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Electrically modulated attachment and detachment of animal cells cultured on an optically transparent patterning electrode

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The purpose of this study was to develop the modulation methods for the attachment and detachment of specifically positioned adhesive animal cells cultured on an electrode surface with the application of a weak electrical potential. A patterned indium tin oxide (ITO) optically transparent working electrode was placed on the bottom of a chamber slide with a counter-(Pt) and reference (Ag/AgCl) electrode. The ITO patterning was formed by a reticulate ITO region and arrayed square glass regions of varying size. Using the 3-electrode culture system, the author succeeded in modulation of the attachment and detachment of animal cells on the working electrode surface. Animal cells suspended in serum or sera containing medium were drawn to and attached on a reticulate ITO electrode region to which a +0.4-V vs. Ag/AgCl-positive potential was applied. Meanwhile, the cells were successfully placed on the square glass regions by −0.3-V vs. Ag/AgCl-negative potential application. Animal cells were detached not only from the ITO electrode but also from the square glass regions after the application of a ±1.0-V vs. Ag/AgCl, 3-MHz rectangular wave potential in PBS(−) for 30–60 min. Rectangular wave potential-induced cell detachment is almost completely noncytotoxic, and no statistical differences between trypsinization and the high frequency wave potential application were observed in HeLa cell growth. The electrical modulation of the specifically positioned cell attachment and detachment techniques holds potential for novel optical microscopic cell sorting analysis in lab-on-chip devices.

[**Key words:** PC12 cell; Normal human dermal fibroblast; HeLa cell; Deep-sea fish cell; Electrical modulation; Cell attachment; Cell detachment]

Micropatterning techniques of animal cells have been reported by numerous groups and fall into 6 major classifications. The first is photolithography, which is used in the semiconductor industry in electronic microcircuits (1,2). Several researchers have made use of photolithography to generate many different chemical micropatterns to assist in the patterning of cell adhesion proteins and cells (2,3). The second is soft lithography, which generates patterns of cell adhesion proteins with feature size ranging from 30 nm to 100 mm using a soft elastomeric stamp with patterned relief structures (2). The third is ink jet printing, which prints animal cells or biomolecules onto target substrates (2,4). The fourth is electron beam writing that controls the spatial distribution of extracellular matrix protein activity by inactivation of the irradiated proteins (5). The fifth is electrochemical desorption of self-assembled monolayers (SAM) (6,7) using an oligo(ethyleneglycol)-terminated SAM to prevent-, and a methyl-terminated SAM to allow-adsorption of proteins and attachment of endothelial cells (6). Electrochemical removal of the oligo(ethyleneglycol)-terminated SAM allowed proteins to adsorb onto areas that had been previously inert and enabled cells to migrate into these areas (6). The sixth is dielectrophoresis that traps mammalian cells onto electrode by weak electric fields (8–10). These six cell micropatterning techniques cannot modulate both the attachment and detachment of animal cells iteratively at the same positions, however. The present work has demonstrated that a weak electrical potential can modulate the attachment and detachment of specifically positioned adhesive animal cells using a patterned indium tin oxide (ITO)/glass electrode culture system (Fig. 1). This new ability has implications for any application that requires cellular and tissue engineering, especially in the fields of cell sorting analysis of microchips (7,11).

Electric effects on living animal cells have long been investigated from a variety of standpoints, which are categorized into (i) dielectric effects, (ii) electrophoretic effects, (iii) electrolytic effects, and (iv) cell/electrode interactive effects (12,13). The dielectric effects induce electropolarization of suspended cells to create pores and to fuse the cells in a low-conductivity solution with a high-voltage pulse of stimulation (12). The electrophoretic effects force cells to migrate in a solution along the potential gradient between a pair of electrodes (11,12), because animal cells have a negative zeta potential resulting from anionic phospholipids in the inner leaflet of the cytoplasmic membrane (14,15). The electrolytic effects induce the electrolysis of electroactive species of either the solution or the cell surface which induces cell necrosis due to irreversible electrochemical reactions (12,16). The cell/electrode interactive effects affect a wide range of cellular functions in animal cells directly cultured on an electrode surface (12,13,17). Animal cells can remain alive if the

