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Light-addressable planar electrode with hydrogenated amorphous silicon and low-conductive passivation layer for stimulation of cultured neurons

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Abstract— **Conventional multielectrode arrays (MEAs) cannot always access desired neurons due to low electrode density and small number of electrode. To overcome this problem, we propose a light-addressable planar electrode on a glass substrate. The electrode has a 3-layer structure, namely a transparent SnO_2 layer, an hydrogenated amorphous silicon (a-Si:H) layer, and a passivation layer. Illumination to the a-Si:H layer increases the conductivity of a-Si:H and creates a virtual electrode at the surface of the illuminated site. In the present study, we developed a low-conductive zinc antimonate-dispersed epoxy layer. This layer could successfully prevent penetration of culture medium and thus deterioration of a-Si:H layer. A fluo-4 calcium imaging demonstrated that, when the whole area of electrode was illuminated, negative-monophasic voltage-controlled pulses could also successfully activate neurons cultured on the electrode. Moreover, the focused illumination to the electrode resulted in the selective activation of neurons around the illuminated area.**

I. Introduction

Conventional multielectrode arrays (MEAs) for monitoring or stimulating cultured dissociated neurons [1]-[3] have limitations in terms of their number and density of electrodes. These limitations lead to the limited accuracy of addressing recording or stimulating sites on electrodes. In order to overcome this problem, some groups have proposed "light-addressable" electrodes, which utilize photo-conductivity of silicon wafers or hydrogenated amorphous silicon (a-Si:H) [4]-[8]. With both types of the electrodes, illumination to photoconductor generates a virtual electrode on the electrode surface, not depending on predefined electrode positions. Monocrystalline silicon can be employed without requiring any special processing [4], [5], but cannot be deposited as a thin film. A-Si:H can be deposited in form of thin film on any kinds of substrates, but is deteriorated and finally dissolved by culture medium with pH of 7.2. Bucher and his colleagues overcame the a-Si:H deterioration by introducing a passivation layer which consisted of laterally insulated sub-μm electrode pads and SiO_x:C-insulator over a-Si:H layer [6]-[8]. But its highly refined structure required complicated fabrication processes.

In the present study, we propose a planar electrode with a simpler structure, employing a-Si:H as photoconductor. First, we develop a low-conductive passivation layer, which can be deposited just by spin-coating. Second we confirm that the layer could prevent penetration of culture medium and thus deterioration of a-Si:H layer. Finally, we demonstrate that illumination can selectively address the stimulation site, using the fluo-4 calcium imaging.

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II. Material and Methods

A. Design of the electrode

Figure 1(a) illustrates the design of the present electrode. The electrode has a 3-layer structure on a glass substrate, namely a transparent SnO_2 layer, an a-Si:H photoconductor layer and a low-conductive passivation layer. Illumination locally increases the conductivity of the a-Si:H layer and generates a virtual stimulating electrode. Then, applied electrical pulses between the SnO_2 layer and a ground electrode in culture medium stimulate neurons around the illuminated site. As shown in Fig. 1(b), the thin low-conductive passivation layer is expected to block current along the plane, and thus, be conductive only along the thickness direction. Thus, the current flow from the stimulator does not diffuse out of the illumination-generated stimulation site and selectively activates the neurons around the illuminated site.

B. Passivation layer

Zinc antimonate-dispersed epoxy film is developed as the low-conductive passivation layer. The developed film is useful in the present electrode passivation for the following reasons. First, epoxy resin used as binder has low water absorption property. The film, thus, can prevent the penetration of culture medium into the a-Si:H layer. Second, this film can be easily deposited on a substrate by the conventional spin-coating method. After being spin-coated in the fluid state, epoxy precursor is baked and polymerized in situ. This formation procedure is effective for covering the

