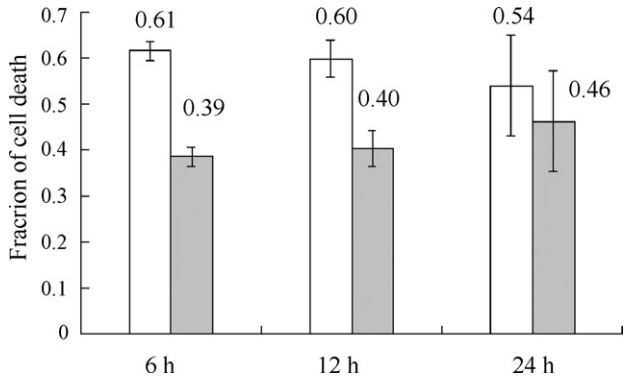

Fig. 6. Fractions of cell death caused by apoptosis (open) and necrosis (filled), determined by flow cytometry-based analysis. HeLa cells that had been treated with FA–TiO_2 (prepared with an FA-to-TiO_2 ratio of 0.2) for 30 min and illuminated with UVA for 5 min were cultured for 6 h, 12 h and 24 h before the flow cytometry analysis.

4. Discussion

The possibility of attaching a wide variety of biomolecules to TiO_2 nanoparticles without the loss of photocatalytic activity enables their use in biological and biomedical applications. Nanoscaled TiO_2 have been tried for the carrier of DNA delivery via the structure of TiO_2-dopamine-DNA nanocomposites [22]. In addition to dopamine, previous studies suggest that surface modification of cycteine [23], Vitamin C [24], ascorbic acid [25], salicylic acid [26], and a variety of bidentate ligands [20] was promising. The present study revealed that folic acid was able to form a stable linkage to photocatalytic TiO_2. As suggested by the IR spectra (Fig. 2), the conjugation was believed to occur by the interaction of carboxylate group of folic acid and OH group on the TiO_2. Since each folic acid molecule contains two carboxylate groups, there might be some free carboxylate groups on bound folic acid molecules that resulted in negatively charged properties of the FA-modified TiO_2, as revealed by Fig. 1.

This kind of linkage between folic acid and TiO_2 nanparticles was different from the coupling of folic acid onto other nanoparticels by a covalent manner. Magnetite nanoparticles were chemically modified with (3-aminopropyl)-trimethozysilane to introduce amino groups on their surface; the resultant amino group-containing nanoparticles were then covalently conjugated with folic acid by the activation of *N*-hydroxysuccinimide and 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide using triethylamine as the catalyst [14]. The amino-modified γ-Fe_2O_3 nanoparticles could also react to folic acid to form a covalent linkage in the presence of 1-hydroxy-1*H*-benzotriazole, benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate and *N*-ethyl-*N*-(1-methylethyl)-2-propanamine [27]. Folic acid could be modified to containing SH or *N*-hydroxysuccinimide group before forming a conjugate with protein or particle via covalent linkage [28]. In comparison with these approaches, the chemistry of surface modification on TiO_2 nanoparticles was much simpler.

The surface attachment of folic acid did not cause a significantly change in particles size, as shown by SEM images (Fig. 3). These results suggest that the modification was in the molecular level. Folic acid modification, however, led to a reduction of photocatalytic activity of TiO_2. Based on the rate constant for the degradation of methyl blue, the photocatalytic activity of FA-modified TiO_2 with FA-to-TiO_2 ratios of 0.2, 0.5 and 1 dropped respectively to 70%, 42% and 19% of that of unmodified TiO_2. This suggests that the presence of folic acid on TiO_2 can shield the degradable substrate from contact with photoexcited TiO_2 nanoparticles and a low FA-to-TiO_2 mass ratio is more suitable for biological application.

Although no TEM image was taken to show the distribution of the TiO_2 particles in cancer cells, results from the flow cytometric assay (Fig. 4) indicated that FA-modified TiO_2 could easily be incorporated to cells by endocytosis (phagocytosis). Most particles might be on the surface of cell membrane for the 5 min incubation. When the incubation time was extended to 30 mim, the endocytosis mediated by the folate receptor became significant. Once TiO_2 particles were cellular internalized, previous report showed that TiO_2 nanoparticles could be incorporated into the cytoplasm [21]. Using a flow cytometer, the amount of particles incorporated to the cells could be quantitatively characterized by the intensity of the side-scattered light (SSC). The exact amount of uptake into cells, however, remained unknown. In comparison with the cells treated with TiO_2 nanoparticles, HeLa cells treated with folic acid-modified TiO_2 nanoparticles for 30 min could possess a markedly high intensity of SSC, suggesting that modification of FA could boost uptake of nanoparticles by cancer cells.