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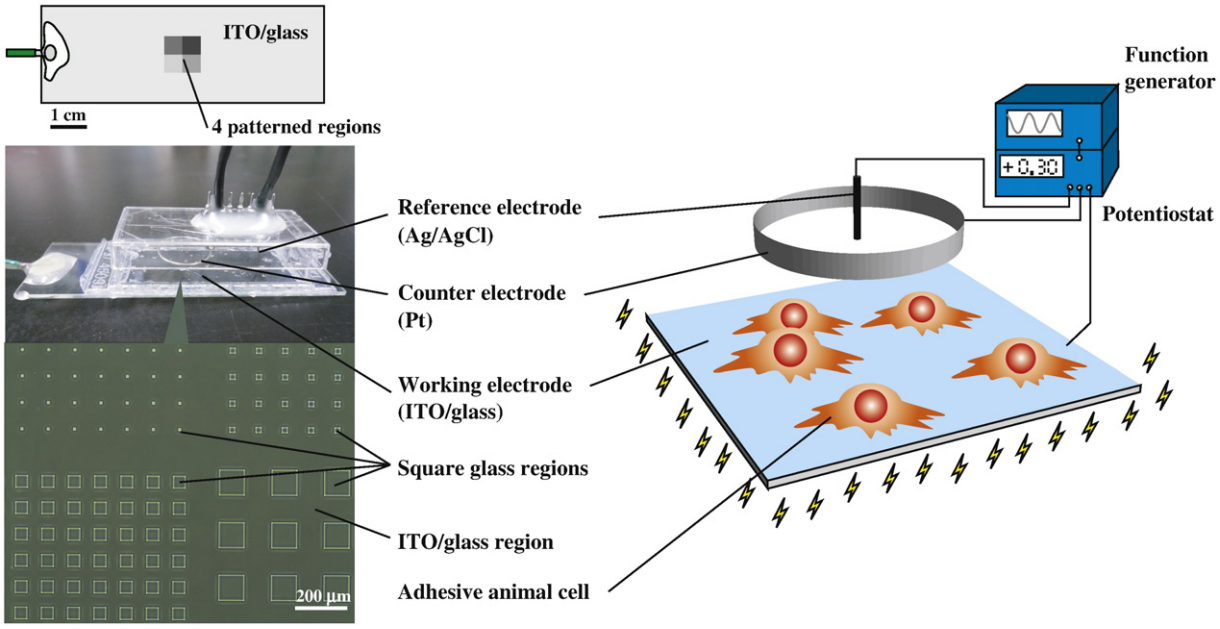

FIG. 1. Schematic illustration of a patterned indium tin oxide (ITO)/glass electrode culture system. The electrode potential is controlled with an Ag/AgCl reference electrode through a potentiostat and a function generator.

electrode potential is controlled properly. Inaba and co-workers reported that the electrochemical desorption of cells uses SAM on the gold electrode (17). The SAM surface was made adhesive by the covalent bonding of Arg–Gly–Asp (RGD)-peptides to the alkanethiol molecules (17). Cellular functions such as proliferation, gene expression, protein production, and differentiation are also modulated by the electrode potential without electrochemical reaction (12,13). When human carcinoma MKN45 cells were cultured directly on an electrode to which a +0.4-V vs. Ag/AgCl potential was applied, the cells stopped proliferating and cell rounding occurred (18). The potential-exposed MKN45 cells grew normally again when the electrode potential shifted to +0.1 V vs. Ag/AgCl (13,18). The MKN45 cells grown on the electrode in the potential range between 0 and +0.3 V vs. Ag/AgCl were the same as those grown on a normal culture plate (18). The results of electrically halted MKN45 cell proliferation strongly suggested a possible mechanism whereby extracellular matrix proteins could be detached from an electrode surface by potential application. The author found that electrical potential application can detach collagen I-FITC and polylysine-FITC from an electrode surface. Furthermore, the potential application can modulate the attachment and detachment of specifically positioned animal cells using both the electrophoretic effects and the cell/electrode interactive effects.

MATERIALS AND METHODS

Electrode preparation Patterned working electrodes were constructed by vacuum evaporation of indium tin oxide (ITO; In_2O_3; 10 Ω/cm^2) onto 76×26-mm^2 silica glass plates (thickness of 1 mm) by Geomatec Co., Ltd. (Yokohama, Japan). The ITO patterning was formed by the reticulate ITO electrode region and arrayed square glass regions of varying size (Fig. 1). The plastic chamber section of a Lab-tek chamber slide system (Cat. 177410, NalgeNunc International, Naperville, IL, USA) was glued to the patterned ITO/glass electrode by silicon bonding (Fig. 1). A Pt counterelectrode and Ag/AgCl reference electrode were placed on the plastic lid of the chamber slide system (Fig. 1).