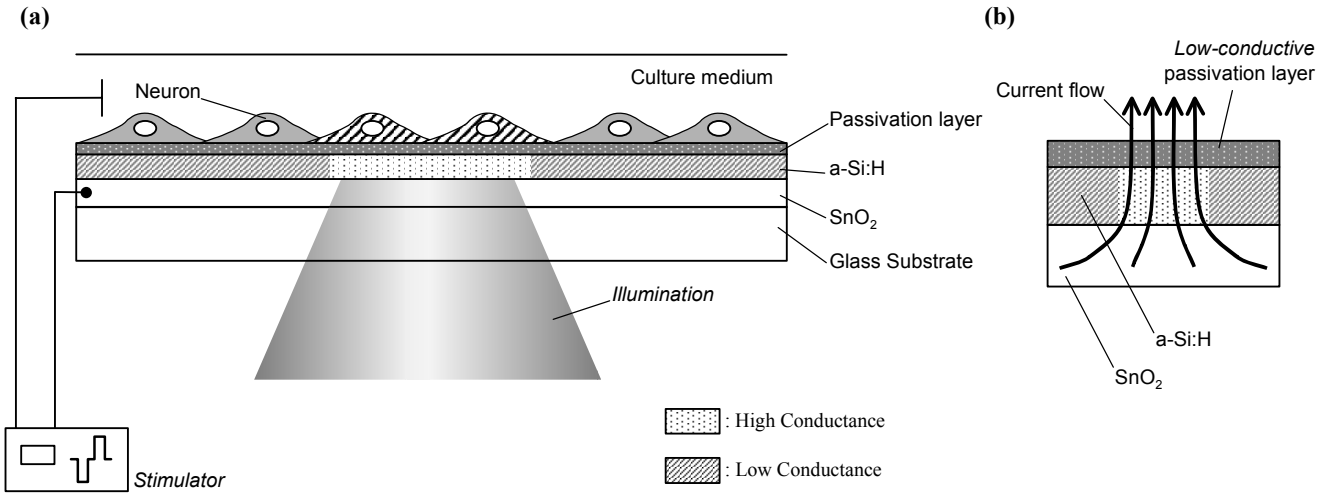

Fig. 1. Design of the proposed electrode. (a) Schematic diagram of the electrode. Illumination increases the conductivity of a-Si:H layer and generates a "virtual" stimulating electrode. (b) Current flow through the low-conductive passivation layer. For its low conductivity, the layer is expected to be conductive only along the thickness direction.

entire electrode surface without defects. Third, its conductivity can be adjusted by changing mixing ratios of zinc antimonate ($ZnSb_2O_6$) sol and epoxy resin. Finally, the formed film has translucency, which makes it possible to obtain phase-contrast images of cultured cells.

C. Fabrication process

A-Si:H was deposited by CVD on a glass substrate covered with 1-μm SnO_2 transparent metal film (Asahi Glass, A110U80). The substrate was cut into 10x20-mm pieces and a part of each piece was masked with polyimide tape. Then, a passivation layer was deposited by spin coating and the mask tape was removed. The precursor of the zinc antimonate-dispersed epoxy resin included epoxy resin (Nippon Kayaku, RE-304S), zinc antimonate sol-dispersed isopropyl alcohol (Nissan Chemical Industries, CX-Z210IP), phenol resin (Nippon Kayaku, GPH-65), and methyl isobutyl ketone. The coated precursor was cured at 120 °C for 2 h and at 150 °C for 6 h. The sheet resistance of finished passivation layer was approximately 10 MΩ/sq. A-Si:H of the masked area was wet-etched with 50 wt.% KOH aqueous solution, resulting in the exposure of the SnO_2 layer for electrical contact to external devices. Finally, a silicone rubber packing and a 35-mm petri dish with a 4-mm through hole were attached to the electrode with silicone adhesive (GE Toshiba

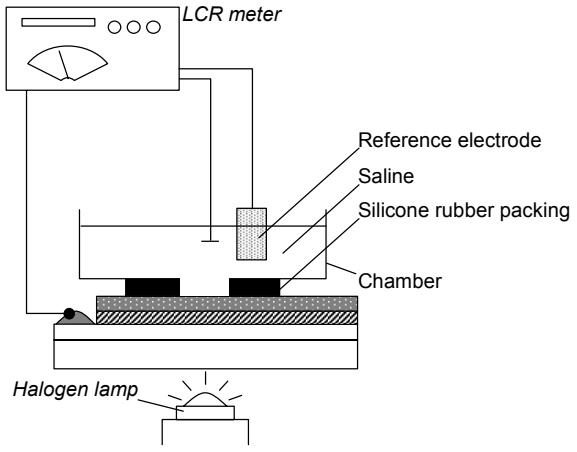

Fig. 2. The impedance measurement setup. The impedance of the 4-mm diameter area facing the electrolyte was measured underneath and without illumination.

