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Photocatalytic effect of anodic titanium oxide nanotubes on various cell culture media

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Abstract The use of titanium dioxide (TiO_2) in photodynamic therapy for the treatment of cancer cells has been proposed following studies of cultured cancer cells. In this work, an ordered channel array of anodic titanium oxide (ATO) was fabricated by anodizing titanium foil. The ATO layer of nanotubes with diameters of 100 nm was made in NH_4F electrolyte by anodization. The photocatalytic effect of ATO was examined on various culture media by ultraviolet A (UV-A) (366 nm) irradiation. After UV-A irradiation of the ATO layer, redox potential of Tris-HCl buffer (pH 7.5) and dilute acrylamide solution increased instantaneously. The redox potential of the serum-containing RPMI1640 medium also increased dramatically, while that of serum-containing MEM and DMEM media increased slightly. The UVA-induced high redox potential was correlated with the greater ability to break down plasmid DNA strands. These phenomena suggest that a culture medium, such as RPMI1640, with a greater ability to produce free radical may be associated with a stronger photocatalytic effect of ATO on cultured cancer cells reported previously.

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1 Introduction

Titanium dioxide (TiO_2) is a photocatalyst that has been extensively adopted for removing unwanted environmental contaminants [1], including harmful compounds in wastewater [2] and microorganisms in air and water [3–5]. The use of TiO_2 in killing human cancer cells has also been reported [6]. TiO_2 is an essential photocatalyst with strong redox properties. It exhibits a spectral photosensitization to ultraviolet A (UV-A) light, and the photocatalytic effect can be achieved by proper surface modification. For example, after its surface was modified by the chemisorption of $H_2[PtCl_6]$, TiO_2 could sterilize the instruments or refresh the air by catalyzing photodegradation using indoor daylight [7]. The absorption energy of ultraviolet radiation exceeds the optical band gap of TiO_2 (3.2 eV for anatase-modified TiO_2), enabling an electron–hole pair to form. After irradiation by UV-A, an aqueous solution containing TiO_2 can release O_2^- or OH^- radicals that could destroy bacteria and organic dyes.

TiO_2 nanoparticles clearly demonstrate a photocatalytic activity that exceeds that of commercial Degussa P-25 [8]. Because of their high surface-to-volume ratio and improved crystallinity, their photocatalytic efficiency can be increased by increasing the number of catalytic sites and also enhancing the delocalization of carriers to reduce electron–hole pair recombination [9]. Thus it indicates that a good photocytoxicity, depends not only on the concentration of the photocatalyst and light dose, but also its crystalline form.

Photodynamic therapy is a relatively new treatment for certain cancers, such as endobronchial and esophageal cancers [10]. The photocytotoxic effects of TiO_2 have been normally studied on cell lines cultured in various media with a pH suitable for cell survival. Most non-cancerous cell lines such as murine macrophages are cultivated in minimum essential medium (MEM) or Dulbecco's Modified Eagle's Medium (DMEM) that is supplemented with calf serum or fetal bovine serum (FBS) [11]; while cancerous cell lines, such as HeLa cells and human adenocarcinoma cells, are generally maintained in RPMI1640 medium that contains calf serum or FBS [12, 13]. Serum included in these media was to provide protein factors that are required for cell growth and attachment [14]. The goal of this investigation is to determine the effects of the photocatalyst activity of anodic titanium oxide (ATO) on several defined media. UVA-induced potential change of the five different solutions and serums including cell culture media were studied in this work. The acrylamide solution was chosen because it is known as a free-radical carrier during gel polymerization reactions [15].

2 Experimental procedure

A self-organized channel array of anodic titanium oxide (ATO) was fabricated by anodizing titanium (Ti) foil. A platinum (Pt) sheet with an area of 2 cm × 2 cm was used as the cathode to anodize the Ti anode in an electrolyte that was comprised of 0.5 wt% NH_4F and ethylene glycol for a period of one hr at 25°C. The distance between the two electrodes was maintained at 1.5 cm. Following anodization, the ATO samples were annealed in a furnace at 450°C for three hrs to form single-crystalline anatase. The structure of the ATO was identified using an X-ray diffractometer (XRD). The band gap of the photoluminescence (PL) of anatase ATO was measured using an He-Cd laser with an excitation wavelength of 325 nm as the light source.

