Selective Killing of a Single Cancerous T24 Cell with TiO_2 Semiconducting Microelectrode under Irradiation

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A titanium dioxide (TiO_2) microelectrode has been applied for selective photo-killing of a single cancerous T24 cell. The anodically polarized TiO_2 microelectrode effectively inactivated the T24 cell while in contact and under UV light irradiation; however, the cell was not killed when the microelectrode was located 10 μm away from the cell surface. Based on these results we concluded that the photogenerated holes and/or active oxygen species with short diffusion length are responsible for the cell death process.

TiO_2 semiconductor particles, electrodes, and films have been applied for various photochemical reactions by utilizing their strong oxidation and reduction power under UV light irradiation, e.g. solar energy conversion,[1,2] and photo-organic synthesis research.[3-5] Recently we applied this high reactivity of the irradiated TiO_2 particles and electrodes to the photodynamic therapy (PDT) of cancer.[6-11] We found that multiplication of human malignant cells was remarkably suppressed both *in vitro* [6-8,11] and *in vivo* [9,10] in the presence of the photo irradiated TiO_2. In addition to the direct oxidation of cell constituents by the photogenerated holes, hydroxyl radical (•OH) and hydrogen peroxide (H_2O_2) produced by the reactions of photogenerated electron-hole pairs with water and/or dissolved oxygen were found to participate in the photo-killing of the cells.[7-9] Additionally, in the PDT using TiO_2 particles, tumor cells can be selectively inactivated by irradiating the UV light selectively to the cancer organ using fiber light source, since TiO_2 has no cytotoxicity to the cells in the dark.

The present study describes the selective photo-killing of a single cancerous T24 cell using an irradiated TiO_2 "*micro*" electrode. The TiO_2 microelectrode with a tip diameter of about 10 μm was brought in contact with the individual T24 cell or detached 10 μm away from the cell membrane with the potential poised anodically, and the effect of the UV light irradiation on the cell viability was observed. Based on those results, the mechanism of the cell death, with emphasis on the contribution of the photogenerated •OH and H_2O_2 for this cell death process is discussed.

The TiO_2 microelectrode was prepared as follows. A tip of 0.2 mm Ti wire (2 mm in length) was dipped into a 2 mol/dm^{-3} NaOH aqueous solution. An alternate current (50 Hz, 20 V) was passed through the solution between the Ti wire and a Pt wire counter electrode to form a conically sharpen Ti microelectrode (~10 μm diameter at the apex) at the air/liquid interface. The TiO_2 microelectrode was obtained by oxidizing the surface of the Ti microelectrode with flame.

Human malignant cell line T24 was used in the experiment. Freshly prepared cell suspension in phosphate buffered saline (PBS, pH 7.4) was transferred to a petrii dish with the concentration being 1 x10^4 cells / ml. A single cell in the suspension (about 30 μm in size) was fixed with a micro pipette, and the TiO_2 microelectrode contacted the cell membrane using a micro manipulator under microscopic observation (Figure 1). The microelectrode was irradiated by UV light while simultaneously being polarized anodically. The irradiated cells were immediately stained by trypan blue and the cell viability was evaluated.[10] Filtered UV light (300 - 400 nm) from a 150 W Hg-Xe lamp was used as light source (4.3 J • s^{-1} • cm^{-2}). A platinum wire and Ag/AgCl were used as a counter and a reference electrode, respectively.

Figure 2 (a) represents the potential-current characteristics of the TiO_2 microelectrode in PBS. Anodic photocurrent due to the hole oxidation of both water and Cl^- contained in PBS solution was observed when the electrode potential was kept at a potential more positive than -0.4 V vs. SCE. No current saturation was observed even at high anodic potentials, suggesting the existence of surface states which remain occupied by electrons.[12] The photocurrent density of the TiO_2 microelectrode at 0.5 V vs. SCE was on the order of 10^2 μA cm^{-2}, almost the same as that of the TiO_2 film electrode which showed high cell killing activity.[10]

The effect of UV light irradiation on the viability of the single T24 cell being in touch with the TiO_2 microelectrode is shown in Figure 2(b). Values of the cell viability at each potential represent the ratio of the survived cells, calculated from the result of 10 of identical experiments. In the dark, when the potential of TiO_2 electrode was kept between -0.5 and +1.2 V vs. SCE, all the cells survived. When the electrode was set at a highly anodic (1.5 V vs. SCE) potential, however, all the cells were killed. These observations suggest that, in the dark, the T24 cell in contact with the TiO_2 microelectrode is inactivated only when the highly anodic potential as positive as 1.5 V vs. SCE is applied at which the cell constituents may be directly oxidized.