The cell specifity of folic acid-modified TiO_2 nanoparticles was evident. Both unmodified and FA-modified TiO_2 nanoparticles were internalized equally well into CHO cells. On the contrary, modification of folic acid significalty promote the uptake of TiO_2 nanoparticles by HeLa cells, on which the folate receptors are overexpressed [29]. Results suggest that the folic acid modified TiO_2 is partically useful for the cancer therapy since most of the cancer cells are rich of folic acid receptor on their surface.

Several *in vitro* studies revealed that TiO_2 nanoparticles were non-toxic but have photocatalytic activity [11,30,31]. As shown in this work, without UV irradiation the surviving fraction of the HeLa cells treated with TiO_2 up to 200 μg/ml was not less than 90%. When the TiO_2-treated cells were exposed to the radiation of UVA, however, the cell survival rate was decreased dramatically as shown in Fig. 5. The survival rate for HeLa cells pretreated with unmodified TiO_2 decreased to 64%, 49% and 19% as the exposure time was 5, 15, and 30 min, respectively. This trends of survival decreasing with exposure time is similar to those in previous reports used a 500W UVA lamp to illuminate HeLa cells pretreated with unmodified TiO_2 [11,32]. The modification of folic acid on TiO_2 with a FA-to-TiO_2 ratio less than one could cause significantly worse cell damage under UV irradiation. The enhancement of cytotoxicity was due to the higher amount of TiO_2 uptake by cells via the recognition of FA to cell surface. By the specificity of FA and receptor on HeLa cells, modified TiO_2 nanoparticles were internalized much more quickly in comparison with unmodified TiO_2 (Fig. 4). However, the higher the amount of folic acid attached on TiO_2 (higher FA-to-TiO_2 ratio), the smaller the photocatalytic activity since the presence of folic acid could shield the illumination of UV light. As shown in Fig. 5, under UV illumination for 15 min, the survival of cells treated with FA-modified TiO_2 (the FA-to-TiO_2 ratio was 0.2) decreased to 25%, which was much lower than that (49%) of cells pretreated with unmodified TiO_2. As the FA-to-TiO_2 ratio increased to 0.5 and 1, the survival rate increased to 30% and 35%, respectively. However, if the modified TiO_2 nanoparticles prepared with a FA-to-TiO_2 ratio of 5 was used to treat HeLa cells, the survival was even higher than the treatment of unmodified TiO_2. These results suggest that a low FA-to-TiO_2 ratio was appropriate for the modification of TiO_2 and could lead to higher cytoxicity.

After FA-modified and unmodified TiO_2 were absorbed by cells, either in the interior of cells or on the cell surface, UV radiation induced the photocatalytic acitivty of TiO_2 nanoparticles and caused cell death. Photocatalytic TiO_2 nanoparticles could not only damage to cellular components including plasma membranes leading to cell nucrosis but also induce the programming cell death. Titanium dioxide photocatalyst can produce reactive oxygen species such as hydroxyl radical, superoxide anion, and hydrogen peroxide under UV illumination. These reactive oxygen species led to the degradation of multiple cellular components

including lipid membranes and eventually caused the cell death by necrosis, a nonspecific process resulting in cell lysis. In the killing process by photoexcited TiO_2, membrane blebbing, DNA fragmentation, DNA laddering, and increasing membrane permeability have been observed [9,30,33]. Some of these were considered to be the characteristics of apoptosis [30]. Previous studies suggested that the intracellular reactive oxygen species could induce apoptosis of neural cells. For example, apoptosis has been associated with increased generation of reactive oxygen species by direct exposure of PC12 cells to oxidative stress such as hydrogen peroxide and lipid hydroperoxide, which triggered apoptotic nuclear condensation and DNA fragmentation in normal PC12 cells [34,35]. Reactive oxygen species have been shown as downstream modulators of p53-dependent apoptosis [36]. Experimental results from the flow cytometry-based analysis, as shown in the present study, suggest that the cell death was most likely contributed by both necrosis and apoptosis.