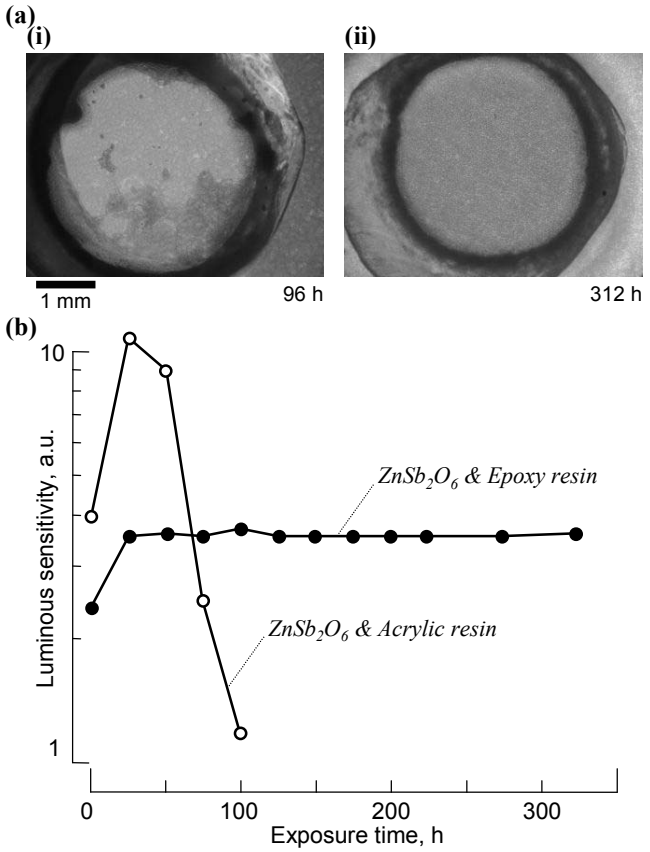

Fig. 3. Exposure test to culture medium. (a) Optical micrographs of the exposed electrodes with the acrylic (i) and epoxy (ii) passivation layer. Total exposure times are shown beneath each photograph. (b) The temporal changes of the luminous sensitivities during exposure.

Silicone, TSE3975) as a culturing chamber.

D. Exposure test

The fabricated electrode was tested for durability against culture medium. The electrode was exposed to Dulbecco's Modified Eagle's Medium (DMEM, Gibco) filled in the culturing chamber and placed in atmospheric air kept at 37 ^{o}C and 100 % humidity. To monitor the electrode condition during exposure, the luminous sensitivity of the exposed area was measured. The luminous sensitivity is defined as the dark to bright impedance ratio at 1 kHz, which was measured every 24 h by the multifrequency LCR meter at amplitude of 50 mV. Figure 2 shows the measurement set up. An optical-fiber-guided 150-W halogen lamp was used for illumination from underneath. In addition to the passivation film with epoxy binder resin, acrylic binder resin was also tested for comparison. The film thickness of tested epoxy passivation layer, acrylic passivation layer and a-Si:H layer were 3 μm, 3.5 μm and 140 nm, respectively.

E. Culturing experiment

In order to evaluate the "light-addressing" capability, dissociated neurons taken from Wistar rat embryos [9], [10] were cultured on the electrode and stimulated under illumination. The fluo-4 calcium imaging with cooled-CCD camera (Hamamatsu Photonics, C8800-21C) [11] was used to monitor the effect of the electrical stimulation. For illumination to the a-Si:H layer, we utilized the excitation light of fluo-4 with the wavelength of 488 nm. The excitation light was focused on the surface of the electrode with the

