Atsushi Yamamoto¹, Jun Suzurikawa², Keisuke Nagato¹, Tadashi Tsukada¹, Aya Tanaka¹, Tetsuya Hamaguchi¹ and Masayuki Nakao¹
¹Department of Mechanical Engineering, Graduate school of Engineering
The University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan
²Department of Assistive Technology,
Research Institute of National Rehabilitation Center for Persons with Disabilities
4-1 Namiki, Tokorozawa, Saitama, Japan

INTRODUCTION

In order to electrochemically and/or electrophysiologically monitor in-situ activities of cultured cells adhering on a substrate, micro-electrode arrays (MEAs) patterned on the substrate have been utilized. MEAs allow non-invasive and multisite monitoring with the fine temporal resolution. However, the spatial resolution of MEAs is limited by their electrode numbers and densities. MEAs, therefore, can access cells only in the vicinity of the electrode pads.

One possible solution to the limited spatial resolution is a light addressing technique, which enables to optically generate a virtual electrode at a desired site on a photoconductive substrate, not depending on predefined electrode positions. There have been many applications of this technique reported, including light addressable potentiometric sensors (LAPS) [1], pH or chemical imaging and cell manipulation. Although these applications have the potential to replace previous cell monitoring methods with the MEAs, they mainly utilize the electrolyte-insulator-semiconductor interface and there have been few reports on light addressed measurement with redox reactions and the induced faradic current at the electrode-electrolyte interface. Such an amperometric measurement with charge transfer is a prerequisite for biosensing of uncharged biological molecules.

In this study we describe a light addressable amperometric sensing (LAAS) [2] for in-situ monitoring of glucose in a biological solution. In order to achieve the sensing with charge transfer at the photo-electrode/electrolyte interface, we utilized titanium dioxide (TiO₂) as a photoconductor. Because TiO₂ has the strong oxidation power under UV illumination, the addressed photo-anode on the TiO₂ electrode will generate a redox current that is proportional to the local concentration of organic compounds as illustrated in Figure 1.

Our final goal is to obtain a distribution image of glucose and can measure the activity level of each cell. For that goal, we made TiO₂ electrodes and examine the characteristics of them. First, we examine the correlation between photocurrent and concentration of glucose, and then attempted to improve the sensitivity of TiO₂ electrodes with Titanium (IV) chloride (TiCl₄) post-treatment. We also examined the light addressing ability. Experiments and results of them are reported.

FIGURE 1. Schematic diagram of light addressable amperometric sensing.

EXPERIMENTAL

Materials
Conductive glass sheets (AGC Fabritech Co., LTD.) were used as substrates for TiO₂ film coating. TiO₂ particulate slurry, STS-21
was prepared. Particles in STS-21 consist of anatase phase. D(+)glucose and titanium (IV) chloride (Wako Pure Chemical Industries, Ltd) were used without further purification. Phosphate buffer saline (PBS, GIBCO 20012-027) was used as an electrolyte solution and solvent of glucose. All other reagents used were of analytical grade unless otherwise stated.

Preparation of TiO_2 Electrodes

TiO_2 particulate slurry STS-21 was stirred one night on VORTEX GENIE2 (M&S Instruments Inc) so that particulates were dispersed. Conductive glass sheets were cleaned first with acetone for 10 min, second with ethanol for 5 min and third with deionized water for 5 min in an ultrasonic bath (W-210R, HONDA ELECTRONICS) and then dried in airflow and washed with corona discharge (CFG-500, Shinko Electric & Instrumentation Co., Ltd.) before the TiO_2 slurry was coated. After these pretreatments the TiO_2 slurry was spin-coated on the glass substrates and then calcinated in an oven at 500 °C. A wire to external devices was bonded on the SnO_2 electrode with conductive epoxy (CW2400, ITW Chemtronics). A plastic dish was attached as a solution chamber. The morphology of the TiO_2 film was characterized by a scanning electron microscope (SEM) (S-4000, HITACHI). The thickness of the film was ca. 1.7 μm. A Pt film and an Ag/AgCl electrode were used as a counter and a reference electrode, respectively (Figure 2).

Titanium (IV) Chloride Post-Treatment

The TiO_2 electrode calcined was immersed in a 0.05 mM aqueous solution of TiCl_4 at 70 °C for 30 minutes, then rinsed with deionized water, and calcined again in an oven at 450 °C for 30 min [3].

**Photocurrent Measurement**

The TiO_2 electrode was set on a microscope (IX71, OLYMPUS) equipped with mercury lamp (USH-1030L, USHIO INC.). UV light was extracted through a band-pass filter (U-MWU2, OLYMPUS). TiO_2, Pt and Ag/AgCl electrodes were connected to the potentiostat (HAL-3001, Hokuto Denko Corporation). Photocurrents were recorded by LabVIEW (National Instruments). The intensity of the illumination was measured at the wavelength of 343 nm with a photodiode power meter (PD300-UV, OPHIR).

Experiment 1: Evaluation of the Concentration Sensitivity

In order to evaluate the concentration sensitivity of the electrode, photocurrents were measured in test solutions with 7 different glucose concentrations from 0 to 10 mM. The light intensity was 2.9 mW/cm^2 and the diameter of UV light spot was 100 μm.

