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A Self-Assembled Monolayer-Based Micropatterned Array for Controlling Cell Adhesion and Protein Adsorption

Dong Jin Kim,[1] Jong Min Lee,[2] Jin-Goo Park,[1] Bong Geun Chung[2]

[1]Department of Materials Engineering, Hanyang University, Ansan, 426-791, Korea; e-mail: jgpark@hanyang.ac.kr

[2]Department of Bionano Engineering, Hanyang University, Ansan, 426-791, Korea; telephone: 82-31-400-5203; fax: 82-31-436-8153; e-mail: bchung@hanyang.ac.kr

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ABSTRACT: We developed a surface micropatterning technique to control the cell adhesion and protein adsorption. This micropatterned array system was fabricated by a photolithography technique and self-assembled monolayer (SAM) deposition. It was hypothesized that the wettability and functional terminal group would regulate cell adhesion and protein adsorption. To demonstrate this hypothesis, glass-based micropatterned arrays with various functional terminal groups, such as amine (NH_2) group (3-aminopropyltriethoxysilane, APT), methyl (CH_3) group (trichlorovinylsilane, TVS), and fluorocarbon (CF_3) group (trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane, FOTS), were used. The contact angle was measured to determine the hydrophilic and hydrophobic properties of materials, demonstrating that TVS and FOTS were hydrophobic, whereas APTs were relatively hydrophilic. The cell adhesion was significantly affected by the wettability, showing that the cells were not adhered to hydrophobic surfaces, such as TVS and FOTS. Thus, the cells were selectively adhered to glass substrates within TVS- and FOTS-based micropatterned arrays. However, the cells were randomly adhered to APTs-based micropatterned arrays due to hydrophilic property of APTs. Furthermore, the protein adsorption of the SAM-based micropatterned array was analyzed, showing that the protein was more absorbed to the TVS surface. The surface functional terminal group enabled the control of protein adsorption. Therefore, this SAM-based micropatterned array system enabled the control of cell adhesion and protein adsorption and could be a potentially powerful tool for regulating the cell–cell interactions in a well-defined microenvironment.

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KEYWORDS: surface micropatterning; self-assembled monolayer; cell adhesion; protein adsorption; wettability

Introduction

Surface micropatterning techniques play an important role in controlling cell adhesion and the cell–microenvironment interactions, such as homotypic and heterotypic cell–cell interactions (Falconnet et al., 2006; Folch and Toner, 2000; Whitesides et al., 2001). Cell adhesion to substrates is of great interest in controlling cell survival, proliferation, and differentiation (Wilson et al., 2005). In general, the wettability, surface charge, and surface roughness enable the control of selective adhesion of the cells to substrates (Bhatia et al., 1997; Lee et al., 1993; Singhvi et al., 1994). To regulate cell adhesion and cell–cell interactions, photo and soft lithography-based surface micropatterning techniques, such as microcontact printing and micromolding in capillaries (MIMIC), have been used (Beebe et al., 2002; Chen et al., 1997; Elloumi Hannachi et al., 2009; Khademhosseini et al., 2006; Shim et al., 2007; Xia and Whitesides, 1998). A microcontact printing technique has been widely used to localize cells and extracellular matrix (ECM) biomolecules in a spatial manner. For example, a microcontact printing technique has been used to generate co-culture transferable cell sheets on a temperature-responsive polymer, poly(*N*-isopropylacrylamide) (PIPAAm; Elloumi Hannachi et al., 2009). Fibronectin-coated poly(dimethylsiloxane) (PDMS) stamps were micropatterned on the substrate, showing the co-culture of hepatocytes and endothelial cells. Cell adhesion and detachment was significantly regulated by temperature, indicating that the cells were removed from the substrates at low temperature (<32°C) without the use of

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enzymes. Thus, the combination of a microcontact printing technique and temperature-responsive polymer enabled the control of selective cell adhesion. Another approach is to use the MIMIC method for patterning cells and immobilizing proteins (Shim et al., 2007). To selectively localize cells and proteins, a MIMIC method was used to generate two surface micropatterned regions; polyethylene glycol (PEG) and polyelectrolyte (PEL) microstructures. Quantitative analysis showed that cells and biomolecules were not adhered to the bio-inert PEG surface, whereas they were selectively patterned on the PEL surfaces with cationic polyallylamine hydrochloride (PAH) layers, as the amine group of PAH layers enhanced cell attachment.

