X-ray Photoactivated Cytotoxicity of Anatase TiO_2 on the CT26 cells Investigated by SR-FTIR

We demonstrate that titanium oxide spherical nanoparticles of 20±3 nm in diameter is proper to be used for enhancements of radiation response by conventional ultraviolet or even high energy X-ray light source. We evaluated the photocatalytic reaction by ultraviolet and X-ray irradiation of methylene blue with photoexcited TiO_2 first to confirm the similar photo-activation effect of X-rays. By comparing these properties with biological assay, such as cytotoxicity indices of mice colorectal adenocarcinoma cells, we confirm the photocatalytic killing effect of TiO_2 nanoparticles on CT26 cells. Synchrotron Radiation Fourier Transform Infrared (SR-FTIR) is shown to be a suitable technique to screen the cell damage by TiO_2 nanoparticles. The observed spectra changed in amide to appear of C=O stretching bonds, induced by photoexcited TiO_2 observed in SR-FTIR spectromicroscopy, is systematically correlated to photoactivated cytotoxicity of TiO_2 on the CT26 cells and also can act as a chemical marker for cell damage. These studies establish that TiO_2 is a good candidate to enhance cancer therapy using x-rays or higher energy ionization radiation.

Beamline

14A IR Microscopy

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Cancer treatment utilizing photo-catalytic process have been realized and practiced since mid-1980s. Most researchers use TiO_2 nanoparticles with ultraviolet (UV) irradiation to enhanced cancer therapy because they believe that TiO_2 is bio-compatible and with high degree of photoactivated cytotoxicity. However, using UV light source for cancer therapy presents several drawbacks due to its low penetration and incapability to be focused. Therefore, X-ray irradiation with higher penetration, could be a promising candidate to allow the treatment of deeper organs without requiring optical fibers or additional surgery. In addition, X-rays are already widely used in standard therapeutical procedures.

The photocatalytic reaction by ultraviolet and X-ray irradiation was evaluated by the photodegradation of methylene blue in solution with anatase TiO_2 nanoparticles measured by ultraviolet-visible spectroscopy. The cell damage was evaluated by the synchrotron radiation Fourier-transform infrared (SR-FTIR) spectroscopy using CT26 cell line to estimate the therapeutical effect. SR-FTIR with high coherence and ultra-high brilliance from synchrotron radiation photon source, provides high spatial resolution and signal to noise ratio to screen various biological studies including investigation of cell membranes, proteins and nucleic acids, as well as tissues engineering.

Understanding the degree of damage by the radiation is quite important for therapeutical applications. In order to extensively test the biology effect of TiO_2 in inert or activated forms, we report here a less common approach using synchrotron based FTIR spectromicroscopy to identify the health condition of cells with different TiO_2 and radiation treatment.

The UV-VIS absorption spectrum was used to determine the

remaining concentration of methylene blue (MB) after UV light or X-ray irradiation. We calculated the photodegradation ratio for C/C_0 (C: remaining concentration of MB; C_0: initial concentration). The corrected peak heights obtained from the bands at 664 nm due to the contribution of MB. It is an exceptionally useful factor to estimate the photocatalytic efficiency of excited TiO_2 as comparison at Fig. 1. These results revealed that nanoparticles TiO_2 excited by X-ray was with higher degradation ratio for C/C_0 than that of ultraviolet light. The hydroxyl radical can form in higher photon concentrations and take part in the degradation process, thus, accelerating the photocatalyzed degradation.

In order to understand the chemical nature of the conditioned investigated, control and irradiated cells were analyzed by SR-FTIR as shown in Fig. 2. Spectral analysis of our samples shows that no difference between control cells and TiO_2 nanoparticles mediated cell existed in terms of the amide I band. However, once CT26 cells treated with TiO_2 and X-ray or UV irradiation, we found that the frequencies of the maximum of the Amide I peaks would shift toward to lower energy. This phenomenon is more evidenced in the irradiated cell mediated by TiO_2 nanoparticles and X-ray.