Next, to evaluate the concentration sensitivity of the electrode at lower concentration, photocurrents were measured in test solutions, whose concentration was changed more minutely; 7 concentrations from 0 to 1.8 mM. The light intensity and the diameter of UV light spot were same as those described above.

Experiment 2: Attempt to Improve the Concentration Sensitivity with TiCl_4 Post-Treatment

In order to improve the electrode sensitivity, we introduced and evaluated the TiCl_4 post-treatment, which is routinely utilized for fabrication of dye-sensitized solar cells [3]. By hydrolysis from a TiCl_4 solution, pure TiO_2 is deposited on a rather impure film from STS-21. The TiCl_4 treatment is, therefore, expected to increase a charge transfer rate at the electrolyte-semiconductor interface and thus the sensitivity of the electrode.

Photocurrents were measured with 11 different concentrations of glucose from 0 to 10 mM. The light intensity and the diameter of UV light spot were same as Experiment 1.

Experiment 3: Measurement of Photocurrent at Different Spots

In order to examine whether the glucose oxidation occurs at the point of the TiO_2 electrode where UV light is irradiated or not, two glucose solutions with different concentrations were prepared on a TiO_2 electrode and photocurrents were measured at each spot. The solutions of 2 mM and 10 mM glucose were
fixed with agarose. Figure 3 shows the schematic of the experiment. The diameter of UV light spot was changed into 3 sizes.

**FIGURE 3.** Schematic diagram of the experiment.

**RESULTS AND DISCUSSION**

**Preparation of TiO₂ Electrodes**

Figure 4 is a cross-sectional surface of TiO₂ particulate film. A homogeneous TiO₂ particulate film was made.

**FIGURE 4.** SEM image of a cross section of the TiO₂ film.

**Experiment 1**

Figure 5 shows the photocurrent profiles induced in solutions with different concentration of glucose. The photocurrent values after the initial capacitive currents seem to correlate with the glucose concentration. Then, we calculated the averaged values of photocurrents between 4.5 and 5 s, and plotted them as the function of glucose concentration in Figure 6. This result demonstrates that the photocatalytic oxidation current can be used as an index of glucose concentration. However, the relationship between the current and concentration was not linear throughout the tested range.

**FIGURE 5.** Photocurrent waveforms for blank PBS and PBS containing glucose at different concentrations. From bottom to top: 0, 0.95, 4, 10 mM glucose.

**FIGURE 6.** Photocurrent as a function of concentration of glucose.

**FIGURE 7.** Photocurrent as a function of concentration of glucose lower than 1.8 mM.

Photocurrents for glucose concentration below 1 mM were plotted in Figure 7. A linear correlation was observed in this concentration range. It seems that oxidative ability of this TiO₂
electrode may saturate higher than 0.77 mM. According to Yang et al. [4], the general equation for complete mineralization of an organic compound, $C_{n}\text{H}_{m}\text{O}_{j}\text{N}_{k}\text{X}_{q}$, on a TiO$_2$ electrode is

$$C_{n}\text{H}_{m}\text{O}_{j}\text{N}_{k}\text{X}_{q} + (2y-j)\text{H}_2\text{O} \rightarrow y\text{CO}_2 + q\text{X}^- + k\text{NH}_3 + (4y-2j+m-3k)\text{H}^+ + (4y-2j+m-3k-q)e^-$$

So, the equation of glucose is

$$C_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} \rightarrow 6\text{CO}_2 + 24\text{H}^+ + 24e^-$$

Following reactions also occurred at each electrode.

- TiO$_2$) 2Cl$^-$ $\rightarrow$ Cl$_2$ + 2e$^-$
- Pt) 2H$^+$ + 2e$^-$ $\rightarrow$ H$_2$
- Ag/AgCl) AgCl + e$^-$ $\rightarrow$ Ag + Cl$^-$

The total reaction consists of electrolysis of water and oxidation of glucose. The reaction rate constant depends on concentration, so the reaction rate of the former reaction is constant and the change of photocurrent was caused by the change of the reaction rate of the latter reaction. The solution contains 0.154 mM of Cl$^-$, which is enough to keep ion conductivity of the electrolyte, and the Ag/AgCl electrode has enough reaction area, so it is thought that the change of the slope of the correlation line means the photocatalytic ability of the TiO$_2$ electrode saturated.

**Experiment 2**

Figure 8 shows the photocurrents for different concentrations of glucose measured with TiCl$_4$ post-treated electrode. The photocurrent values increased compared with those by the untreated. This means that TiCl$_4$ post treatment enhanced reaction rate, and so photocurrent. The slope of the correlation line was also larger than that by the untreated (compared with Figure 6); almost twice. This means that both the reaction rate of electrolysis of water and that of oxidation of glucose were increased.

**Experiment 3**

Figure 9 shows the result. Photocurrent was larger at higher concentration spot. This shows that the point to conduct glucose oxidation can be designed with UV light spot on our TiO$_2$ electrode, and so the researched sensor has light-addressing ability.

**CONCLUSION**

The TiO$_2$ electrode we researched has sensitivity to glucose at the range of 0~10mM, and it was higher below 1mM. TiCl$_4$ post-treatment increased the photocurrent and improved the sensitivity. It was also proved that our sensor has an ability of light-addressing through an experiment.

**REFERENCE**


