Photocatalytic killing effect of TiO$_2$ nanoparticles on Ls-174-t human colon carcinoma cells

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MATERIALS AND METHODS

**Reagent preparation**

TiO$_2$ colloid solutions were prepared$^{[13,14]}$ by hydrolysis of titanium isopropoxide, Ti[OCH(CH$_3$)$_2$]$_4$ (97%, Aldrich Chemical Co). In brief, 12.5 mL of Ti[OCH(CH$_3$)$_2$]$_4$ was added to 2 mL isopropanol, then the mixture was added to 150 mL of distilled deionized water containing 2 mL of 700 mL/L nitric acid and vigorously stirred for 6 h at 75 °C. Approximately 150 mL of TiO$_2$ colloid solution being stable for several months at 4 °C was obtained after the organic layer was removed. The average diameter determined by Zetasizer 3000HS (USA) was 21.2 nm.

The pH value of TiO$_2$ colloid solutions used in the subsequent experiment had to be adjusted from 1.8 to 5.5-6.5 in order not to damage the normal growth of cells. Therefore, 1 mol/L NaOH aqueous solution and 1.5 mL/L polyvinyl alcohol were added to the colloid solutions before the pH adjustment to prevent the TiO$_2$ from precipitation. The final TiO$_2$ colloid solutions were sterilized by autoclaving and then diluted to the required concentration. Other chemical reagents used were all of analytical purity from commercial sources.

**Cell culture and treatment**

Human colon carcinoma cell lines Ls-174-t were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences, Shanghai, China. The cell lines were cultured in RPMI 1640 (Gibco) medium supplemented with calf serum 100 mL/L, penicillin (100×10$^3$ U/L) and streptomycin (100 mg/L). pH was maintained at 7.2-7.4 by equilibration with 50 mL/L CO$_2$. Temperature was maintained at 37 °C. Cells were sub-cultured with a mixture of ethylenedinitrile tetraacetic acid (EDTA) and trypsin. All experiments were performed using cells during the exponential growth phase. Cell concentration was determined by using a hemocytometer and the cell density was adjusted to the required final concentration.

Ls-174-t cells were treated with TiO$_2$ diluted in RPMI 1640 medium for 2 h at 37 °C. Then the solutions were irradiated with a GGZ-300W high pressure Hg lamp (E$_{vis}$ = 365 nm) at room temperature. A UV pass filter was used to obtain a light wavelength between 300-400 nm. The light intensity at the liquid surface was measured by a VLX-3W radiometer-photometer (USA). The incident light intensity was 3.7 mW/cm$^2$. In our study, three groups of tests were carried out. One group was treated in the absence of TiO$_2$. Another group was treated in the absence of UVA. The third group was treated with different TiO$_2$ concentrations and irradiated by UVA.

**RESULTS:** The photocatalytic killing of Ls-174-t cells was carried out in vitro with TiO$_2$ nanoparticles. The killing effect was weak by using UVA irradiation without TiO$_2$ nanoparticles. In our studies, the photocatalytic killing effect was correlated with the concentration of TiO$_2$ and illumination time. Once TiO$_2$ was added, Ls-174-t cells were killed at a much higher rate. In the presence of 1 000 μg/mL TiO$_2$, 44% of cells were killed after 10 min of UVA irradiation, and 88% of cells were killed after 30 min of UVA irradiation.

**CONCLUSION:** When the concentration of TiO$_2$ is below 200 μg/mL, the photocatalytic killing effect on human colon carcinoma cells is almost the same as that of UVA irradiation alone. When the concentration of TiO$_2$ is above 200 μg/mL, the remarkable killing effect of photoexcited TiO$_2$ nanoparticles can be found.

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**INTRODUCTION**

The application of TiO$_2$ photocatalysis has received increasing attention since the first report of microbicidal effects by Matsunaga et al. in 1985$^{[1]}$. In recent years, in contrast to many studies using TiO$_2$ powder for photodecomposition of organic pollutants$^{[12]}$, few studies have investigated the application of TiO$_2$ in life science, especially in the field of cancer treatment$^{[10,12]}$. The incidence of colon cancer is rising in China. Despite that surgical operation is used currently, people have recognized its limitation. The way to treat cancer usually includes radiation therapy and chemical therapy, which may generate severe side effects in human body. Therefore, this study tried to investigate a new therapy for cancer. Ls-174-t cells were used as experiment objects in this study. The photocatalytic killing effect of TiO$_2$ nanoparticles on malignant cells and its killing mechanism were investigated.