Acknowledgments

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References

[1] A. Fujishima, K. Honda, Electrochemical photolysis of water at a semiconductor electrode, Nature 238 (1972) 37–38.
[2] J.A. Byrne, B.R. Eggins, N.M.D. Brown, B. McKinnery, M. Rouse, Immobilisation of TiO_2 powder for the treatment of polluted water, Appl. Catal. B Environ. 17 (1998) 25–36.
[3] K.-H. Wang, Y.-H. Hsieh, R.-C. Ko, C.-Y. Chang, Photocatalytic degradation of wastewater from manufactured fiber by titanium dioxide suspensions in aqueous solution, Environ. Int. 25 (1999) 671–676.
[4] S. Hager, R. Bauer, Heterogeneous photocatalytic oxidation of organics for air purification by near UV irradiated titanium dioxide, Chemosphere 38 (1999) 1549–1559.
[5] R. Franke, C. Franke, Model reactor for photocatalytic degradation of persistent chemicals in ponds and waste water, Chemosphere 39 (1999) 2651–2659.
[6] T. Matsunaga, R. Tomada, T. Nakajima, H. Wake, Photoelectrochemical sterilization of microbial cells by semiconductor powders, FEMS Microbiol. Lett. 29 (1985) 211–214.
[7] T. Matsunaga, R. Tomada, Y. Nakajima, N. Nakamura, T. Komine, Continuous-sterilization system that uses photosemiconductor powders, Appl. Environ. Microbiol. 54 (1998) 1330–1333.
[8] Y. Kubota, T. Shuin, C. Kawasaki, M. Hosaka, H. Kitamura, R. Cai, H. Sakai, K. Hashimoto, A. Fujishima, Photokilling of T-24 human bladder cancer cells with titanium dioxide, Br. J. Cancer 70 (1994) 1107–1111.
[9] D.M. Blake, P.-C. Maness, Z. Huang, E.J. Wolfrum, W.A. Jacoby, J. Huang, Application of the photocatalytic chemistry of titanium dioxide to disinfection and the killing of cancer cells, Sep. Purif. Methods. 28 (1999) 1–50.
[10] P.-C. Maness, S. Smolinski, D.M. Blake, Z. Huang, E.J. Wolfrum, W.A. Jacoby, Bactericidal activity of photocatalytic TiO(2) reaction: toward an understanding of its killing mechanism, Appl. Environ. Microbiol. 65 (1999) 4094–4098.
[11] R. Cai, K. Hashimoto, K. Itoh, Y. Kubota, A. Fujishima, Photokilling of malignant cells with ultrafine TiO_2 powder, Bull. Chem. Soc. Jpn. 64 (1991) 1268–1273.
[12] I.G. Campbell, T.A. Jones, W.D. Foulkes, J. Trowsdale, Folate-binding protein is a marker for ovarian cancer, Cancer Res. 51 (1991) 5329–5338.
[13] C.P. Leamon, P.S. Low, Folate-mediated targeting: from diagnostics to drug and gene delivery, Drug Discov. Today 6 (2001) 44–51.
[14] Y. Zhang, N. Kohler, M. Zhang, Surface modification of superparamagnetic magnetite nanoparticles and their intracellular uptake, Biomaterials 23 (2002) 1553–1561.
[15] B. Stella, S. Arpicco, M.T. Peracchia, D. Desmaele, J. Hoebake, M. Renoir, J. D'Angelo, L. Cattel, P. Couvreur, Design of folic acid-conjugated nanoparticles for drug targeting, J. Pharm. Sci. 89 (2000) 1452–1464.
[16] A. Quintana, E. Raczka, L. Piehler, I. Lee, A. Myc, I. Majoros, A.K. Patri, T. Thomas, J. Mule, J.R. Baker Jr., Design and function of a dendrimer-based therapeutic nanodevice targeted to tumor cells through the folate receptor, Pharm Res. 19 (2002) 1310–1316.
[17] T.C. Long, N. Saleh, R.D. Tilton, G.V. Lowry, B. Veronesi, Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): Implications for nanoparticle neurotoxicity, Environ. Sci. Technol. 40 (2006) 4346–4352.
[18] S. Lakshmi, R. Renganathan, S. Fujita, Study on TiO_2-mediated photocatalytic degradation of methylene Blue, J. Photochem. Photobiol. A: Chem. 88 (1995) 163–167.
[19] N. Viola-Villegas, A. Vortherms, R.P. Doyle, Targeting gallium to cancer cells through the folate receptor, Drug Target Insights 3 (2008) 13–25.