Polymeric membranes have been previously used to pattern cells and biomolecules (Chang et al., 2007; Lee et al., 2003; Suh et al., 2004; Wright et al., 2008). For instance, parylene-C, a bio-inert material, has been used to pattern cells and proteins (Chang et al., 2007). The cell adhesion and protein adsorption on parylene-C surfaces was compared with different substrates, such as PDMS, polystyrene, and glass. Although the parylene-C and PDMS showed hydrophobic properties, oxygen plasma treatment made their surface hydrophilic, resulting in enhanced cell adhesion. In contrast, cells were not adhered to parylene-C or PDMS surfaces that were not treated by oxygen plasma, and the surface wettability played an important role in controlling cell adhesion. The effect of the surface hydrophobic property on protein adsorption was also investigated, showing that protein was more adhered to hydrophobic surfaces than hydrophilic surfaces. Another study used PEG microstructures that was fabricated using the capillary force lithography (Suh et al., 2004). Micropatterned PEG substrates were used as a physical and biological barrier for adhesion of cells and ECM molecules. As a result, the cells and proteins were selectively adhered within PEG hydrogel micropatterns. In contrast, cells and proteins were not attached to PEG surfaces. Furthermore, hydrophobic polymer thin films developed by a photolithographic lift-off technique have been used to pattern proteins (Lee et al., 2003). Proteins (i.e., fluorescent conjugated bovine serum albumin, BSA) were selectively immobilized within the micropatterns on chemical vapor-deposited silicon nitride substrates. The wettability of hydrophobic thin films was unchanged during protein patterning, and hydrophobic thin films prevented nonspecific protein binding, indicating that the hydrophobic thin film was a physical barrier of nonspecific protein binding. This system has some advantages over conventional micropatterning methods (i.e., robotic spotter, inkjet dispenser), such as inexpensive microfabrication-based protein patterning.

Self-assembled monolayers (SAMs) of alkanethiols have also been used to regulate cells and biomolecules (Arima and Iwata, 2007a,b; Tan et al., 2002). For example, the microcontact printing technique and SAMs, consisting of aliphatic and polar alkanethiols, have been used to pattern proteins (Tan et al., 2002). Proteins absorbed on a PDMS microstamp were transferred to SAM surfaces. The wettability difference between a PDMS stamp and SAM surface were investigated with protein adsorption, indicating that the minimum wettability of SAMs was reduced by decreasing the wettability of PDMS stamps. The wettability and surface functional groups of mixed SAMs enabled the control of cell adhesion and protein adsorption (Arima and Iwata, 2007a,b). SAMs with different wettability and terminal functional groups were employed as a model surface to understand cell and protein behavior, demonstrating that the cell adhesion was significantly increased with decreasing contact angles (40–60°; Arima and Iwata, 2007a). Furthermore, protein adsorption was analyzed using surface plasmon resonance (SPR), showing that the functional terminal groups of SAMs played an important role in controlling the proteins adsorbed from a cell culture medium with serum (Arima and Iwata, 2007b). Previous SAM approaches enabled the control of micropatterning, cell adhesion, and protein adsorption, however, they used gold-based substrates. Despite their potential, the gold-based substrates did not allow for direct cell imaging and monitoring.

To address this limitation, a glass-based SAM micropatterning technique containing different functional terminal groups; amine (NH2), methyl (CH3), and fluorocarbon (CF3), was developed. As compared with previously developed micropatterning methods, our micropatterning system, which was developed using photolithography and a SAM deposition method, enabled the control of the surface wettability and functional terminal groups. In this study, the effect of wettability and surface terminal functional group on cell adhesion and protein adsorption was demonstrated by a SAM-based micropatterning technique. Mouse fibroblast cells and embryonic stem cells were selectively adhered within glass-based SAM micropatterns. Therefore, this SAM-based micropatterning on a glass substrate could be a potentially powerful tool for biosensors, cell-based microfluidic devices, and tissue engineering applications.

Materials and Methods

Fabrication of a Self-Assembled Monolayer (SAM)-Based Micropatterning

SAM-based micropatterns were fabricated on a glass substrate with different functional terminal groups, such as 3-aminopropyl-triethoxysilane (APT) 99%, Sigma-Aldrich, St. Louis, MO), trichlorovinylsilane (TVS 97%, Sigma–Aldrich), and trichloro(1H, 1H, 2H, 2H-perfluorooctyl)silane (FOTS 97%, Sigma–Aldrich). To fabricate an APTs-based micropatterned array (Fig. 1A), glass was cleaned with piranha solution (4:1 H_2SO_4:H_2O_2) for 10 min and subsequently rinsed in deionized (DI) water for 3 min. For the APTs deposition, the glass surface was sequentially treated with a liquid SAM method and 6% APTs in ethanol, resulting in chemical bonding between hydroxyl groups on the glass and silane groups of APTs. The glass

