Communication

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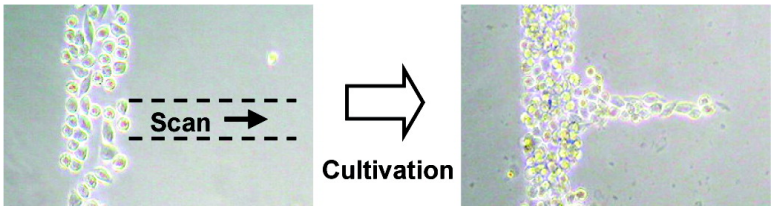
J. Am. Chem. Soc., **2004**, 126 (46), 15026-15027• DOI: 10.1021/ja045702t • Publication Date (Web): 29 October 2004



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Published on Web 10/29/2004

In Situ Control of Cellular Growth and Migration on Substrates Using Microelectrodes

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Controlling the interfaces between cells and solid substrates is an important theme[1−3] pertinent to a wide range of research fields such as fundamental cellular biology,[4,5] tissue engineering,[6,7] and cell-based chip devices.[8,9] In particular, the recent advances in the understanding of the molecular mechanism of cell adhesion has promoted the development of self-assembled monolayer (SAM)-based techniques for precise cell patterning, including microcontact printing (μCP).[10−16]

We present herein a strategy for the real-time local manipulation of the cell-adhesive properties of a substrate even in the presence of attached living cells, which enables the arbitrary modification of cellular micropaterns by directing the cell growth in situ. The strategy is based on our recent report[17] that the cytophobic nature of albumin-coated substrates rapidly switched to a cell-adhesive one when exposed to an oxidizing agent such as hypobromous acid, which can be produced by the electrochemical oxidation of bromide ion in aqueous solution. We have also reported that the local production of the oxidizing agent using a microelectrode provides cellular micropatterns. In this communication, the technique will progress into an unprecedented one that allows the in situ guidance of cellular growth and migration on substrates during cultivation.

We first investigated the relationship between the size of the cellular pattern and the distance between the electrode tip and the substrate surface. The Pt disk-type microelectrode (tip diameter, 15 μm) was placed at various distances above the bovine serum albumin (BSA)-coated glass substrate, and a potential pulse of 1.7 V vs Ag/AgCl with a 30 s period was applied to generate Br_2 (subsequently HBrO) in a 0.1 M phosphate buffer solution containing 25 mM KBr (pH 7.4). Figure 1a shows the cultured HeLa cells forming circular patterns within the electrochemically treated areas, the sizes of which become smaller as the electrode tip is removed from the substrate surface. As can be seen in the plots in Figure 1b, the radius of the cell adhesion area against the electrode height corresponded to an expansion of the diffusion layer from the electrode tip (solid curves in Figure 1b; see Supporting Information for details)[18] for electrolysis periods of both 10 and 30 s. This result indicated that the surface reaction was fast enough to satisfy the diffusion-limited case.

To achieve a stepwise multipatterning of the cells, we evaluated the cytotoxicity of the present method of electrochemical surface processing. The microelectrode was scanned ca. 30 μm above a substrate surface on which HeLa cells were confluently cultured, and the resulting damaged HeLa cells stained by propidium iodide (PI) are visible as red areas in Figure 2a. While the lower scan rate <100 μm s^{-1} caused line patterns of damaged cells, almost no damage was discernible for higher scan rates. Figure 2b depicts the patterns of HeLa cells adhered on the BSA-coated substrate pretreated by a scanning microelectrode; evidently, even a scan

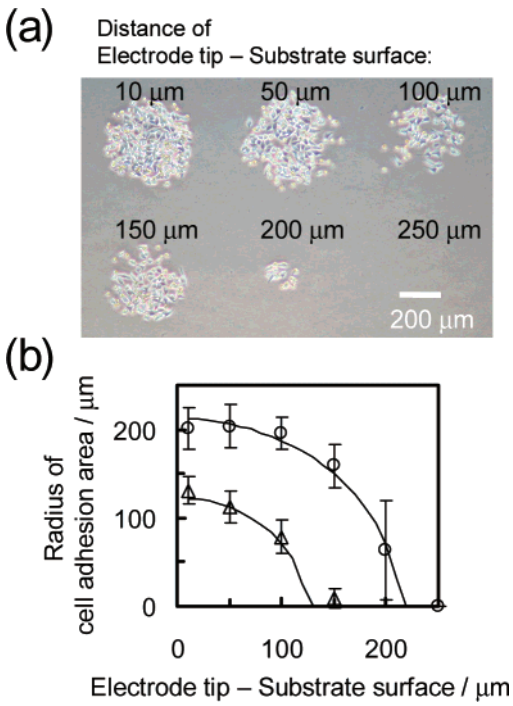

Figure 1. Size of cellular adhesion area at various distances between the electrode tip and the substrate surface during the electrochemical treatment. (a) Phase contrast micrograph of HeLa cells cultured for 24 h on the BSA-coated glass substrate, which was pretreated by a Br^- oxidation pulse of 30 s at the tip−surface distance indicated in the micrograph. (b) Plots of the radius of the cell adhesion area versus the distance of electrode tip−substrate surface for the electrolysis periods of 10 s (△) and 30 s (○). Error bars for the plots were calculated from the standard deviation of at least four cellular patterns. Solid curves were calculated assuming a diffusion-limited surface reaction (see Supporting Information).

rate faster than 100 μm s^{-1} results in clear cell patterns. The width of the line patterns of cell adhesion was roughly proportional to the reciprocal square root of the scan rate, a result in agreement with a model that predicts that the pattern size corresponds to the width of the diffusion layer of the oxidizing agent. A comparison of Figures 2a and 2b indicates that the oxidizing agent's threshold concentration for cell damage is higher than the concentration required for the surface processing of the BSA-coated substrate. Similar results were obtained from experiments using a fixed microelectrode with various electrolysis periods (see Supporting Information).

