Supporting Information

Electrochemical topography of a cell monolayer with an addressable microelectrode array

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Fabrication of microelectrode array

An electrode array with 6 platinum microbands was constructed on a glass substrate using a photolithographic method. The glass substrates were cleaned by ultrasonication in acetone and 2-propanol. It was then spin-coated (3000 rpm for 30 s) with a primer and positive photoresist (S1818, Shipley Far East Ltd., Japan), and subsequently baked at 95°C for 5 min. This was followed by irradiation with UV light for 11 s through a chromium mask with microelectrode patterns. After developing with a developer, Ti and Pt were deposited on the substrate by sputtering to create a Ti/Pt multilayer. The electrode pattern was revealed with a lift-off technique after immersing the electrode substrate in an acetone bath.

Each band was 100 nm thick and 50 μm wide and was separated by a distance of 250 μm from the adjacent band electrodes. A rectangle wall without one side was fabricated on the substrate by photolithography using an SU-8 sheet (thickness: 15-μm; TR61965, Taiyo Ink MFG Co., Tokyo, Japan). As shown in Fig.1b, another glass substrate without the SU-8 sheet (act as column electrodes) was covered on the top of the glass substrate with the SU-8 sheet (act as row electrodes) so that the two microelectrodes arrays one the glass substrates were orthogonal and faced each other,

24 the column electrodes and row electrodes forming 36 orthogonal crossing points.
25 These points will act as the addressing points in this study. The open space held by the
26 SU-8 sheet between the two glass substrates served as the working area.

27 **Instrumentation**

28 Three channels (W1 to W3) of a multichannel potentiostat (HA-1010mM4,
29 Hokuto Denko, Corp., Tokyo, Japan) were used for potential control and current
30 acquisition. The potentiostat was connected to the electrodes by a multiplexer
31 (PXI-2529 combined with TB-2634, National Instrument, Austin, USA) and the data
32 was controlled and collected by a program developed by Labview (National
33 Instrument, Austin, USA) through an AD/DA converter (PXI-6723, National
34 Instrument, Austin, USA). Ag/AgCl and a Pt wire were used as the reference and
35 counter electrodes, respectively.

36 **2.3 Cell pattern preparation**

37 HeLa cells, derived from a human cervix epithelial cell line, were routinely
38 cultured in a RPMI1640 medium (Gibco) containing 10% fetal bovine serum (Gibco)
39 and 1% penicillin in a humidified incubator (37°C in an atmosphere of 5% CO_2). The
40 HeLa cells were harvested and treated with a 0.25% trypsin solution before being
41 seeded into the wells. The concentration of the cell suspension was approximately $1 \times$
42 10^6 cells/mL.

43 A PDMS (Sylgard 184, Dow Corning, Co.) stencil with a 500 μm × 500 μm hole
44 was fabricated using a CO_2 laser engraving system (Universal Laser System Inc.) and
45 the surface of the PDMS was treated with O_2 plasma [1，2]. After sterilizing in 70%

46 ethanol, the stencil was attached to the substrate with the microelectrode array. Then,
47 the cell suspension solution was dropped on the surface of the PDMS stencil. After
48 cultivation for 6 h, the stencil was removed to leave the patterned cells adhered to the
49 surface of the substrate. The substrate combined with another electrode substrate as
50 described above to form the addressable device.

51 **Detection procedure**

52 The detection procedure has been described in our earlier reports [3]. In brief, the
53 row electrode substrate with the patterned cells was rinsed carefully with PBS(-)
54 solution. Then a 4.0 mM $Fe(CN)_6^{3-}$ solution was applied to the substrate, which was
55 then covered with the column electrode substrate. Electrochemical detection was
56 performed as follows: a voltage (V_C = 0 V) was applied to all the column electrodes
57 through W1 of the potentiostat. At the same time a different voltage (V_R = 0.5 V) was
58 applied to the row electrode 1 (R1) through W3 of the potentiostat for 2 s for
59 preconditioning purposes to stabilize the current, while the other row electrodes
60 (R2–R6) were set at V_C through W1. Then, the column electrode 1 (C1) was
61 connected to W2 set at V_C, and the current data were transferred to a PC. This
62 read-out process was sequentially repeated from C1 to C6 with an interval of 20 ms
63 for the read-out time in each step. All the current responses through W2 were read out,
64 while W1 was used only for the potential control. Since all of the row electrodes were
65 connected to W1 (set at V_C) during the above process, no redox cycling was expected
66 at the crossing points on R2–R6 during scanning. The same measurements were
67 sequentially repeated for the other row electrodes (R2–R6) to address and acquire all

68 the responses at every crossing point. The total detection time for the 36 addressing
69 points was less than 15 s. After the measurement, the substrate was rinsed with PBS(-)
70 solution and the patterned cells were again cultivated in the cultivation medium.

71 After detection, the dada at all addressable point were collected and applied to
72 construct the cell images with a Matlab program (MathWorks, Natick, Massachusetts,
73 USA).

74 **Reference**

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