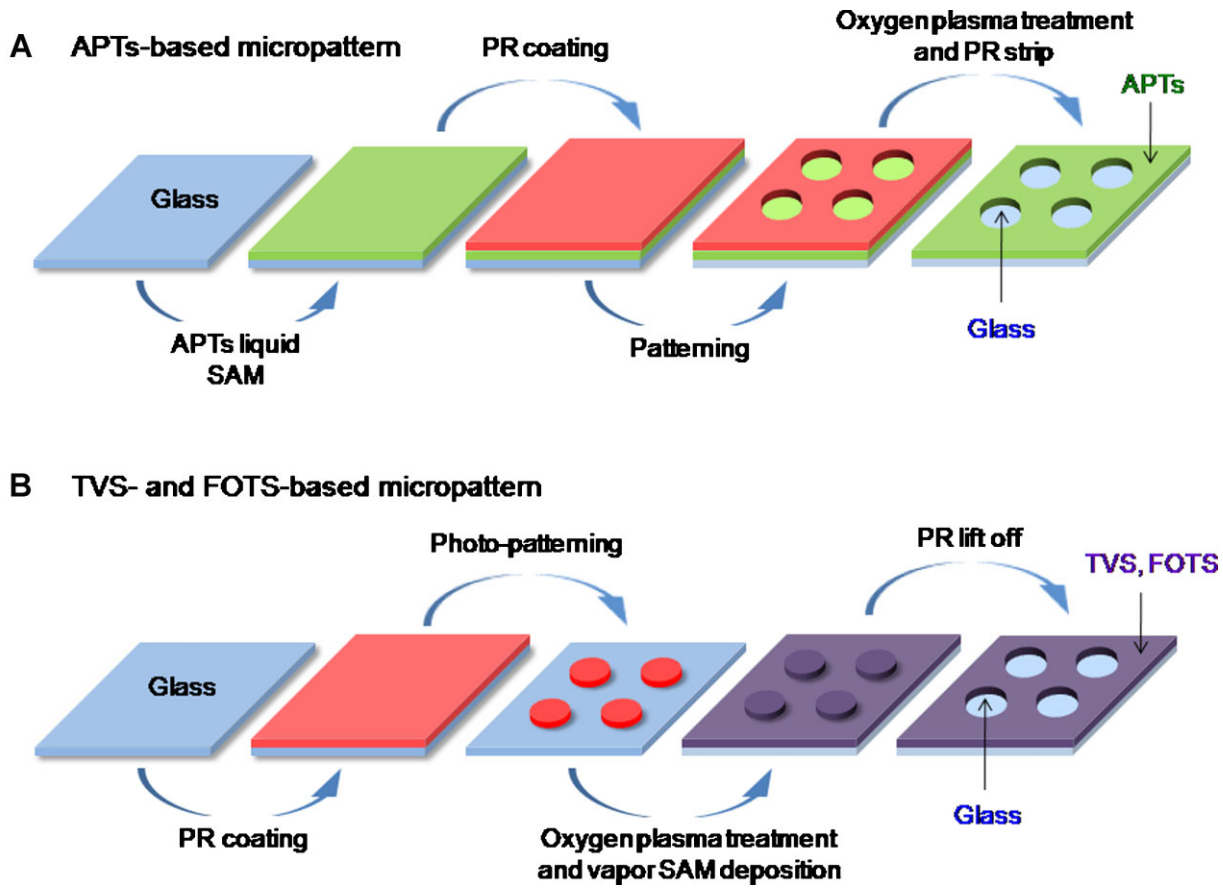

Figure 1. Schematic process of the SAM-based micropatterning fabrication. **A**: APTs-based micropatterning fabrication process. A glass substrate was treated using the deposition of APTs-liquid SAMs and photolithography technique in a sequential manner. **B**: The TVS- and FOTS-based micropatterning fabrication process. In contrast to the APTs-based micropatterning fabrication process, PR was patterned on a glass substrate and then TVS and FOTS were subsequently deposited using a vapor SAM method on a PR patterned substrate. As a result, a glass-based SAM micropatterned array system was fabricated with different functional terminal groups (i.e., APTs, TVS, and FOTS) as a background. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]

was then spin-coated with photoresist (PR; AZ1512, AZ Electronic Materials, Charlotte, NC) at 3,000 rpm for 1 min (1–2 μm thickness) and was baked for 1 min at 100°C. The PR on the glasses was subsequently photo-crosslinked by a UV light (13 mJ/cm^2, 365 nm $< \lambda <$ 436 nm, Midas, Daejon, Korea) through a film mask embedding with circular micropatterns (300 μm diameter). Glass slides were then developed for 1 min by dipping in a developer (AZ300mif; AZ Electronic Materials) for generating PR-patterned surfaces. After the photolithography process, glasses were treated with oxygen plasma (100 mTorr and 300 W, Glen-R3A; Yield Engineering System, San Jose, CA) for 1 min to remove organic residues and induce hydrophilic property. For the PR strip, the glasses were treated with acetone and ethanol using an ultrasonicator for 1 min, rinsed with DI water for 3 min, and then dried by blowing nitrogen. The fabrication process of TVS- and FOTS-based micropatterned arrays was slightly different than APTs-based micropatterning (Fig. 1B). For TVS- and FOTS-based micropatterns, PR was first patterned. 6 μL TVS and FOTS hydrophobic precursors were subsequently deposited on PR-patterned glasses using vapor SAM deposition in an oven for 30 min at 85°C. In this process, the hydroxyl groups on glass were chemically bonded with the hydrophobic silane groups. The PR lift off process was followed to produce micropatterns containing TVS and FOTS molecules. As a result, a glass-based micropatterned array containing different functional groups (i.e., APTs, TVS, and FOTS) as a background was generated.