Figure 3 demonstrates the level of control of cellular growth and migration possible with the microelectrode technique. The BSA-coated substrate was processed to make the first cellular pattern by a microelectrode scan (Figure 3a) and by an incubation for 1 h in a serum culture medium containing suspended HeLa cells to promote cell attachment (Figure 3b). As illustrated in Figure 3c,

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10.1021/ja045702t CCC: $27.50

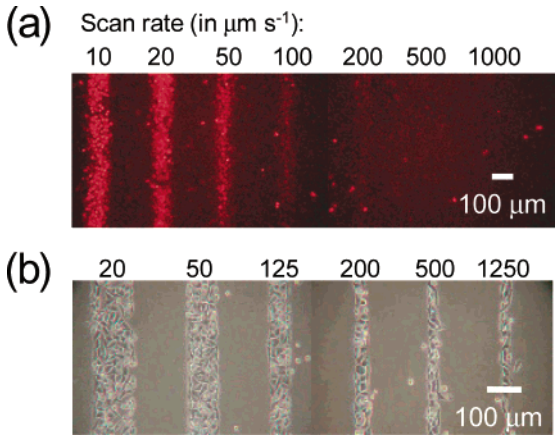

Figure 2. (a) Fluorescence micrograph of micropatterns of damaged HeLa cells stained by PI. The microelectrode electrogenerating HBrO was scanned closely above the substrate surface on which HeLa cells were confluently cultured. (b) Phase contrast micrograph of patterned HeLa cells. The cells were cultured for 24 h on the BSA-coated substrate, which was pretreated by scanning the microelectrode.

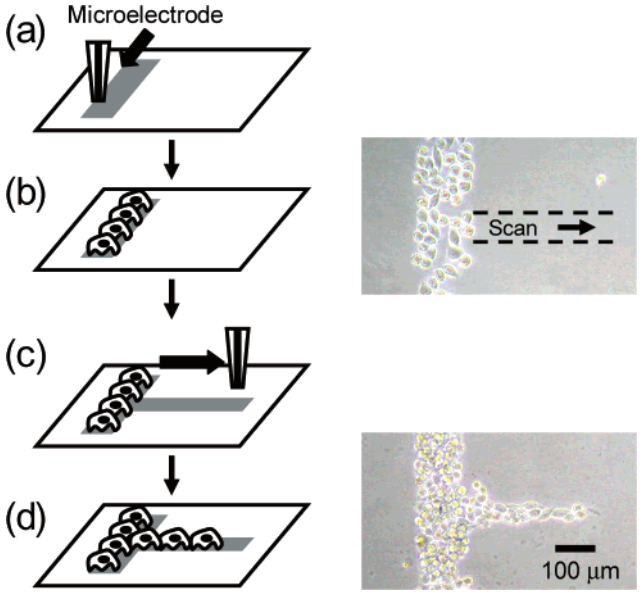

Figure 3. Direction of cellular growth and migration by the microelectrode technique. (a) The BSA-coated substrate was locally treated to make the first cellular pattern by scanning the microelectrode at 50 μm s^{-1}. (b) HeLa cells attached only to the treated area after 1 h of cultivation. (c) The microelectrode was scanned at 125 μm s^{-1} from a position above the first cellular pattern. (d) Cultivation for 24 h without seeding of additional cells resulted in cellular migration and growth along the added pattern.

the scanning microelectrode technique was again applied to extend the cellular line pattern perpendicularly. To avoid the damage on the prepatterned cells, the electrode potential (1.7 V vs Ag/AgCl) was applied simultaneously with the start of electrode scan at 125 μm s^{-1}. In fact, no damaged cells appeared with PI staining after the electrochemical treatment (data not shown), although there may be slight damage to cell surface. The substrate was further incubated for 24 h in a serum-containing medium without the seeding of additional cells. The micrograph in Figure 3d shows that the HeLa cells proliferated and migrated along the region electrochemically treated with the microelectrode, a finding indicating that the oxidizing agent can change the property of the BSA-coating near the cells without damaging the preexisting cells.

Some other research groups have reported on μCP-based electrochemical methods for in situ control of cell adhesion. Mrksich and co-workers have developed a technique that uses the electrochemical oxidation of a hydroquinone-terminated to a quinine-terminated SAM on a Au substrate; this step is followed by a Diels–Alder reaction to immobilize a cyclopentadiene-conjugated cell-adhesive peptide sequence to activate inert surfaces for the attachment of cells.[12,13] Whitesides and co-workers have adopted the electrochemical desorption of an ethylene glycol-terminated SAM on Au to induce cellular attachment onto the SAM-desorbed area.[14]

The method we describe here is a simple electrochemical technique that, as demonstrated in Figure 3, enables the in situ drawing of patterns for cell growth and migration. We believe that such a unique technical feature would be useful to simplify bioassays based on cellular motility. Since stepwise multipatterning is possible, this technique could also facilitate the formation of micropatterned cocultures of different kinds of cell types and thus contribute to cell and tissue engineering.

Acknowledgment. This study was supported by a Grant-in-Aid for Young Scientist (A) (No. 15681008) from the Ministry of Education, Science and Culture, Japan. H.K. acknowledges support from a research fellowship of the Japan Society for the Promotion of Science.

Supporting Information Available: Experimental details and additional data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA045702T