Algal biosensor array on a single electrode

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An algal array was prepared on a single transparent electrode, and photosynthetic activity of each algal channel and its inhibition by a toxin were monitored with a single-channel potentiostat by successive light irradiation with a LED array.

Introduction

Algae have been used in bioassays for aquatic risk assessment and environmental monitoring.[1–4] In particular, microalgae have been widely used for toxicity tests because of their high sensitivity and reproducibility. However, the toxicity tests based on monitoring of algal growth take several days in general. On the other hand, algal biosensors have been developed for quick assessment of water toxicity. In general, inhibition of photosynthetic activity is monitored on the basis of electrochemical reduction of evolved oxygen[5–13] or fluorescence of chlorophyll contained in chloroplasts.[14–18] Alternatively, respiratory oxygen consumption[19] or preoxidative activity of algal protoplast[20] and gravitaxis[21] or phototaxis[22] of flagellate algae is monitored electrochemically.

Although those sensors give information about the presence and strength of a toxin in a sample, it is generally difficult to identify the toxin. Qualitative analysis of toxins is possible in principle on the basis of different sensitivities of different algal species and strains to toxins.[3,23–26] Podola *et al.*[27] have developed algal sensor arrays based on fluorescence measurement. Algae of different strains were immobilized and arrayed on a membrane filter and fluorescence was measured by a CCD camera. Although the fluorescence-based sensors have better detection limit than the electrochemical sensors in general, the latter does not suffer from turbid sample solutions. In order to fabricate an electrochemical biosensor array, an electrode array coupled with a multi-channel potentiostat is necessary. Alternatively, a scanning microelectrode probe may be used. In either event, equipment would be expensive. In the present work, we developed a more inexpensive electrochemical sensor array with different algal species.

Here we arrayed algae on a single transparent electrode by means of photocatalytic lithography.[28] The electrode, which is in contact with a sample solution, is connected to a normal single-channel potentiostat, and each algal channel is irradiated in turn with a red light from a LED (Fig. 1). Photosynthetic activity of each channel is measured by potentiostatic reduction of oxygen generated as a result of photosynthesis. The recorded cathodic current reflects the total photosynthetic activity of the irradiated algal channel. Thus, successive measurement of the channels is possible with a single electrode by successive irradiation of the channels.

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Experimental

A glass plate coated with indium-tin oxide (ITO) was used as a transparent electrode. The electrode surface was cleaned and hydrophilized by argon sputtering. The electrode was then treated with 5% ethanol solution of octadecyltriethoxysilane (ODS) and annealed at 120 °C for 1 h.

An alkaline-free glass plate with a chromium photomask (Fig. 2a, Dainippon Printing Co.) was coated by spin-coating technique (1500 rpm, 10 s) with anatase TiO_2 sol (ST01, Ishihara Sangyo) diluted with ethanol (1:3 by vol) and annealed at 400 °C for 1 h. The obtained TiO_2–coated photomask was irradiated with UV light (125 mW cm^{-2}) in 50 vol% ethanol–water mixed solution containing 150 μM H_2PtCl_6 for 1 h so as to deposit Pt nanoparticles onto the TiO_2 film[28c] as a consequence of photocatalytic reduction of Pt(IV) and oxidation of ethanol.

Unicellular algae *Chlorella vulgaris* strain C-27 (Institute of Applied Microbiology Culture Collection (IAMCC), Japan), *Pseudokirchnerilla subcapitata* strain C-139 (IAMCC, Japan) and *Chlamydomonas reinhardtii* strain C-9 (IAMCC, Japan) were

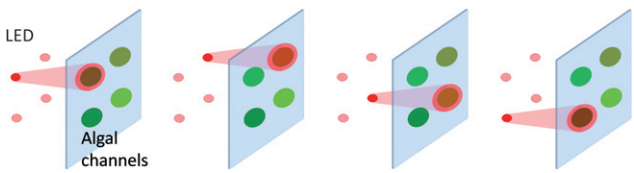

Fig. 1 Schematic illustration for the monitoring of photosynthetic activities of algal channels on a single electrode by successive light-irradiation with a LED array.

