Supporting Information for

Electrochemical Detection of Epidermal Growth Factor Receptors on a Single Living Cell Surface by Scanning Electrochemical Microscopy

Yasufumi Takahashi[a], Takeshi Miyamoto[a], Hitoshi Shiku*[a], Ryutaro Asano[b], Tomoyuki Yasukawa[c], Izumi Kumagai[b], and Tomokazu Matsue*[a]

[a]Graduate School of Environmental Studies, Tohoku University, 6-6-11-604 Aramaki, Aoba-ku, Sendai 980-8579, Japan, matsue@bioinfo.che.tohoku.ac.jp

[b]Graduate School of Engineering Studies, Tohoku University, 6-6-11-607 Aramaki, Aoba-ku, Sendai 980-8579, Japan

[c]Graduate School of Material Science, University of Hyogo, 3-2-1 Kouto, Kamigori-cho, Ako-gun, Hyogo 678-1297, Japan

Flow cytometric analysis.

Figure S1 shows the epidermal growth factor receptor (EGFR) expression level of normal Chinese hamster ovary (CHO), CHO transfected with EGFR (EGFR/CHO), and A431, as measured by flow cytometry. The cells (1×10^6) were fluorescently labeled by incubating on ice with mouse anti-EGFR IgG-FITC conjugation (1 μg/ml) for 30 min. After washing with PBS containing 0.1% NaN_3, the cells were analyzed by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA). The details of the flow cytometric analysis were reported previously.[5]

SECM images of EGFR on microstenciled, patterned living cells

Figure S2 shows scanning electrochemical microscopy (SECM) images of the two types of CHO cell patterns that comprise 300-μm-diameter circles formed by the microstencil method, together with corresponding optical microscope images. As described in the manuscript, the SECM images, which are based on the oxidation of PAP yielded by the catalytic reaction of ALP, reflect the amount of EGFR on the cell surfaces. EGFR/CHO cells showed large current responses, while no current response was observed for normal CHO cells, indicating that EGFR-expression level at the cell surface can be clearly imaged with SECM. Since normal CHO cells show no current responses, undesired influences caused by physical absorption of enzyme-labeled antibodies on the cell surface should be negligible. The EGFR-expression level of the patterned cells was estimated by SECM single-scan measurements and shown in Fig. S3. The expression level of EGFR/CHO cells was considerably higher than that of

normal CHO cells. For comparison, a large current response was observed for A431 cells. The current response of normal CHO, EGFR/CHO, and A431 cells were 10 pA, 65 pA, and 2.4 nA, respectively. The currents were statically distinguishable ($p < 0.001$, normal CHO and EGFR/CHO; $p < 0.001$, EGFR/CHO and A431) with SECM. Inappropriate pH caused morphology changes. We circumvented this problem by adding 10% fetal bovine serum (FBS) in an HEPES-based saline solution of pH 9.5. Figure S4 shows the cells 3 h after exchanging the RPMI-1640 medium with an HEPES-based saline solution of pH 9.5. By adding FBS, the cells maintained an adhesion state.

Membrane penetration effects of $FcCH_2OH$

Figure 4 shows the SECM image of the Dp-labeled EGFR/CHO cells. Previously, Bard et al. used $FcCH_2OH$ to monitor cellular respiration.[19] In our experiment, an oxidation current increase (positive feedback) was observed above the CHO cellular pattern in cells that were not enzyme-labeled (Fig. S5). Interestingly, we found that the current increased when the tip scan rate increased. This phenomenon may be explained by a very large partition coefficient of $FcCH_2OH$ between the cell membrane and the aqueous solution ($Kp = [FcCH_2OH]^{cell\ membrane}/[FcCH_2OH]^{water}$). In Figure 4, we succeeded in measuring EGFR activity using Dp as the labeling enzyme and $FcCH_2OH$ as the mediator. However as shown here, the ferrocenemethanol ($FcCH_2OH$) oxidation current monitored in this system can also be affected by cellular respiration and membrane permeation properties.

Figure S1. Comparison of EGFR expression level by flow cytometry.

Figure S2. Microstencil-patterned CHO cells SECM image in substrate generation/tip collection mode. (a) EGFR/CHO cells (b) normal CHO cells. ALP was used as labeling enzyme. The measurement was conducted in pH 9.5 HEPES + 10 % fetal bovine serum (FBS), 4.7 mM PAPP. The electrode was set at 20 μm above the substrate, and the scan rate was 20 μm/s. The scan range was 700 μm × 700 μm, and the step size was 10 μm.

Figure S3. Relative EGFR expression levels of the microstencil-patterned cells measured using substrate generation/tip collection mode SECM.

Figure S4. Effect of the solution pH on the shape of the adhesion cells. EGFR/CHO cells were incubated for 3 h in (a) pH 9.5 HEPES buffer, (b) pH7.0 HEPES buffer, (c) RPMI-1640, and (d) pH 9.5 HEPES + 10% FBS.