Contact Angle Measurement

Contact angles were measured for three different substrates; APTs, TVS, and FOTS. As a control, the contact angle of a glass surface was measured using a contact angle measurement system (Phoenix3000 Plus, Suwon, Korea). Each set of data was obtained from at least three different measurements, and the statistical significance of the data were analyzed with a Student's *t*-test, where $P < 0.05$ and $P < 0.01$ were considered statistically significant.

Cell Culture in a SAM-Based Micropatterned Array

Fibroblast cells were cultured on a tissue culture flask with Dulbecco's modified Eagle media (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin in an incubator (5% CO_2, 37°C). Trypsin was used to detach the cells adhered to a tissue culture flask. Cells were resuspended after centrifuging at 1,200 rpm for 3 min and a 200 μL of cell suspension (1×10^6 cells/mL) was seeded on a SAM-based micropattern. After 30 min, 3 mL culture medium was added to a SAM-based micropattern, and the adhered cells were cultured for 24 h. We also cultured murine embryonic stem cells (R1 cell line). The cells were cultured with DMEM medium with 15% embryonic stem cell qualified FBS (Invitrogen, Auckland, NZ) and 1,400 U/mL leukemia inhibitory factor (Millipore, Billerica, MA).

Cell Adhesion Analysis

Cell adhesion to a SAM-based micropattern with a 300 μm diameter was analyzed at 6, 12, and 24 h after cell seeding. Ten images of each micropattern were randomly selected, and the numbers of adherent cells were analyzed. For TVS- and FOTS-based micropatterns, the cells adhered within SAM-micropatterned substrates were counted. However, for the APTs-based micropatterned array and glass control, only the cells adhered within the 300 μm diameter circle were analyzed, because the cells were not selectively patterned.

Immunocytochemistry

Cells were fixed with 4% paraformaldehyde after culturing for 24 h within SAM micropatterns, were washed with phosphate-buffered saline (PBS), and were subsequently permeabilized by 1% triton X-100 in PBS for 3–5 min. After washing with PBS three times, the cells were subsequently blocked by 1% BSA (Sigma, St. Louis, MO) for 30 min at room temperature. The cells were immunostained by Alexa fluor 594 phalloidin (Invitrogen) for 30 min and DAPI (0.1 μg/mL) for 3 min at room temperature. Immunostained cells were stored at 4°C until image analysis.

Protein Adsorption Assay and Image Analysis

To analyze protein adsorption on a glass-based micropatterned array, a fluorescent isothiocyanate (FITC) conjugated BSA (Sigma–Aldrich) was used. FITC-BSA solution was dissolved in PBS to make 100 μg/mL. Fifty microliters of FITC-BSA solution was placed on micropatterned surfaces containing APTs, TVS, and FOTS-functional terminal groups. After incubation for 20 min at room temperature, SAM-based micropatterns were washed three times with PBS. Fluorescent images were obtained from an inverted microscope (Olympus IX71, Tokyo, Japan) and were analyzed using an Image J program. The fluorescent intensity of each sample was compared with a glass control.

Results and Discussion

Fabrication of Self-Assembled Monolayer (SAM)-Based Micropatterns

A SAM-based micropatterned array was developed by photolithography and liquid/vapor SAM deposition techniques (Fig. 1). Briefly, SAM containing three different functional terminal groups (i.e., amine(NH_2)-APTs, methyl(CH_3)-TVS, and CF_3-FOTS) was deposited onto glass substrates for controlling hydrophilic and hydrophobic properties of a micropatterned array. Given the difference in surface properties of these functional groups, the fabrication process of APTs-based micropatterns (Fig. 1A) was slightly different as compared with TVS- and FOTS-based micropatterns (Fig. 1B), showing that the APTs-based micropatterns were generated using the liquid SAM process, whereas TVS- and FOTS-based micropatterns were developed by the vapor SAM process. TVS and FOTS groups were deposited after PR coating in the vapor SAM method. In contrast, amine group was deposited before PR coating in the liquid SAM method, because PR was dissolved by ethanol that was used for liquid SAM process. To overcome this problem, amine group-based SAM was first deposited on the glass substrate before PR coating. In contrast, TVS- and FOTS-based micropatterns were fabricated by the vapor SAM and PR lift-off method, because PR was not damaged by vapor SAM and PR lift-off process. We did not observe any damage or removal of TVS- and FOTS-based micropatterns after PR lift-off process. APTs, TVS, and FOTS were deposited as a background, whereas the glass substrate was remained inside the micropatterns (300 μm diameter). As a result, a SAM-based micropatterned array containing different functional terminal groups was generated to regulate hydrophilic and hydrophobic surfaces. As compared with previous micropatterning techniques (i.e., microcontact printing, MIMIC, polymeric membrane, and SAM), our SAM-based surface micropatterning approach showed several advantages; the wettability and functional terminal group were easily controlled by a simple microfabrication process, and a glass substrate was used, instead of a gold surface, for improving cell imaging. Therefore, this glass-based SAM micropatterned array could be a potentially powerful tool to selectively pattern the cells and biomolecules in a well-defined microenvironment.