Five solution and culture media, Tris (hydroxymethyl) aminomethane hydrochloride (Tris-HCl) buffer, acrylamide solution, Dulbecco's Modified Eagle's Medium (DMEM), minimum essential medium (MEM) and Rosewell Park Memorial Institute (RPMI1640) medium, were employed to elucidate the photocatalytic effect of ATO in these media. After disinfection in 95% ethanol, the ATO nanotube layer was set into each well of a tissue culture plate that was full of each one of the above solution or media. Then various solutions and media were irradiated with ultraviolet A (UV-A) light for 100 s exposure after stabilization for a period of 100 s. The distance between the sample and the UV-A illuminant was approximately 10 cm. The intensity of the UV-A light was 0.1 mW/cm^2, as measured using a UV meter, and its wavelength was 366 nm. Three electrodes—a Ag/AgCl reference electrode, a working Pt-wire electrode, and a Pt-wire auxiliary electrode—were immersed into each ATO containing a well of a tissue culture plate, filled up with either a specific solution or medium. The electrical potential of the well was measured and recorded with and without exposure to UV-A irradiation. Whether the plasmid DNA strand in Tris-HCl buffer could be broken down by the high redox potential of ATO induced by UV-A, was checked with 1.2% agarose gel electrophoresis and ethidium bromide staining.

3 Results and discussion

The top and side view of TiO_2 nanotube layers were shown in Fig. 1. The average diameter and thickness of nanotubes are around 100 nm, and 1.8 μm, respectively (Fig. 1). The crystalline structure of ATO that had been annealed at 450°C for 3 hrs mainly comprises of anatase, and a very small amount of titanium, as revealed by XRD in Fig. 2. To verify its photocatalytic property of TiO_2 nanotube array, the photoluminescence (PL) spectra were obtained at a low temperature of 10 K, and are presented in Fig. 3. The band-gap energy appears to be at about 3 eV, close to that of the anatase, which has superior photocatalytic ability. The energy of spectra at 2.5 eV is caused by emission from defects, because the emission energy is less than the gap energy [16].

As shown in Fig. 4, the redox potential of the media changed after UV-A irradiation, the redox potential (photocatalytic reaction potential) of Tris-HCl buffer, acrylamide solution, and RPMI1640 + 10%FBS media increased substantially, whereas the redox potential of DMEM + 10%FBS and MEM + 10%FBS only increased slightly after UV-A irradiation on ATO. Finally, supercoiled plasmid was used for testing whether the photocatalytic reaction potential of the ATO in TiO_2 array was correlated with the ability to break down DNA (photocytotoxicity). Irradiation of UV-A caused plasmid DNA strand breakage in Tris-HCl buffer in the presence of ATO, as revealed by the smeared appearance of this plasmid DNA shown in agarose gel electrophoresis pattern, while this plasmid DNA remained intact when it was irradiated in Tris-HCl buffer in the absence of TiO_2 (Fig. 5). Anatase phase of TiO_2 in annealed ATO has an ability to photocatalyze and activate culture media to release free hydroxyl radicals or oxygen radicals effectively after UV-A ir-