Figure S5. One-line scan of nonlabeled microstencil-patterned EGFR/CHO cells using ferrocenemethanol ($FcCH_2OH$) as an electron mediator in feedback mode SECM. The electrode was set at 15 μm above the substrate, and the scan rate was 10, 20, or 50 μm/s.

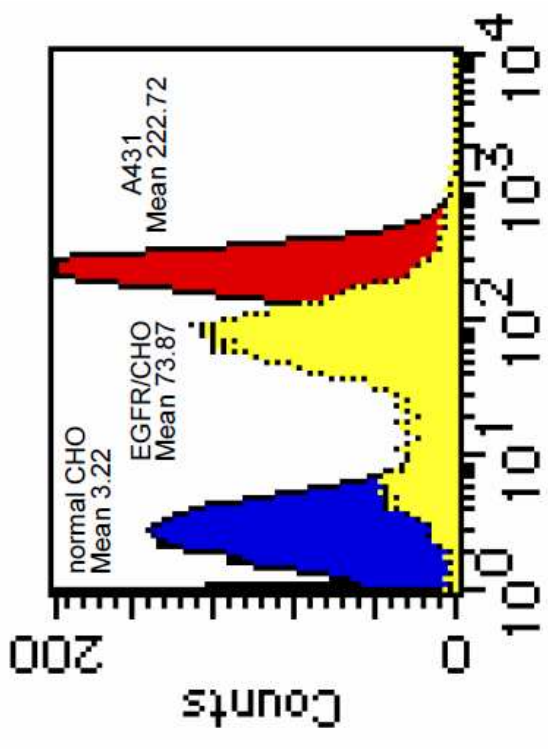
normal CHO
Mean 3.22
EGFR/CHO
Mean 73.87
A431
Mean 222.72
Counts
200
0
10^0
10^1
10^2
10^3
10^4

Figure S1.

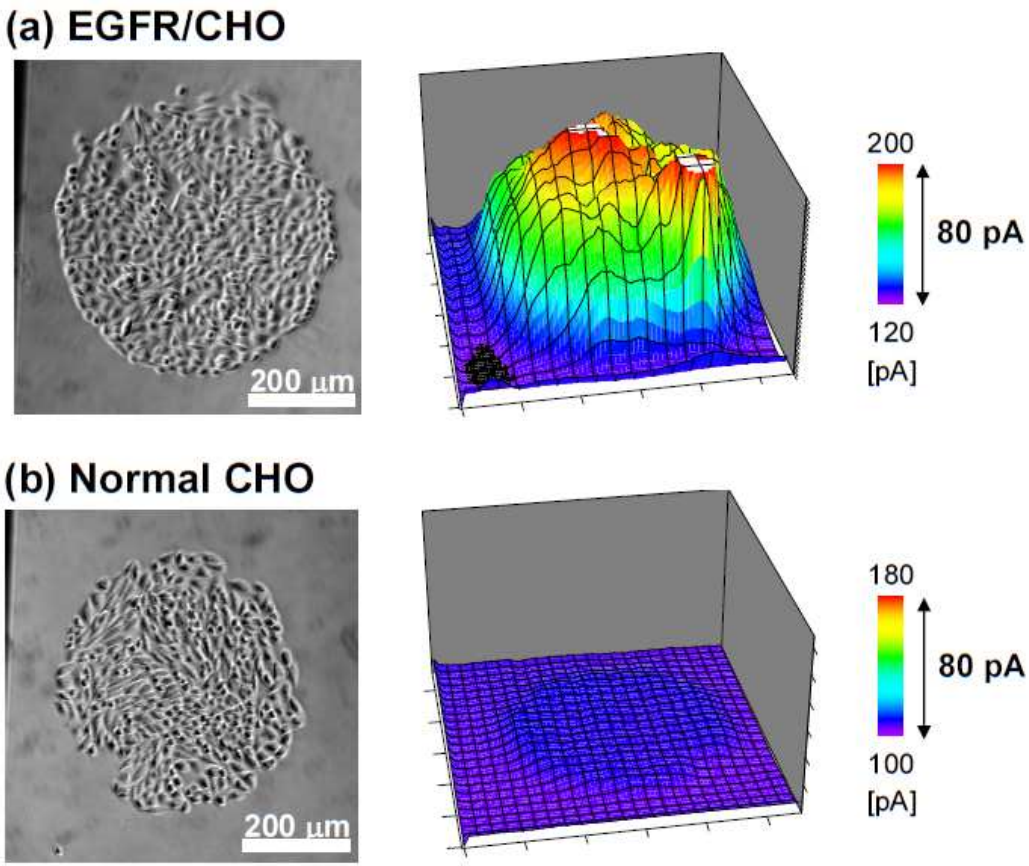
(a) EGFR/CHO
200 μm
200
80 pA
120
[pA]
(b) Normal CHO
200 μm
180
80 pA
100
[pA]

Figure S2.