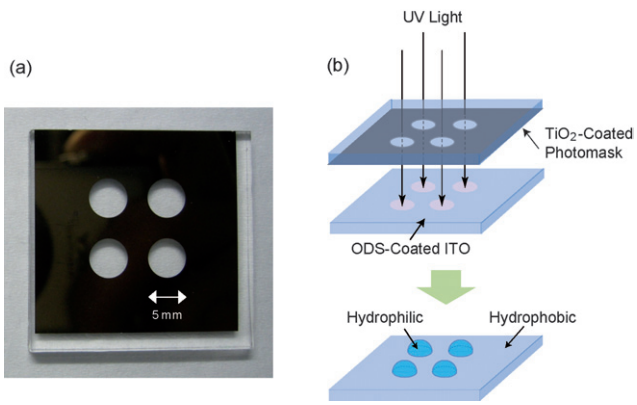

Fig. 2 (a) Photomask coated with TiO_2, which was used for the patterning of an ITO surface. (b) Procedure of the patterning by means of photocatalytic lithography.

cultured in Kessler medium[29] (pH 7.5) in 1 L culture bottles at 25 °C. The culture bottles were aerated through a membrane filter and illuminated by fluorescent light periodically (12 h illumination, 12 h dark).

The Pt–TiO_2-coated photomask was faced with the ODS-coated ITO with an intervening gap of 12.5 μm and irradiated with UV light (100 mW cm^{-2}) for 2 min to obtain a hydrophilic/hydrophobic pattern. A 0.5 μL aliquot of a 1 wt% aqueous solution of poly (L-lysine) (PLL) was cast onto each hydrophilic region, and left for 10 min. Each hydrophilic region was treated as follows so as to immobilize an alga onto the region. An algal suspension (10^{10} cell mL^{-1}) was mixed with an equivolume of 2 wt% sodium alginate aqueous solution, and a 14 μL aliquot of the suspension was applied onto the region. The electrode was left for 30 min and an aqueous 1 wt% $CaCl_2$ solution (14 μL) was cast onto the surface and left for 30 min. Finally, a 1 wt% PLL solution (2 μL) was applied and left for 10 min.

Amperometric measurements were performed in a 6.7 mM phosphate buffer (pH 7.4, 23 °C) with a Ag/AgCl reference electrode and a coiled Pt wire counter electrode. The algal array working electrode was polarized at −0.7 V *vs.* Ag/AgCl and a cathodic current for oxygen reduction was monitored. Each algal channel was irradiated with red light from a LED.

Results and discussion

The surface of the argon-sputtered ITO electrode showed good hydrophilicity (water contact angle (WCA) ~ 5°). The treatment with ODS made the surface hydrophobic (WCA ~ 105°). The ITO electrode modified with ODS was subjected to photocatalytic lithography (Fig. 2b). Two minutes irradiation was sufficient to change the irradiated region from hydrophobic to hydrophilic. It is known that the alkyl chain of ODS is removed in the photocatalytic lithography.[28] Algae were immobilized on the hydrophilized regions.

The prepared algal array electrode was subjected to electrochemical monitoring of their photosynthetic activities. In the present system, it is important to irradiate each channel independently, without leaking of light, so as to monitor a response from a single channel. The leaking might stimulate not only the target algal channel but also other channels, resulting in crosstalk between the channels. To examine this potential issue, we fabricated a four-channel array with three identical *Chlorella* channels and one blank channel without alga (Fig. 3a). Experiments were carried out with or without crossed fins that shield light from a channel to other channels.

In a pH 7.4 phosphate buffer, the electrode was polarized at −0.7 V *vs.* Ag/AgCl, and each channel was irradiated with a red LED in turn. When an algal channel was irradiated, a cathodic current response was observed, due to reduction of oxygen generated as a result of algal photosynthesis. The current decreased upon removal of the light. However, a similar amperometric response was observed even when the blank channel was irradiated, in the absence of the shield (Fig. 3b). The current response when the blank channel was irradiated was *ca.* 70% of those obtained when an algal channel was irradiated. This suggests that the LED for the blank channel illuminates not only the blank channel but also algal channels, resulting in the crosstalk.

On the other hand, when the light shield was mounted onto the LED array, irradiation of the blank channel gave no response (Fig. 3c). These results indicate that the employed shield is effective in preventing the crosstalk.