**Abstract**

**AIM:** To investigate the photocatalytic killing effect of photoexcited TiO$_2$ nanoparticles on human colon carcinoma cell line (Ls-174-t) and to study the mechanism underlying the action of photoexcited TiO$_2$ nanoparticles on malignant cells.

METHODS: Ls-174-t human colon carcinoma cells were cultured in RPMI 1640 medium supplemented with 199 mL/L calf serum in a humidified incubator with an atmosphere of 50 mL/L CO$_2$ maintained at 7.2-7.4 by equilibration with 50 mL/L CO$_2$. Viable cells in the samples were measured by using the MTT method. A GGZ-300 W high pressure Hg lamp with a maximum ultraviolet-A (UVA, 320-400 nm) irradiation peak at 365 nm was used as light source in the photocatalytic killing test.

**RESULTS:** The photocatalytic killing of Ls-174-t cells was carried out in vitro with TiO$_2$ nanoparticles. The killing effect was weak by using UVA irradiation without TiO$_2$ nanoparticles. In our studies, the photocatalytic killing effect was correlated with the concentration of TiO$_2$ and illumination time. Once TiO$_2$ was added, Ls-174-t cells were killed at a much higher rate. In the presence of 1 000 μg/mL TiO$_2$, 44% of cells were killed after 10 min of UVA irradiation, and 88% of cells were killed after 30 min of UVA irradiation.

**CONCLUSION:** When the concentration of TiO$_2$ is below 200 μg/mL, the photocatalytic killing effect on human colon carcinoma cells is almost the same as that of UVA irradiation alone. When the concentration of TiO$_2$ is above 200 μg/mL, the remarkable killing effect of photoexcited TiO$_2$ nanoparticles can be found.

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Measurement of the viability of Ls-174-t cells
Viable cells in the samples were measured by using the MTT staining method. MTT [3-(4, 5-dimethylthiazol-2-yl) -2, 5-diphenyl tetrazolium bromide] was dissolved in phosphate-buffered saline (PBS, pH 7.4) at 5 mg/mL and filtered to be sterilized. Twenty microliters of stock MTT solution were added to all wells for an assay, and plates were incubated at 37 °C for 4 h. One hundred and fifty microliters of DMSO were added to all wells and mixed thoroughly to dissolve the blue-violet crystals. After a few minutes at room temperature to ensure that all crystals were dissolved, the plates were read on a Bio-Rad Novapath™ microplate reader (Japan), using a test wavelength of 595 nm, taking the solution without MTT as control. Then optical absorptions [A] were obtained. Plates were normally read within 1 h after DMSO was added. The survival rate could be calculated according to [A]/[A]i, where [A]i is the optical absorption of untreated cells.

RESULTS

Determination of the average diameter of TiO2 nanoparticles
Zetasizer 3000HSA (USA) was used to determine the average diameter of TiO2 nanoparticles. The result is shown in Figure 1. The average volume size of TiO2 nanoparticles was 21.2 nm.

Cytotoxicity of TiO2 nanoparticles
The cytotoxicity of TiO2 nanoparticles (without UVA irradiation) was determined by exposing cells to various concentrations of TiO2 in RPMI 1640 (Gibco) medium for 24 h. The surviving fraction of the cells was greater than 90% when the concentration of TiO2 was in the range of 1 000 µg/mL, as shown in Figure 2. The result confirmed that nonirradiated TiO2 nanoparticles were not toxic to the Ls-174-t cells. It was consistent with those in literatures.

Effect of photoexcited TiO2 nanoparticles on Ls-174-t cells
The fact that the surviving fraction was greater than 90% after 30 min as shown in Figure 3 (A) indicated that TiO2 nanoparticles without UVA irradiation showed little toxicity to living cells. The killing effect of UVA without TiO2 is shown in (B) with the surviving fraction of Ls-174-t cells given as a function of the UVA light irradiation time. About 20% cells were killed after 30 min exposure, whereas after 20 min exposure, more than 90% of the cells survived. Once TiO2 was added, the Ls-174-t cells were killed at a much higher rate as shown in (C). For example, in the presence of 1000 µg/mL of TiO2, 44% of the cells were killed after 10 min of UVA irradiation, and after 30 min irradiation, 80% of the cells were killed. Therefore it was concluded that photoexcited TiO2 nanoparticles had an active killing effect on Ls-174-cells.

Effect of TiO2 concentration on Ls-174-t cells activity
The effect of the TiO2 concentration ranging from 200 to 1 000 µg/mL on the rate of cell killing by UVA light is shown in Figure 4. The light intensity was 3.7 mW/cm² and kept constant. The experimental results demonstrated that cell viability decreased monotonically as TiO2 concentration increased and cell viability decreased with time.