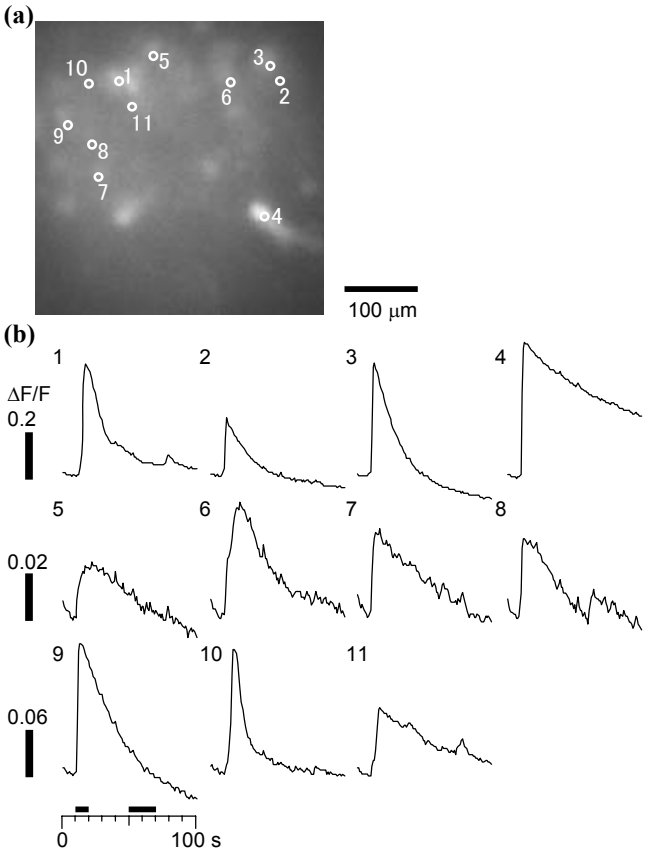

Fig. 4. The fluorescence transients recorded during the whole-area stimulation of 5 V. (a) The fluorescence raw image obtained after the stimulation. (b) The fluorescence transients of the neurons indicated in (a). The stimulating periods are shown by black bars.