Characterization of Hydrophilic and Hydrophobic Properties

To analyze the hydrophilic and hydrophobic properties of SAM-based micropatterned substrates, the contact angle of APTs-, TVS-, and FOTS-based SAM surfaces were measured

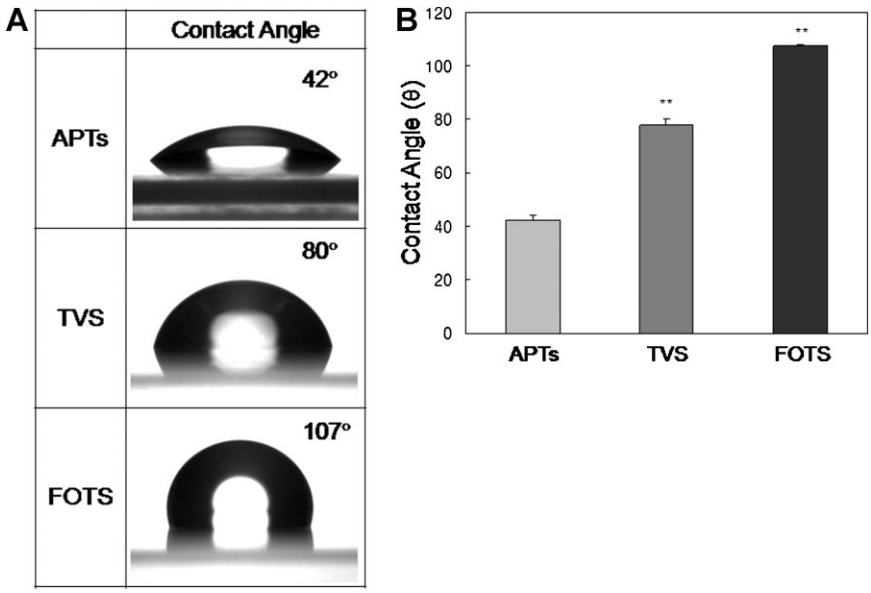

Figure 2. Contact angle measurements to determine hydrophilic and hydrophobic properties of materials. Contact angle images (**A**) and analysis (**B**) of APTs, TVS, and FOTS surfaces, indicating that the contact angles were 42°, 80°, and 107°, respectively. The contact angles of TVS and FOTS surfaces were compared with the APTs surface ($^{**}P < 0.01$).

(Fig. 2A). The surface properties (i.e., wettability, functional terminal group) of a SAM micropatterned array system were believed to play an important role in controlling cell adhesion and protein adsorption. As a control, the contact angle of a glass substrate was measured at approximately 8°, indicating a hydrophilic surface. The glass was employed as a substrate inside the SAM-based micropatterns for cell adhesion and protein adsorption experiments. The wettability analysis showed that the contact angles of APTs, TVS, and FOTS surfaces were approximately 42°, 80°, and 107°, respectively (Fig. 2B). The contact angles of APTs, TVS, and FOTS were much greater than the glass control. In addition, FOTS showed the greatest contact angle, thereby resulting in a more hydrophobic property than the APTs- and TVS-based surfaces. In particular, the contact angle of the FOTS surface was approximately 2.5 times greater than the APTs surface. We also found that the contact angles of the TVS and FOTS surfaces were statistically different from those of the APT surface ($^{**}P < 0.01$).

Cell Adhesion Within a SAM-Based Micropatterned Array

The cell adhesion within APTs-, TVS-, and FOTS-based SAM micropatterned arrays with various shapes (i.e., circle, square, and rectangle) was examined (Fig. 3). We observed that the smallest pattern sizes were significantly affected by the interval between micropatterns, showing that we fabricated the smallest size patterns with circular shapes (200 μm in diameter, 200 μm interval), square shapes (100 μm × 100 μm, 100 μm interval), and rectangular shapes (100 μm × 200 μm, 100 μm interval). However, for circular patterns with 300 μm in diameter, we observed that the smallest interval between patterns was 30 μm. The highest density of micropatterns per unit surface area was 12 × 9 micropatterns/4.14 mm^2 in the square micropatterns (100 μm × 100 μm, 100 μm interval). When the patterns were designed less than 100 μm size and interval, the patterning was easily broken or peeled off from substrates after PR lift-off process. Therefore, we demonstrated that the design of SAM-based micropatterns was affected by micropatterning shape and interval (>100–200 μm size and interval).

