Evaluation of Differentiation State of an Embryonic Stem Cell using Scanning Electrochemical Microscopy

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We will report the evaluation of differentiation state of a single embryonic stem (ES) cell by scanning electrochemical microscopy (SECM). ES cells have properties of self-renewal and pluripotency that make them invaluable tools for regenerative medicine. But monitoring of the differentiation state of ES cells is still difficult. Quantitative and non-invasive measurement technique is required to monitor the differentiation state of ES cells for promoting the research of tissue engineering. SECM, which uses a microelectrode for detecting localized electroactive chemical concentration as faradaic current with non-invasive, is an effective tool for estimating oxygen consumption of a single living cell and detecting enzyme activity. [1-5] To evaluate differentiation state of ES cells, we measured intracellular alkaline phosphatase (ALP) expression level. ALP is known as a differentiation-marker and the expression level of ALP decreases during differentiation process. The oxidation current of pAP (p-aminophenol), produced by the ALP-catalyzed reaction, was monitored by SECM. A faradaic current image corresponding to the differentiation state is available at the single-cell level. In previous works, we succeeded to detect intracellular ALP activity using SECM[4,6] and developed electrochemical device. [7]

The mouse ES cell line was purchased from DS Pharma Biomedical. Co., Ltd., strain 129/SV, passage 11. To differentiate the ES cells, ES cells were cultured in medium containing retinoic acid (5 μM), the most commonly used neuralizing agent during in vitro ES cell differentiation. Pt microelectrode (diameter, 20 μm) was positioned 30 μm above the cell. The microelectrode position was controlled by motor-driven XYZ stage (K701-20RMS, Suruga Seiki). Schematic illustration of SECM measurement shows in Figure 1. The potential of electrode was held at 0.3 V vs. Ag/AgCl to measure oxidation current of pAP. SECM measurement was performed in HEPES buffer solution including 4.7 mM p-aminophenyl phosphate (pAPP).

Figure 2 shows optical and SECM images of undifferentiated and differentiated ES cells at single cell level. The current response of the undifferentiated ES cell (29.4 ± 14.3 pA) was considerably larger than that of differentiated ES cell (0.49 ± 0.25 pA) ($p < 0.001$). This result indicates that SECM can distinguish the differentiation state of a single ES cell without labeling. Next we evaluated the ES cells which were cultured for 3 days in medium containing retinoic acid. Figure 3 shows optical and SECM images. Most cells show neuritis with no or only small electrochemical responses; however some of those cells show round in shape and high electrochemical responses. Since the electrochemical responses are induced by ALP activity, this result indicates that SECM is useful to discriminate differentiation state which is difficult to recognize from morphological change.

References
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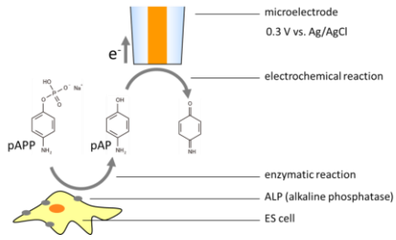

Figure 1 Schismatic illustration of SECM measurement for evaluating the differentiation state of an ES cell.

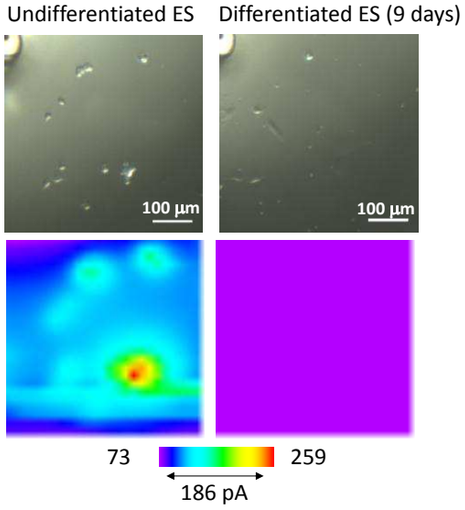

Figure 2 SECM images of undifferentiated ES cells and differentiated ES cells. The differentiated ES cells were cultured for 9 days in medium containing retinoic acid.

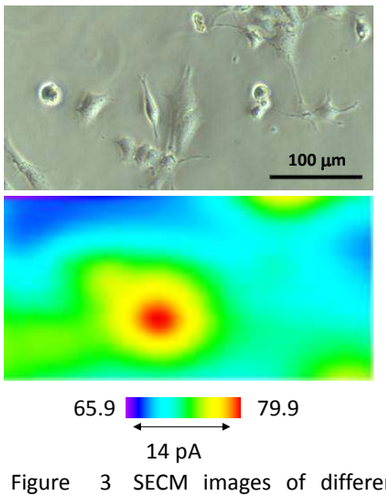

Figure 3 SECM images of differential processing ES cells. The ES cells were cultured for 3 days in medium containing retinoic acid.