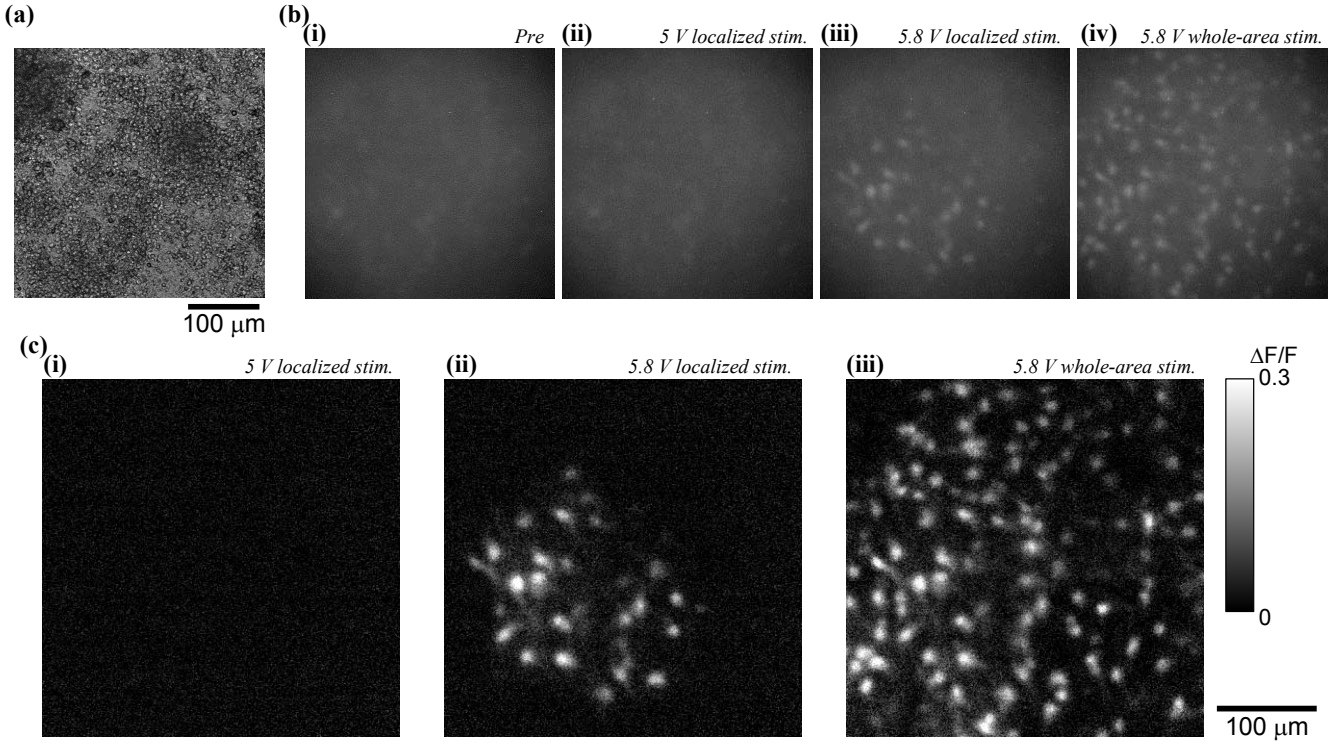

Fig. 5. Neurons activated by the localized stimulation. (a) The optical micrograph of the observed area. (b) The fluorescence raw images before stimuli (i), after the localized stimuli of 5 V (ii) and 5.8 V (iii), and the whole-area stimulation of 5.8 V (iv). (c) The post-stimulus differential images after the localized stimuli of 5 V (i) and 5.8 V (ii), and the whole-area stimulation of 5.8 V (iii), calculated with the pre-stimulus image shown in (b, i).

objective lenses of the inverted microscope (Olympus, iX70). First, voltage-controlled electrical pulses were applied at the same time as fluo-4 imaging. By the excitation light, the imaged area was entirely light-addressed and stimulated (hereinafter called "whole-area stimulation"). We estimated the stimulation threshold with the present electrode by varying the stimulation amplitude. Second, the electrical pulsed were applied under the excitation light focused with the higher magnification objective lens (hereinafter called "localized stimulation"). Before and after the stimulation, fluorescence images were obtained with the lower magnification objective lens. The differential image between the pre- and post-stimulus fluorescence image revealed the coverage of activated neurons. The electrical pulses were negative- monophasic rectangular waves with 1-3 ms duration and 100 Hz frequency and were applied between the SnO_2 layer and the 50-μm-diameter platinum wire in culture medium.

III. Results and Discussion

Figure 3(a) show the optical images of the electrodes with the acrylic and epoxy passivation layer after the 96-h and 312-h exposure to DMEM, respectively. The a-Si:H layer covered with the acrylic passivation layer was deteriorated and diluted, while that with the epoxy passivation layer was stable over 2 weeks. Figure 3(b) shows the temporal changes of the electrode luminous sensitivities. The sensitivity of the electrode with the epoxy passivation layer was stably kept around 3.5. These results indicate the impermeable property of the developed zinc-antimonate dispersed epoxy film.

Figure 4(a) shows the fluorescence raw image after whole-area stimulation with the voltage of 5 V. As shown in Fig. 4(b), the fluorescence transients of the neurons indicated in Fig. 4(a) rose in synchronization with the electrical stimulation. The stimulation threshold was thus estimated approximately at 5 V with this electrode.

Localized stimuli with voltages around the estimated threshold were administered to the area shown in Fig. 5(a). Figure 5(b) shows fluorescence raw images before and after the stimuli. Figure. 5(c) shows the post-stimulus differential images, which were calculated with the pre-stimulus image shown in Fig. 5(b, i). Fig. 5(c, ii) shows the differential image after the localized stimulation of 5.8 V. As compared with the differential image after the whole-area stimulation of 5.8 V shown in Fig. 5(c, iii), the coverage of the localized stimulation-activated neurons is narrower. This result demonstrates that the focused illumination successfully addressed the stimulating site and the neurons under the illuminated area were stimulated selectively.

Neurons on the passivation layer seemed to grow normally. The cell densities on glass and passivation layer showed no significant difference as of 5 DIV. This suggests that the developed zinc antimonate-dispersed epoxy film has no toxic influence on cultured neurons.

IV. Conclusion

In this study, we proposed and developed a light-addressable planar electrode with a-Si:H. The present electrode consisted of 3-layer structure on a glass substrate, namely, a transparent SnO_2 layer, an a-Si:H photoconductor layer and a low-conductive passivation layer. We developed the low-conductive passivation layer with zinc antimonate-dispersed epoxy resin. The exposure test demonstrated that the developed passivation layer has impermeablility to culture medium. The fluo-4 calcium imaging revealed that the localized stimulation of 5.8 V selectively activated the cultured neurons around the illuminated area. This result indicates that the focused illumination to the electrode successfully addressed the stimulating site.

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