A Porous Membrane-based Culture Substrate for Studying Chemical and Electrical Local Stimulation of Cell Culture

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Introduction

In-vitro assays using cultured cells have been generally carried out for cytological diagnosis and for evaluating the cytotoxic effect of chemicals as an alternative to animal experiments. For the studies evaluating the effect of chemicals on adherent cells, local dose technique has a merit to provide simultaneous control experiment. In this presentation, we describe a culture system applicable for subjecting cells to local stimulation and the subsequent Scanning Electrochemical Microscopy (SECM)-based bioassay. As the other application, we studied cell-cell interaction in the cellular sheets by integrating this culture system with the cellular micropattern technique. The capable use of this substrate as the in situ electroporation device for adherent cells has also been studied.

Experimental

Fig. 1 shows the experimental setup for the local stimulation. The micromask made of PDMS film was prepared using a columnar photoresist pattern as the template [1]. The resulting PDMS film (40 µm thickness) having a 50–200 µm diameter hole was stuck to the bottom of a culture insert (Nalge), consisting of a microporous membrane (pore size 0.02 µm). Cells (HeLa, KMST-6 (human embryonic fibroblast cell line) and primary cardiac myocytes obtained from herts of chick embryos at 8-9days [2]) were suspended in medium, transferred into assembled culture substrate precoated with collagen to promote cell adhesion and grown for 2 days to reach confluent monolayer. Chemicals for local dose were added to the solution outside the culture insert. Electric fields were applied by using Pt plate electrodes set inside and outside the culture insert. SECM measurements [3] were conducted in HPEPS buffer solution.

Results and discussion

After 20 minutes local dose of ethanol, HeLa cells cultured on right above a hole of the micromask were stained by Propidium Iodide (PI; membrane impermeable fluorescent dye) (Fig. 2a). The structural features of the porous membrane (high density, regular cylindrical pores) were responsible for the area-limited transport of the chemicals without lateral diffusion. Also, the elastomeric character of the PDMS was suitable to make a tight attachment and prevent lateral soak up at the boundary between the mask and the membrane. As shown in Fig. 2b, the reduction current values measured around stimulated cells (●) were increased during the electrode approach, while the currents for non-stimulated cells (○) were almost independent of electrode height, indicating the exudation of some reducible species from the stimulated cells.

When 1-octanol (300 µM) was dosed to a part of cardiac myocyte sheet, oscillations of intercellular [Ca^{2+}] were disappeared around the hole. Local dose of 1-octanol to patterned myocyte network aligned across the stimulation hole was found to the break synchronous oscillation of [Ca^{2+}] along the cell pattern.

Electrically pulsed cells increased the uptake of membrane impermeable LY (Fig. 3a), while there were some cells irreversibly damaged in plasma membrane (Fig. 3b). Dye uptake were observed only at the PMDS hole, indicating that PDMS mask was effective to localize electrical field by insulating the other part of culture substrate. Un-masked area (3.1 x 10^{-2} mm^2) was significantly small against electrode surface (2.3 x 10^2 mm^2), forming highly concentrated electric field near the PDMS hole. As a result, electroporation was achieved by lower voltage than that for the conventional electroporation with hundreds-thousands volt.

Refernces

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[2] H. Kaji, K. Takoh, M. Nishizawa, T. Matsue, *Biotechnol. Bioeng.*, **81**, 748 (2003).
[3] M. Nishizawa, K. Takoh, T. Matsue, *Langmuir*, **18**, 3645 (2002).

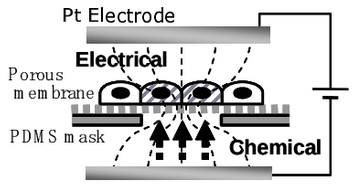

Fig. 1 Schematic illustration describing the device for local stimulation of cell culture.

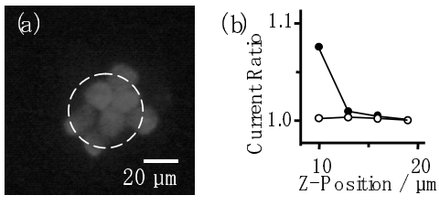

Fig. 2. Local dose of ethanol to HeLa cell culture. (a) Fluorescence image of PI-stained cells taken after 20 min of ethanol stimulation. Dotted circle represents the location of the PDMS-hole. (b) Electrochemical characterization of locally dosed HeLa cells. After 20 min of local dose, a microelectrode probe (-0.5 V vs. Ag/AgCl) was scanned over the stimulated (●) and un-stimulated (○) cells at the various heights (10, 13, 16 and 19 µm above from the substrate). The ratio of the reduction current value to that at 19 µm height was plotted against electrode heights.

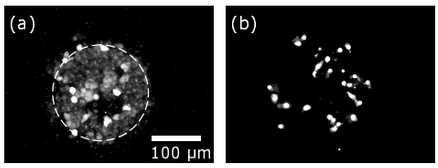

Fig. 3 Local electroporation to cell culture. KMST-6 cells were exposed to single square pulse (4 V, 10 msec duration) in D-MEM media (without phenol red) supplemented with Lucifer Yellow (LY; 2 mg mL^{-1}). The cells were left in incubator for 30 min and were then thoroughly washed with PBS. (a) Fluorescence image for KMST-6 cells taken after LY electroporation. Dotted circle represents the location of the PDMS-hole. (b) Fluorescence image of the cells stained with PI.