The most striking disorder in the spectra variation is that a new peak is appeared at around 1730 cm^{-1} corresponding to the C=O resonance in the spectra of irradiated cell system, but not shown in the spectra of non-irradiated cell system. It may be attributed to the formation of unsaturated aldehyde during the breakdown of hydroperoxides or lipid endoperoxides or early state apoptosis and cell death.

To minimize the individual cell thickness effect during FTIR measurement, the deduced absorbance values of band area are estimated after normalization to the protein Amide II band area as shown in Fig. 3. Indeed, the ratio was significant higher for irradiated CT26 cells, especially for the X-ray irradiation. These findings may provide a conceptual background for the development of radiotherapy with TiO_2 nanoparticles irradiated by X-ray light source.

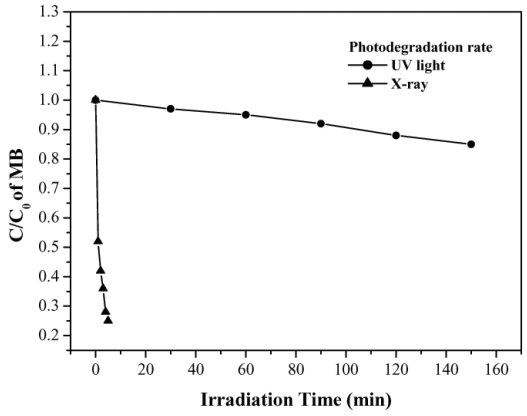

Fig. 1: The photodegradation ratio C/C_0 (C = remaining methylene blue concentration; C_0 = initial concentration) derived from the normalized 664 nm peak heights obtained from UV spectra as a function of the irradiation time.

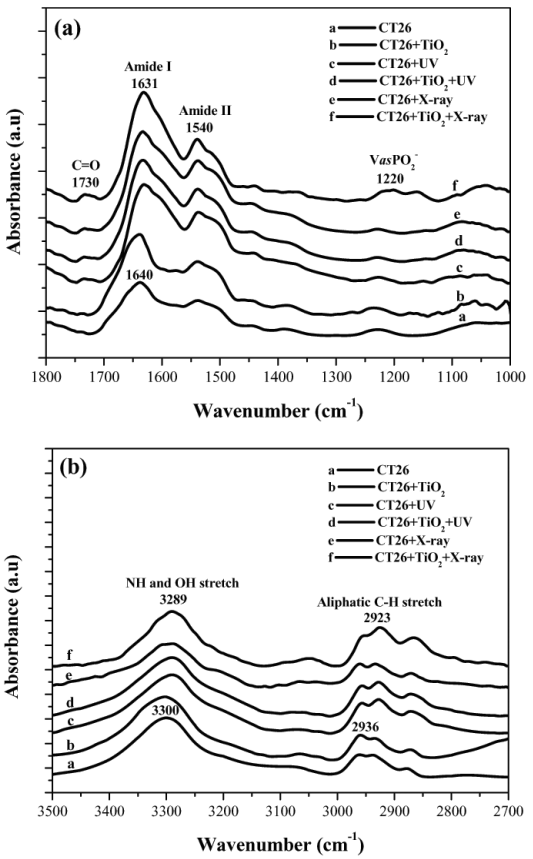

Fig. 2: Synchrotron radiation FTIR spectra of CT 26 cells in the range (a) 1800-1000 and (b) 3500-2700 cm^{-1} for different treatments.

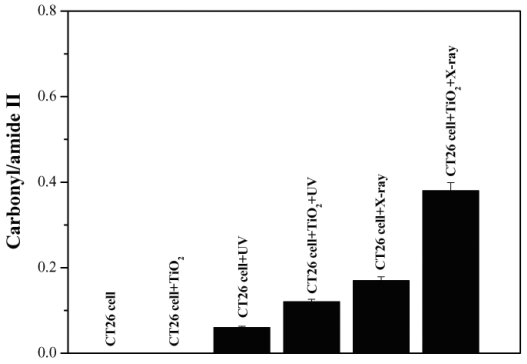

Fig. 3: Carbonyl/amide II ratio of CT26 cells for different treatments.

Experimental Station

IR microscopy end station

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