Although a higher concentration of TiO2 could achieve a higher reaction rate, the difficulties in separation and measurement had to be considered. So the effect of a higher TiO2 concentration (C>1 029 µg/mL) on Ls-174-t cell activity was not investigated. The concentration of TiO2 was set at <1 029 µg/mL, with which the dark cytotoxicity was considered to be negligible. The range of TiO2 concentrations was close to that used previously.

Morphological changes of Ls-174-t cells
Untreated and TiO2-treated cells were collected by centrifugation and resuspended in RPMI 1640 medium. The samples were pipetted into a 24-well plate, which was directly observed with an inverted phase-contrast microscope. When treated by photoexcited TiO2, the cellular shape was condensed and nuclei were dispersed in fragments.

DISCUSSION
In this experiment, the TiO2 nanoparticle system was used, displaying its superiority. TiO2 nanoparticles were easy to attach
to the cellular membranes and accumulate. They were also easy to enter into the cytoplasm via phagocytosis\[19\]. It could lead to accumulation of ROS on the surface of cell membranes and in the cytoplasm. Hence under light irradiation, TiO2 nanoparticles had more significant cell killing effect in vitro.

Human colon carcinoma cells treated with photoexcited TiO2 nanoparticles (C>200 μg/mL) were effectively damaged, with cells contracted and a lot of cell fragments simultaneously observed by an inverted phase-contrast microscope\[19\]. According to these characteristics, we assumed that the mechanism of photoexcited TiO2 in killing human colon carcinoma cells might be through a series of oxidized chain reactions and in inducing cell death by reactive oxygen species\[20-23\]. Human colon carcinoma cell damages occurred in two stages. The initial oxidative damage took place on the cell membranes, where the TiO2 photocatalytic surface had its first contact with intact cells, the membranes became somewhat permeable. At this stage the cells did not lose their viability. Photocatalytic action made the cell membranes permeable, intracellular components began to leak from the cells and free TiO2 nanoparticles might also diffuse into the damaged cells and directly attack intracellular components, eventually leading to cell death. It is different from the bactericidal effect of TiO2 photocatalytic reaction. Bacteria are simple prokaryotic cells that do not contain the nucleus characteristics of eukaryotic cells. Whereas human colon carcinoma cells are eukaryotic cells and their structure is complex. Based on their structural differences, we assumed that killing cancer cells might be more difficult than killing bacteria by the photocatalytic reaction of TiO2 nanoparticles.

In the present study, cultured human colon carcinoma cells were effectively killed by photoexcited TiO2 nanoparticles in vitro. The concentration of TiO2 affected the photocatalytic killing effect. When the concentration of TiO2 was below 200 μg/mL, there was only a slight decrease in survival ratio after UVA irradiation for more than 30 min. It was almost the same as that of UVA irradiation alone. It indicated that minor cell membrane leakage might occur and the cell viability was not lost. When the concentration of TiO2 was above 200 μg/mL, the survival ratio decreased rapidly with increasing TiO2 concentration. It indicated that major rupture of cell membranes and decomposition of essential intracellular components might take place, thus accelerating cell death. It verified the mechanism of TiO2 nanoparticles in killing human colon carcinoma cells.

The photocatalytic killing effect of TiO2 nanoparticles on human colon carcinoma cells suggested the idea of cancer treatment using TiO2 nanoparticles and light irradiation. Under these conditions, it could be adapted to an anticancer modality by the local or regional treatment of the tumor with TiO2 nanoparticles, followed by light irradiation focusing on the tumor. Although UVA light (320-400 nm) cannot penetrate the human body deeply, it may be possible that the modality will be applied to several human tumors in the future.

REFERENCES

1 Matsunaga T, Tomoda R, Nakajima T, Wake H. Photoelectrochemical sterilization of microbial cells by semiconductor powders. *FEMS Microbiol Lett* 1985; 29: 211-214

2 Byrne JA, Eggins BR, Brown NMD, Mckinney B, Rouse M. Immobilisation of TiO2 powder for the treatment of polluted water. *Appl Catal B Environ* 1998; 17: 25-36

3 Wang KH, Hsieh YH, Ko RC, Chang CY. Photocatalytic degradation of wastewater from manufactured fiber by titanium dioxide suspensions in aqueous solution. *Environ Int* 1999; 25: 671-676

4 Byrne JA, Eggins BR, Byers W, Brown NMD. Photoelectrochemical cell for the combined photocatalytic oxidation of organic pollutants and the recovery of metals from waste waters. *Appl Catal B Environ* 1999, 2: L85-L89