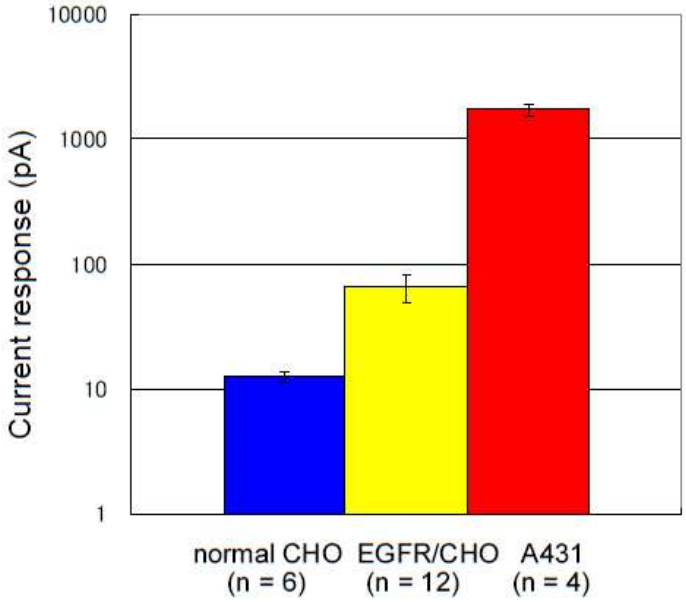
10000
1000
100
10
1
Current response (pA)
normal CHO
(n = 6)
EGFR/CHO
(n = 12)
A431
(n = 4)

Figure S3.

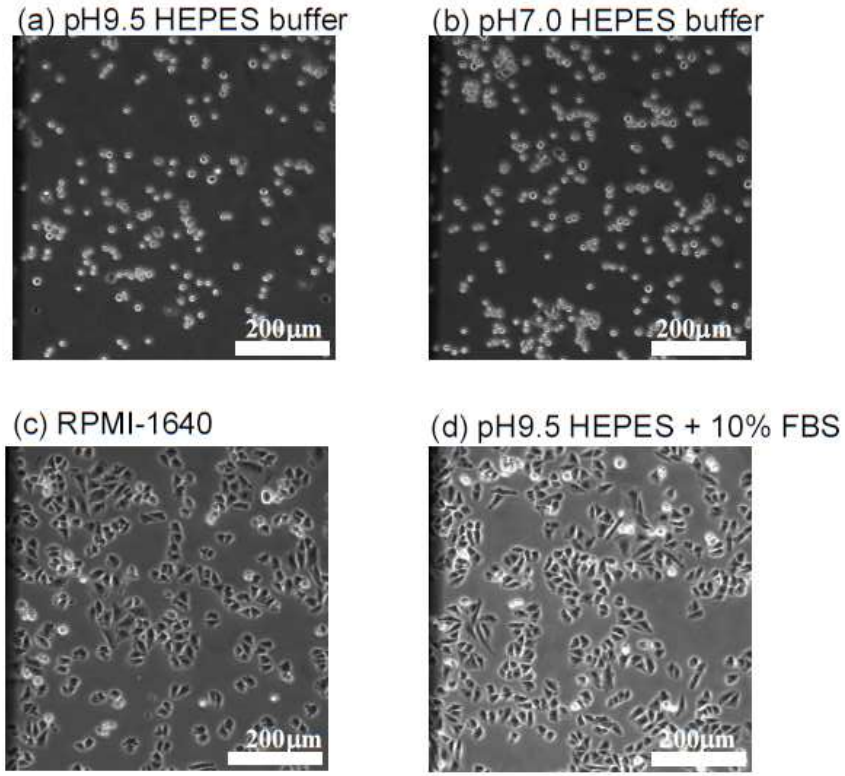
(a) pH9.5 HEPES buffer
200μm
(b) pH7.0 HEPES buffer
200μm
(c) RPMI-1640
200μm
(d) pH9.5 HEPES + 10% FBS
200μm

Figure S4.

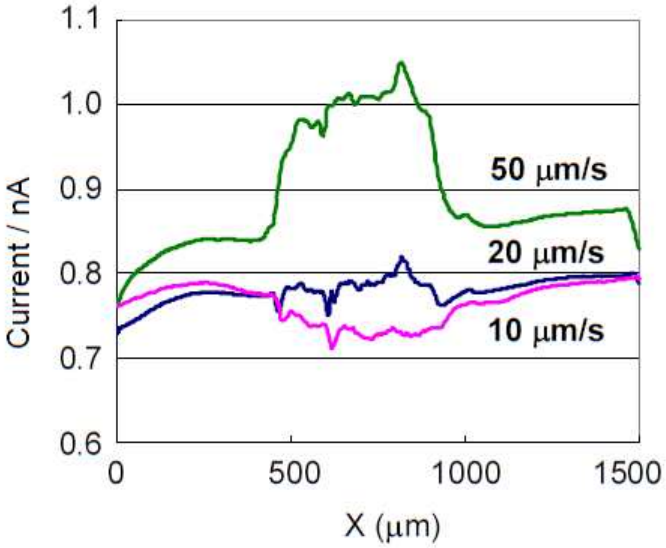
1.1
1.0
0.9
0.8
0.7
0.6
Current / nA
0
500
1000
1500
X (μm)
50 μm/s
20 μm/s
10 μm/s

Figure S5.