The selective cell patterning and adhesion was hypothesized to be significantly affected by the surface wettability properties. A cell suspension was seeded on micropatterned arrays and cell adhesion was observed over time (~24 h; Fig. 4A). The fibroblast cells patterned within 12 × 10 SAM-based microarrays were cultured for 24 h, because cells were completely occupied within SAM-based micropatterns and were also overgrown after 24 h. The fibroblast cells were immunostained by phalloidin to confirm cytoskeleton of cells adhered within 8 × 6 SAM micropatterns (Fig. 4B and C). We also observed that the cells cultured within micropatterned arrays for 24 h remained highly viable (>95%; data not shown). Fluorescent images of cell adhesion also demonstrated that the cells were not adhered well to the TVS and FOTS surfaces, but they were selectively attached to glass substrates inside TVS- and FOTS-based micropatterns. In contrast, the cells seeded on the APTs-based micropatterned arrays were evenly distributed, and selective cell patterning was not observed. Therefore, the feasibility was demonstrated with cell adhesion results, indicating that the

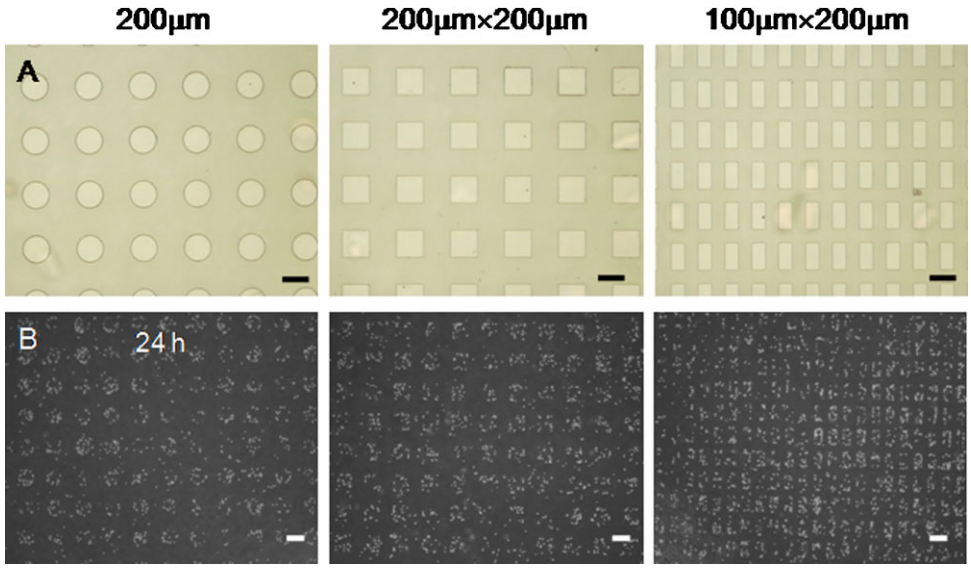

Figure 3. SAM micropatterns containing various shapes to culture the fibroblast cells. **A**: SAM micropatterns with various shapes, such as circular (200 μm in diameter, 200 μm interval), square (200 μm × 200 μm, 200 μm interval), and rectangular patterns (100 μm × 200 μm, 100 μm interval). **B**: Phase contrast images of fibroblast cell patterning. Scale bars are 200 μm. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]