On the other hand, under UV irradiation, the cells attached to the electrode were killed effectively when the electrode potential was as positive as 0.0 V vs. SCE. In particular, a potential more positive than +0.8 V resulted in all the cells being inactivated after 3 minutes of UV irradiation. By comparing Figure 2 (a) and (b), we found that cells were killed when the anodic photocurrent flowed. This indicates that the photogenerated holes are responsible for the cell killing in this TiO_2 microelectrode system. The surrounding T24 cells, which were not in contact with the TiO_2 microelectrode, were not inactivated at all with the 3 minutes UV irradiation even the electrode was polarized anodically enough.

To consider the contribution of the photogenerated active oxygen species to the photokilling effect, the TiO_2 microelectrode was located 10 μm away from the cell. In this case, the decrease of the cell viability was not observed even when the electrode was

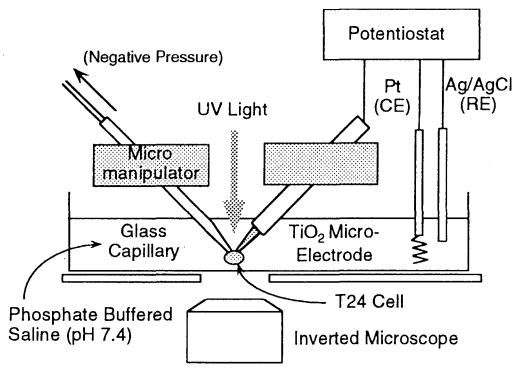

Figure 1. Schematic illustration of the experimental setup for the selective photo-killing of a single cancer cell using a TiO_2 microelectrode

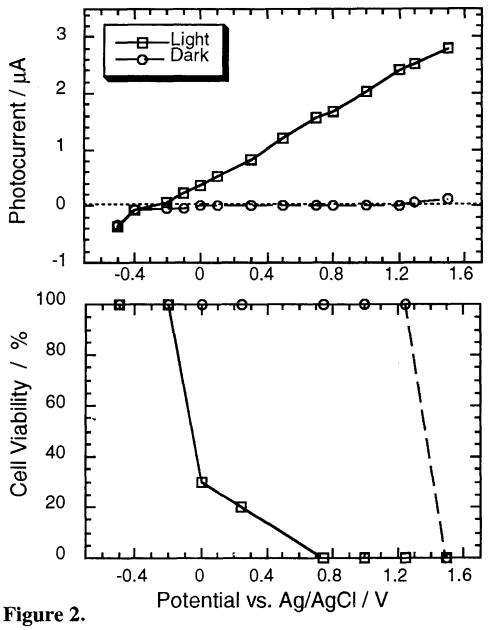

Figure 2.
(a) I-V characteristics of the TiO2 microelectrode
(b) The effect of UV light irradiation (3 min.) on the viabiliity of the T24 cell contacted by the TiO_2 microelectrode as a function of the electrode potential.

polarized anodically to +1.5 V vs. SCE under UV irradiation (Table 1). Due to instrumental restriction, more precise control of the distance could not be achieved. Nevertheless these results clearly indicate that the T24 cell was inactivated under UV light irradiation only by photo-generated holes in TiO_2 and/or short lived intermediate species which do not diffuse at least 10 μm.

In the photocatalytic reactions in an oxygenated aqueous suspension of TiO_2 *particles*, hydrogen peroxide (H_2O_2), hydroxyl radical (•OH), and super oxide (O_2^{-}•), are produced by either the oxidative water degradation with the holes (shown in eqs. (1) and (2)) or the reduction of dissolved oxygen with the photogenerated electrons (eqs. (3) and (4)).[13,14]

$$H_2O + h^+ \rightarrow \cdot OH + H^+ \quad (1)$$
$$2H_2O + 2h^+ \rightarrow H_2O_2 + 2H^+ \quad (2)$$
$$O_2 + e^- \rightarrow O_2^{-}\cdot \quad (3)$$
$$O_2^{-}\cdot + e^- + 2H^+ \rightarrow H_2O_2 \quad (4)$$