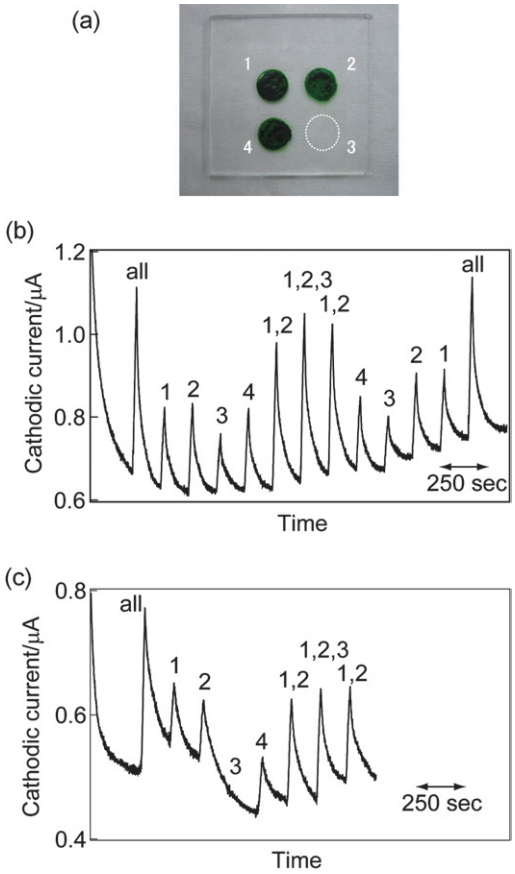

Fig. 3 (a) Algal array electrode with three *Chlorella* channels and one blank channel and (b, c) its cathodic current responses when channels are light-irradiated successively or simultaneously, (b) without or (c) with the light shield.

We also fabricated an array with four identical *Chlorella* channels on a single electrode (Fig. 4a). The electrode was polarized at −0.7 V *vs.* Ag/AgCl in a pH 7.4 phosphate buffer and one of the channels was irradiated, or two to four channels were irradiated simultaneously, with the red LED array equipped with the light shield.

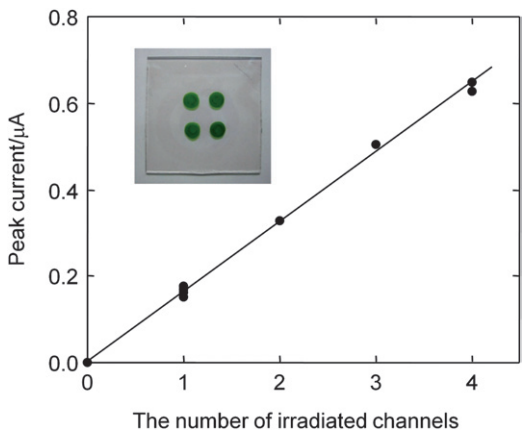

Fig. 4 (a) Algal array electrode with four *Chlorella* channels and (b) its cathodic current responses when 1–4 of the channels are light-irradiated simultaneously with the light shields.

Table 1 Cathodic current responses of an algal array electrode with three different cell channels before and after addition of 10 mM formaldehyde

	Response/μA	
Cell	Before addition	After addition
Chlorella vulgaris	0.4	0.2
Pseudokirchnerilla subcapitata	0.7	0.1
Chlamydomonas reinhardtii	0.7	0.1

Dependence of the peak current on the number of channels irradiated are shown in Fig. 4b. The peak current was almost perfectly proportional to the number of channels.

Next, a four-channel array with three different algae was prepared. Three channels were modified with *Chlorella*, *Pseudokirchnerilla* and *Chlamydomonas*. The other channel was a blank one. All the algal channels exhibited current responses to the LED irradiation, although the peak currents were different due to different total photosynthetic activities of the channels (Table 1). Then, formaldehyde (final concentration, 10 mM) was added to the buffer, and the peak currents were suppressed because formaldehyde inhibits photosynthesis. Although the *Chlorella* channel exhibited the smallest current before the addition of formaldehyde, it exhibited the largest current after the addition. This indicates that *Chlorella* is less sensitive (*i.e.* more resistive) to formaldehyde than the other two algae. For more precise measurements, successive collection of the responses from the different channels may be repeated until constant responses are obtained. If algal species are carefully selected, one may predict a type of an unknown toxin present in a sample, on the basis of inhibition of photosynthetic activities of the different algae.

Conclusions

An algal array was prepared on a single electrode, by means of photocatalytic lithography. Photosynthetic activity of each algal channel was monitored with a single-channel potentiostat by successive light irradiation with a LED array. This can be applied to multi-channel toxin sensing based on inhibition of algal photosynthetic activities.

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