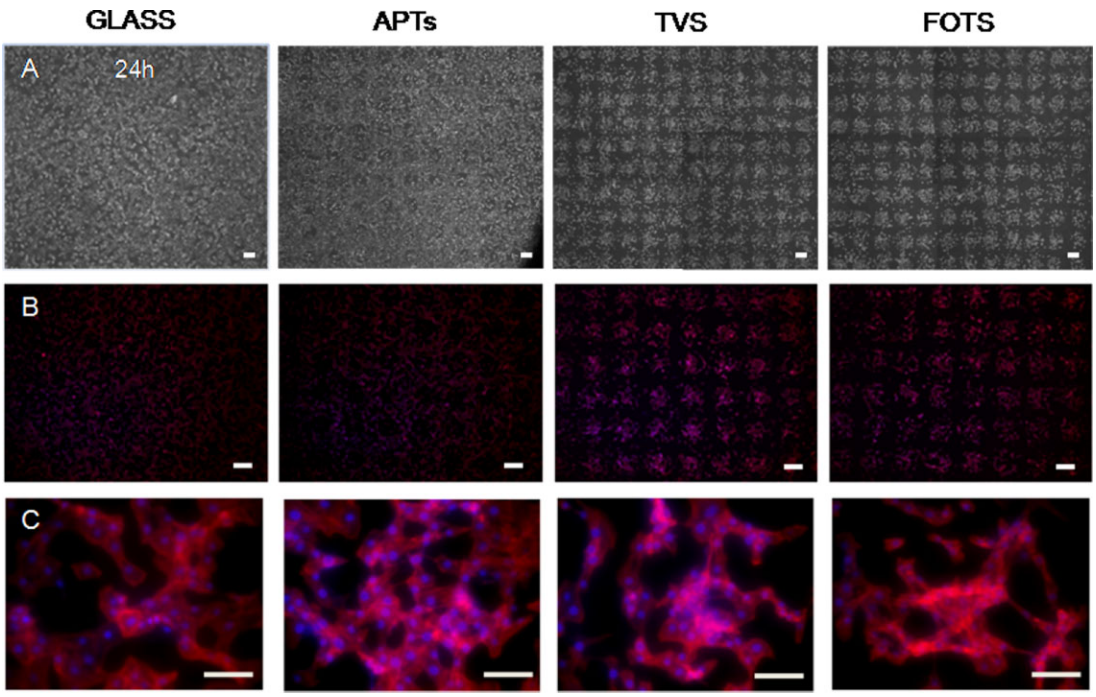

Figure 4. Cells adhered within SAM micropatterns. **A**: Phase contrast images of the fibroblast cells cultured for 24 h on APTs-, TVS-, and FOTS-based 12 × 10 SAM micropatterns with circular shape (300 μm in diameter). A glass substrate was used as a control. Scale bars are 300 μm. **B**: Fluorescent images of the fibroblast cells within 8 × 6 SAM micropatterns. The cells were immunostained by phalloidin (red, cytoskeleton) and DAPI (blue, cell nucleus). Fibroblast cells were selectively adhered to glass substrates inside TVS- and FOTS-based micropatterns. In contrast, the cells were randomly attached to the APTs-based micropatterned substrate and glass surface. Scale bars are 300 μm. **C**: Fluorescent images of fibroblast cells with high magnification. Scale bars are 100 μm. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]

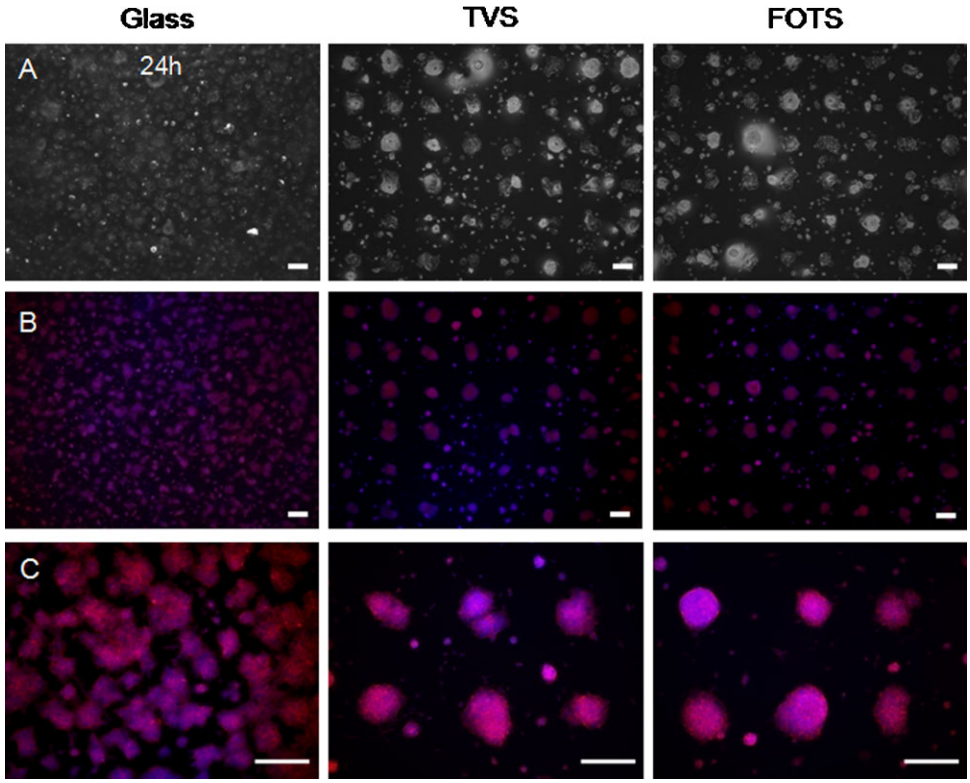

Figure 5. Embryonic stem cells cultured on TVS- and FOTS-based SAM micropatterns. **A**: Phase contrast images of the embryonic stem cells cultured for 24 h on 8 × 6 TVS- and FOTS-based SAM micropatterns. Scale bars are 300 μm. **B**,**C**: Fluorescent images of the embryonic stem cells on TVS- and FOTS-based micropatterns. The embryonic stem cells were immunostained by phalloidin (red, cytoskeleton) and DAPI (blue, cell nucleus). Scale bars are 300 μm. [Color figure can be seen in the online version of this article, available at http://wileyonlinelibrary.com/bit]

fibroblast cells were preferentially adhered to hydrophilic regions, such as glass and APTs, whereas they were not adhered well to either TVS- or FOTS-based hydrophobic regions. We also cultured murine embryonic stem cells on TVS- and FOTS-based micropatterns (Fig. 5). Murine embryonic stem cells were adhered on glass substrates inside TVS- and FOTS-based micropatterns. As compared with the patterning of fibroblast cells (Fig. 4), murine embryonic stem cells were aggregated within SAM micropatterns, because they tended to be aggregated to form embryoid body.