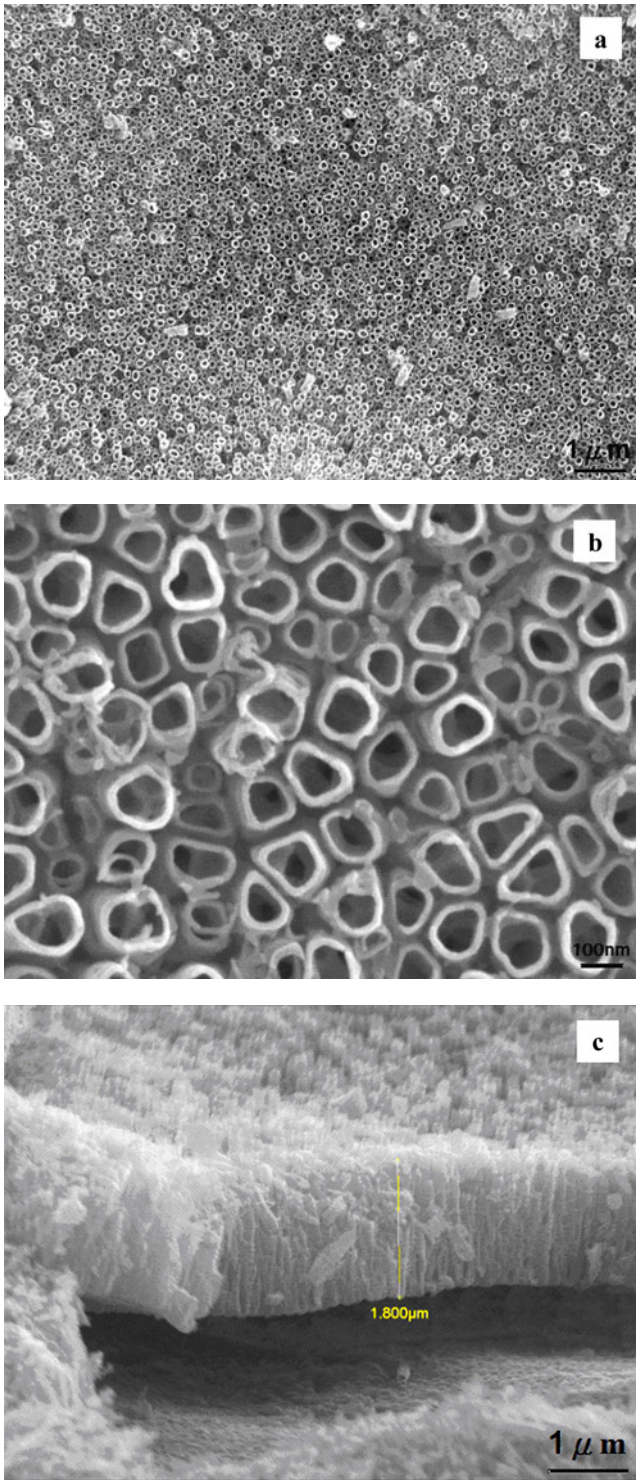

Fig. 1 SEM images of TiO_2 nanotube layer: (**a**) low magnification of top-view image, (**b**) high magnification of top-view image, and (**c**) side view of 1.8 μm-thick layer

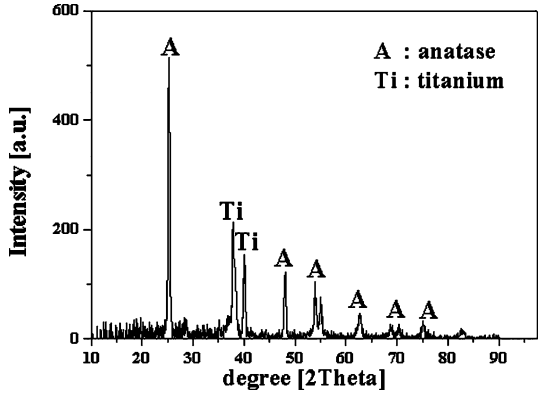

Fig. 2 XRD patterns of an annealed (3 hrs at 450°C) ATO in TiO_2 nanotube layer. *A*: anatase, *Ti*: titanium