Before extracellular matrix coating, the patterned ITO/glass electrodes were sonicated in ultrapure water for 5 min and immersed in 1 M NaOH for 5 min to remove any unwanted deposits. After the procedure, the ITO/glass electrodes were washed with ultrapure water, dried, and irradiated with UV light for 5 min for sterilization. Thereafter, the ITO/glass electrodes were coated with either 0.1 mg/mL of collagen type I-FITC conjugate from bovine skin (collagen I-FITC), 0.1 mg/mL of collagen from rat tail (collagen I), or 0.1 mg/mL of poly-L-lysine-FITC (Sigma, St. Louis, MO, USA) for 10 min at 37°C.

Experimental apparatus Constant and rectangular potentials were applied to the working ITO/glass electrode using the Ag/AgCl reference and the Pt counter electrode (Fig. 1). The potentials were delivered via a function generator (AD-8624A, A&D Company, Tokyo, Japan) and a potentiostat (PS-14, Toho Technical Research, Tokyo, Japan).

Cell culture HeLa cells, PC12 cells, and normal human dermal fibroblasts were obtained from Dainippon Pharmaceutical Co., Ltd. (Osaka, Japan). The 25-cm^2 culture flasks were obtained from NalgeNunc International. The HeLa cells and normal human dermal fibroblasts were cultured in 25-cm^2 culture flasks in Dulbecco's modified Eagle's medium (DMEM; ICN Biomedicals, Aurora, OH, USA) containing 10% (v/v) fetal bovine serum (FBS; Gibco, Grand Island, NY, USA) and 1% (v/v) penicillin and streptomycin mixture (5000 IU/mL and 5000 μg/mL, respectively, ICN Biomedicals). The PC12 cells were cultured in 25-cm^2 culture flasks in RPMI-1640 medium with L-glutamine (ICN Biomedicals) containing 5% (v/v) FBS, 10% (v/v) horse serum donor (Sigma), and 1% (v/v) penicillin and streptomycin mixture (5000 IU/mL and 5000 μg/mL, respectively, ICN Biomedicals). The HeLa, PC12, and normal human dermal fibroblast cells were grown at 37°C in a humidified atmosphere of 5% CO_2.

Culture of pectoral fin cells from the deep-sea eel *Simenchelys parasiticus*, collected at a depth of 1162 m, was described elsewhere (19–22). The deep-sea eel cells were cultured in 25-cm^2 culture flasks in L-15 medium containing extra addition of NaCl 4 g/L, 1% antibiotic solution consisting of penicillin and streptomycin (5000 IU/mL and 5000 μg/mL, respectively, ICN Biomedicals), and 20% (v/v) FBS. The salt-enriched L-15 medium was adjusted to pH 7.3. The deep-sea eel cells were grown at 15°C in a humidified atmosphere.

Potential application To attach the PC12 cells to the reticulate ITO region of the patterned electrode (Fig. 1), a +0.4-V vs. Ag/AgCl constant potential was applied to the collagen I-FITC-coated electrode for 66 h in Dulbecco's PBS(−) (Wako, Osaka, Japan) at 37°C and 5% CO_2, washed twice with PBS(−), and thereafter 1×10^7 cells/well of PC12 cell suspension in the sera containing RPMI-1640 medium were seeded on the patterned electrode. After cell seeding, a +0.4-V vs. Ag/AgCl constant potential was applied for a further 24 h in the sera containing RPMI-1640 medium at 37°C in a 5% CO_2 atmosphere.

For attachment of the HeLa cells on the square glass regions of the patterned electrode (Fig. 1), a −0.3-V vs. Ag/AgCl constant potential was applied to the collagen I-coated electrode for 24 h in PBS(−) at 37°C and 5% CO_2, washed twice with PBS(−), and thereafter 1×10^5 cells/well of the cell suspension in the serum containing DMEM were seeded on the patterned electrode. After seeding, a −0.3-V vs. Ag/AgCl constant potential was applied for a further 24 h in the serum containing medium at 37°C and 5% CO_2.

For attachment of the deep-sea eel cells on the glass region (Fig. 1), a −0.3-V vs. Ag/AgCl potential was applied to the collagen I-coated electrode for 24 h in PBS(−) at 37°C and 5% CO_2, washed twice with cold PBS(−), and thereafter 1×10^5 cells/well of the deep-sea fish cell suspension at 15°C in serum containing L-15 medium was seeded on the patterned electrode. After seeding, a −0.3-V vs. Ag/AgCl constant potential was applied for a further 24 h in the serum containing medium at 15°C and ambient CO_2 conditions.

To detach the extracellular matrix proteins from the electrode surface, the constant potential was applied to the electrode in PBS(−) at 37°C in a humidified atmosphere of 5% CO_2. A ±1.0-V vs. Ag/AgCl, 3-MHz rectangular wave potential was applied to detach the adhesive animal cells or the collagen I-FITC from the electrode surface. The