5 Horikoshi S, Satou Y, Hidaka H, Serpone N. Enhanced photocurrent generation and photooxidation of benzene sulfonate in a continuous flow reactor using hybrid TiO2 thin films immobilized on OTE electrodes. *J Photochem Photobiol A* 2001; 146: 109-119

6 Molinari R, Grande C, Drioli E, Palmisano L, Schiavello M. Photocatalytic membrane reactors for degradation of organic pollutants in water. *Catal Today* 2001; 67: 273-279

7 Shephard GS, Stockenstrom S, de Villetters D, Engelbrecht WJ, Wessels GE. Degradation of microcystin toxins in a falling film photocatalytic reactor with immobilized titanium dioxide catalyst. *Water Res* 2002; 36: 140-146

8 Muneer M, Singh HK, Bahnemann D. Semiconductor-mediated photocatalysed degradation of two selected priority organic pollutants, benzo[α]pyrene and 1, 2-diphenylhydrazine, in aqueous suspension. *Chemosphere* 2002; 49: 193-203

9 Alhakimi G, Studnicki LH, Al-Chazali M. Photocatalytic destruction of potassium hydrogen phthalate using TiO2 and sunlight: application for the treatment of industrial wastewater. *J Photochem Photobiol A* 2003; 154: 219-228

10 Fujishima A, Cai RX, Otsuki J, Hashimoto K, Itoh K, Yamashita T, Kubota Y. Biochemical application of photoelectrochemistry: photokilling of malignant cells with TiO2 powder. *Electrochem Acta* 1993; 38: 153-157

11 Mills A, Hunte SL. An overview of semiconductor photocatalysis. *J Photochem Photobiol A* 1997; 108: 1-35

12 Fujishima A, Rao TN, Tryk DA. Titanium dioxide photocatalysis. *J Photochem Photobiol C* 2000; 1: 1-21

13 Kormann C, Bahnemann DW, Hoffmann MR. Preparation and characterization of quantum-size titanium dioxide. *J Phys Chem* 1988; 92: 5196-5201

14 O'Regan B, Moser J, Anderson M, Gratzel M. Vectorial electron injection into transparent semiconductor membranes and electric field effects on the dynamics of light-induced charge separation. *J Phys Chem* 1990; 94: 8720-8726

15 Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; 65: 55-63

16 Bernard BK, Osheroff MR, Hofmann A, Mennen JH. Toxicology and carcinogenesis studies of dietary titanium dioxide-coated mica in male and female Fischer 344 rats. *J Toxicol Environ Health* 1990; 29: 417-429

17 Linnainmaa K, Kivipensas P, Vainio H. Toxicity and cytogenetic studies of ultratine titanium dioxide in cultured rat liver epithelial cells. *Toxicol In Vitro* 1997; 11: 329-335

18 Xu MH, Huang NP, Xie ZD, Lu ZH. Photoexcited TiO2 nanoparticles through OH- radicals induced malignant cells to necrosis. *Supramole Sci* 1998; 5: 449-451

19 Cai RX, Hashimoto K, Itoh K, Kubota Y, Fujishima A. Photokilling of malignant cells with Ultrafine TiO2 powder. *Bull Chem Soc Jpn* 1991; 64: 1268-1273

20 Jaeger CD, Bard AJ. Spin trapping and electron spin resonance detection of radical intermediates in the photodecomposition of water at TiO2 particulate systems. *J Phys Chem* 1979; 83: 3146-3152

21 Rao MV, Rajeshwar K, Pal Verneker VR, DuvBow J. Photosynthetic production of H2 and H2O2 on semiconducting oxide grains in aqueous solutions. *J Phys Chem* 1980; 84: 1887-1991

22 Harbour JR, Hair ML. Superoxide generation in the photolysis of aqueous cadmium sulfide dispersions. Detection by spin trapping. *J Phys Chem* 1977; 81: 1791-1793

23 Harbour JR, Tromp J, Hair ML. Photogeneration of hydrogen peroxide in aqueous TiO2 dispersions. *Can J Chem* 1985; 63: 204-208

24 Hong AP, Bahnemann DW, Hoffmann RR. Cobalt (II) tetrasulfophthalocyanine on titanium dioxide: a new efficient electron relay for the photocatalytic formation and depletion of hydrogen peroxide in aqueous suspensions. *J Phys Chem* 1987; 91: 2109-2117

25 Hidaka H, Horikoshi S, Serpone N, Knowland J. In vitro photochemical damage to DNA, RNA and their bases by an organic sunscreen agent on exposure to UVA and UVB radiation. *J Photochem Photobiol A* 1997; 111: 205-213

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