[20] T. Rajh, D.M. Tiede, M.C. Thurnauer, Surface modification of TiO_2 nanoparticles with bidentate ligands studied by EPR spectroscopy, J. Non-Crystal. Solids 205–207 (1996) 815–820.
[21] H. Suzuki, T. Toyooka, Y. Ibuki, Simple and easy method to evaluate uptake potential of nanoparticles in mammalian cells using a flow cytometric light scatter analysis, Environ. Sci. Technol. 41 (2007) 3018–3024.
[22] T. Paunesku, T. Rajh, G. Wiederrecht, J. Maser, S. Vogt, N. Stojicevic, M. Protic, B. Lai, J. Oryhon, M. Thurnauer, G. Woloschak, Biology of TiO_2-oligonucleotide nanocomposites, Nat. Mater. 2 (2003) 343–436.
[23] T. Rajh, A.E. Ostafin, O.I. Micic, D.M. Tiede, M.C. Thurnauer, Surface modification of small particle TiO_2 colloids with cysteine for enhanced photochemical reduction: an EPR study, J. Phys. Chem. 100 (1996) 4538–4545.
[24] T. Rajh, J.M. Nedeljkovic, L.X. Chen, O. Poluektov, M.C. Thurnauer, Improving optical and charge separation properties of nanocrystalline TiO_2 by surface modification with vitamin C, J. Phys. Chem. B 103 (1999) 3515–3519.
[25] T. Rajh, O. Poluektov, A.A. Dubinski, G. Wiederrecht, M.C. Thurnauer, A.D. Trifunac, Spin polarization mechanisms in early stages of photoinduced charge separation in surface-modified TiO_2 nanoparticles, J. Chem. Phys. Lett. 344 (2001) 31–39.
[26] S.X. Li, F.Y. Zheng, W.L. Cai, A.Q. Han, Y.K. Xie, Surface modification of nanometer size TiO_2 with salicylic acid for photocatalytic degradation of 4-nitrophenol, J. Hazard. Mater. B 135 (2006) 431–436.
[27] S. Taira, T. Hatanaka, Cellular recognition of functionalized with folic acid nanoparticles, e-J, Surf. Sci. Nanotech. 5 (2007) 23–28.
[28] S.F. Atkinson, T. Bettinger, L.W. Seymour, J.-P. Behr, C.M. Ward, Conjugation of folate via gelonin carbohydrate residue retains ribosomal inactivating properties of the toxin and permits targeting to folate receptor positive cells, J. Biol. Chem. 276 (2001) 27930–27935.
[29] R.J. Lee, P.S. Low, Folate-mediated tumor cell targeting of liposome-entrapped doxorubicin in vitro, Biochim. Biophys. Acta 1233 (1995) 134–144.
[30] N.-P. Huang, M.-H. Xu, C.-W. Yuan, R.-R. Yu, The study of the photokilling effect and mechanism of ultrafine TiO_2 particles on U937 cells, J. Photochem. Photobiol. A: Chem. 108 (1997) 229–233.
[31] A.-P. Zhang, Y.-P. Sun, Photocatalytic killing effect of TiO_2 nanoparticles on Ls-174-t human colon carcinoma cells, World. J. Gastroenterol. 10 (2004) 3191–3193.
[32] R. Cai, Y. Kubota, T. Shuin, H. Sakai, K. Hashimoto, A. Fujishima, Induction of cytotoxicity by photoexcited TiO_2 particles, Cancer Res. 52 (1992) 2346–2348.
[33] H. Sakai, E. Ito, E.-X. Cai, T. Yoshioka, Y. Kubota, K. Hashimoto, A. Fujishima, Intracellular Ca^{2+} concentration change of T24 cell under irradiation in the presence of TiO_2 ultrafine particles, Biochem. Biophys. Acta 1201 (1994) 259–265.
[34] H. Aoshima, T. Satoh, N. Sakai, M. Yamada, Y. Enokido, T. Ikeuchi, H. Hatanaka, Generation of free radicals during lipid hydroperoxide-triggered apoptosis in PC12 cells, Biochem. Biophys. Acta 1345 (1997) 35–42.
[35] T. Satoh, Y. Enokido, H. Aoshima, Y. Uchiyama, H. Hatanaka, Changes in mitochondrial membrane potential during oxidative stress-induced apoptosis in PC12 cells, J. Neurosci. Res. 50 (1997) 413–420.
[36] T.M. Johnson, Z.X. Yu, V.J. Ferrans, R.A. Lowenstein, T. Finkel, Reactive oxygen species are downstream mediators of p53-dependent apoptosis, Proc. Natl. Acad. Sci. USA 93 (1996) 11848–11852.