In a previous report, we have shown that these three active oxygen species are responsible for the cell killing effect induced by the TiO_2 *particles*, by observing the change in the cell viability when scavengers of those active species were added to the cell suspension.[7] In the cell killing with TiO_2 micro*electrode* under anodic polarization where only photogenerated holes can participate in the reactions, O_2^{-}• cannot be produced on the TiO_2 electrode. Therefore only •OH and H_2O_2 are considered to be formed through eqs. (1) and (2). •OH is a very reactive species and its diffusion length in aqueous solution is less than 10 nm at neutral pH.[15] On the other hand, H_2O_2 is stable in the bulk of solution. Thus, if there is no further reactions, H_2O_2 can diffuse to the T24 cell which is located 10 μm away from the TiO_2 surface, and it should show cytotoxicity when its concentration is higher than 1×10^{-5} M.[16] In the present study, however, the cell viability did not decrease under UV irradiation when the microelectrode was detached from the cell, suggesting that the formation of H_2O_2 through eq.(2) by oxidative water decomposition is inefficient, or the H_2O_2 formed by this reaction is immediately decomposed with •OH and/or holes to water and oxygen. Recently we have shown that, in the photocatalyzed reaction of the TiO_2 suspension, H_2O_2 is primarily produced by the reduction of the dissolved oxygen via eq. (4) rather than the oxidation of water via eq. (2).[17] The results obtained here agree with those observations.

Table. The change in the cell viabiliy of the T24 cell after 3 minutes UV light irradiation when the TiO_2 microelectrode was detached from the cell

Distance between the TiO_2 microelectrode and the cell / μm	Potential of the TiO_2 microelectrode / V vs.Ag/AgCl	Cell viability /%
0	0	30
	1.5	0
10	0	100
	1.5	100

It can be concluded that, in the photo-killing effect by the TiO_2 microelectrode, the direct oxidation of the cell components with the holes and/or the attack of the active oxygen species with short lifetime, e.g. •OH, are the main reason for cell inactivation. Additionally, in the photodynamic therapy of cancer, it is of great importance that the cancer cells are inactivated selectively by the irradiation without damaging normal cells. The present micro TiO_2 electrode technique may become a useful tool for such selective photo-killing of the cancer cell.

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References and Notes

1 A. Fujishima and K. Honda, *Nature*, **238**, 37 (1972).
2 E. Borgarello, J. Kiwi, E. Pelizzetti, M. Visca, and M. Grätzel, *J. Am. Chem. Soc.*, **103**, 6324 (1981).
3 H. Reiche and A. J. Bard, *J. Am. Chem. Soc.*, **101**, 3127 (1979).
4 M. Fujihira, Y. Satoh, and T. Osa, *Nature*, **293**, 206 (1981).
5 J. Onoe and T. Kawai, *J. Chem. Soc., Chem. Commun.*, **401**, 1480 (1987).
6 R.-X.Cai, K. Hashimoto, K. Itoh, Y. Kubota, and A. Fujishima, *Bull. Chem. Soc. Jpn.*, **64**, 1268 (1991).
7 R.-X.Cai, K. Hashimoto, Y. Kubota, and A. Fujishima, *Chem. Lett*, **1992**, 427.
8 R.-X.Cai, H. Sakai, K. Hashimoto,Y. Kubota, and A. Fujishima, *Denki Kagaku*, **60**, 314 (1992).
9 R.-X.Cai, Y. Kubota, T. Shuin, H. Sakai, K. Hashimoto, and A. Fujishima, *Cancer Res.*, **52**, 2346 (1992).
10 A. Fujishima, R.-X.Cai, J. Otsuki, K. Hashimoto, K. Itoh, T. Yamashita, and Y. Kubota, *Electrochim. Acta*, **18**, 153 (1993).
11 H. Sakai. E. Ito, R.-X. Cai, T. Yoshioka, Y. Kubota, K. Hashimoto, and A.Fujishima, *Biochim, Biophys. Acta,* in press.
12 M. P. Dare-Edwards and A. Hamnett, *J. Electroanal. Chem.*, **105**, 283 (1979).
13 J. R. Harbour, J. Tromp, and M. L. Hair, *Can. J. Chem.*, **63**, 204 (1985).
14 C. D. Jaeger and A. J. Bard, *J. Phys. Chem.*, **83**, 3146 (1979).
15 G. Czapski, *Method Enzymol.*, **105**, 209 (1984).
16 T. Tatsuma, Thesis, The University of Tokyo (1988).
17 R.-X.Cai, K. Hashimoto, and A. Fujishima, *J. Electroanal. Chem.*, **326**, 345 (1992).