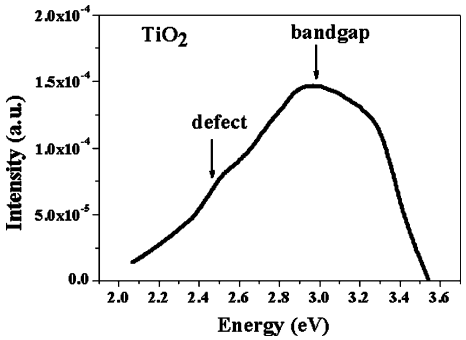

Fig. 3 PL spectrum of an ATO in TiO_2 nanotubes which were excited at low temperature 10 K

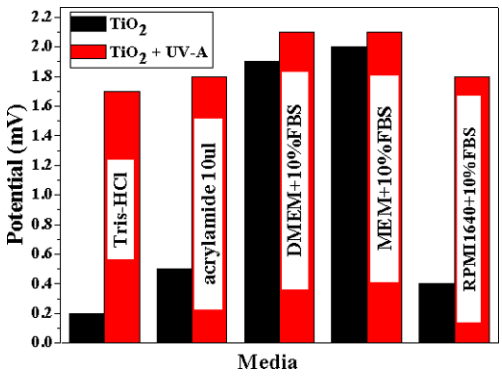

Fig. 4 Redox potential of the media changes after UV-A irradiation on ATO in TiO_2 nanotube layer

radiation [17]. The increase of the redox potential after UV-A irradiation in the acrylamide solution in the presence of ATO could be explained by the electron-accepting capacity of acrylamide ($CH_2CHCONH_2$). This is shown in the following:

$$TiO_2 \xrightarrow{UV} e^- + h^+$$

$$e^- + CH_2 = \underset{\displaystyle CONH_2}{\underset{|}{CH}} \longrightarrow CH_2 - \underset{\displaystyle CONH_2}{\underset{|}{\underset{\bullet}{CH}}}$$

Free Radical

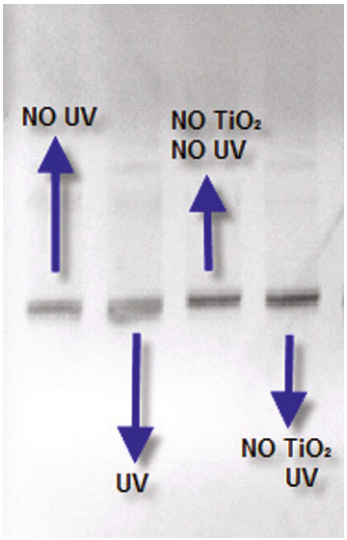

Fig. 5 Agarose gel electrophoresis pattern of plasmid DNA in Tris-HCl buffer with or without UV-A irradiation (400 J m^{-2}) in the presence or absence of ATO

Consequently many free radicals are brought into intensive contact with acrylamide molecules [15]. The electron-accepting capacity of RPMI1640 + 10%FBS may be very similar to that of the acrylamide ($CH_2CHCONH_2$). RPMI1640 contains more L-arginine free base, which is structurally more similar to acrylamide than those of MEM and DMEM [14]. Previous studies have demonstrated that the photocytotoxicity of TiO_2 depends on the concentration of the photocatalyst, the dose of irradiating light, the morphology of TiO_2, and the incorporation of the photosensitizer into the target cells [1, 18]. The results suggest that the electron-accepting capacity of the aqueous environment around or within target cells might influence the photocytotoxic activity of TiO_2 on target cells.

4 Conclusions

The self-ordered TiO_2 anatase nanotube layer prepared in this study effectively absorbed UV-A light and photocatalyzed Tris-HCl buffer, acrylamide solution and various cell culture media. UV-A irradiation of ATO drastically increased the redox potential in serum-containing RPMI1640 medium, while UV-A irradiation slightly increased the redox potential in serum-containing MEM and DMEM media. The redox potential induced by UV-A was related to the ability to break down DNA strands of a bacterial plasmid, suggesting that culture media with a strong electron-accepting ability, such as RPMI1640, may enhance the photocatalytic effect of ATO on cultured cancer cells.

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