The number of fibroblast cells attached to the SAM-based micropatterned arrays was analyzed (Fig. 6). The cell number inside TVS- and FOTS-based micropatterned arrays was measured. For the APTs-based micropattern and control glass substrate, the fibroblast cell number inside the 300 μm diameter circle was counted, because the fibroblast cells were not selectively patterned within both the APTs-based micropatterned array and control glass substrate. Cell attachment analysis showed that the fibroblast cells were selectively adhered to glass substrates inside TVS- and FOTS-based micropatterned arrays, and the cell numbers were slightly increased with increasing culture times. In contrast, an increase in cell number was not observed on the APTs-based micropattern and control glass substrate, because the fibroblast cells were already occupied on the APTs and glass-based substrates after 6 h. The significant differences in fibroblast cell numbers were observed on TVS- and FOTS-based micropatterned arrays

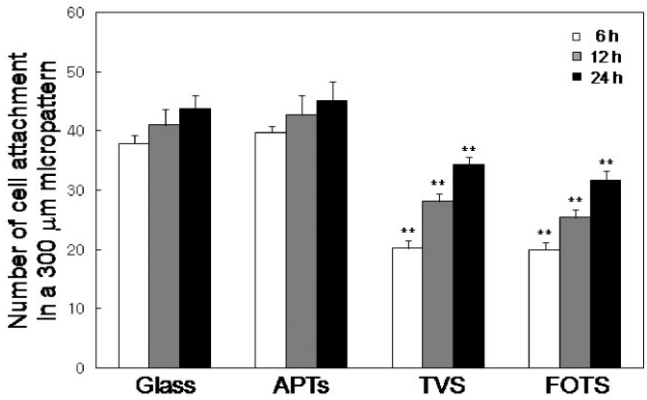

Figure 6. Analysis of cell adhesion within APTs-, TVS-, and FOTS-based SAM micropatterned substrates with a 300 μm diameter. The fibroblast cells were selectively adhered to glass substrates within both TVS- and FOTS-based micropatterns. The number of fibroblast cell adhesions within TVS- and FOTS-based micropatterns were compared with a glass substrate ($^{**}P < 0.01$).

as compared with those on the APTs-based micropattern and glass substrate ($^{**}P < 0.01$). Interestingly, the fibroblast cell attachment on TVS- and FOTS-based micropatterned arrays at 24 h was greater than at 6 h, indicating 20 cells at 6 h and 32–34 cells at 24 h, because floating cells were likely adhered inside the micropatterns, or the cells adhered within micropatterns were divided after culturing for 24 h. Cell attachment analysis demonstrated that hydrophobic TVS and FOTS surfaces showed more cell repellent properties, as compared with the hydrophilic APTs-based substrate, indicating that the fibroblast cells were more selectively adhered to glass substrates inside hydrophobic TVS- and FOTS-based micropatterns. Therefore, the hydrophilic and hydrophobic surface properties played an important role in controlling the cell adhesion in a SAM-based micropatterned array system.

Protein Adsorption on a SAM-Based Micropatterned Array

The effect of protein adsorption on APTs-, TVS-, and FOTS-based micropatterns was analyzed (Fig. 7). To characterize the protein adsorption, the surface of SAM-based micropatterned arrays was treated with FITC-BSA, and the protein adsorption was analyzed by measuring the fluorescent intensity of FITC-BSA. The protein adsorption analysis demonstrated that protein adsorption on the TVS surface was significantly greater than on APTs, FOTS, and glass substrates. In contrast, the FOTS surface showed protein repellent behavior, due to the $–CF_3$ functional group, as previously described (Tang et al., 1998). The protein adsorption on FOTS was approximately 78% of that on the glass substrate. The cells were selectively patterned within a TVS-based micropatterned array, because the cells were not adhered to hydrophobic TVS surfaces. In contrast, BSA proteins were highly adhered to hydrophobic TVS substrates. For the FOTS surface, the cell adhesion behavior of FOTS surface was similar to the TVS surface, due to hydrophobic properties of FOTS and TVS surfaces. However, the protein was not adhered well to the FOTS surface, as compared with the TVS surfaces, likely due to the difference of functional terminal groups; FOTS ($–CF_3$) and TVS ($–CH_3$) surface. Interestingly, the protein adsorption of the APTs surface was slightly greater than the glass substrate, although proteins are known to more adhere to an amine ($–NH_2$) surface (Arima and Iwata, 2007b; Tang et al., 1998). This behavior was likely due to the SAM deposition method and microfabrication process. For the APTs-based SAM micropatterns, APTs was first deposited using the liquid SAM method before the photolithography process. In contrast, for the TVS- and FOTS-based SAM micropatterns, TVS and FOTS were deposited using vapor SAM deposition after the photolithography process. Thus, the functional terminal groups of APTs-based SAM micropatterns might be slightly changed during the liquid SAM deposition and microfabrication process. As a result, we demonstrated that the protein adsorption was not affected by the surface wettability properties, but the surface functional terminal group significantly influenced the protein adsorption.

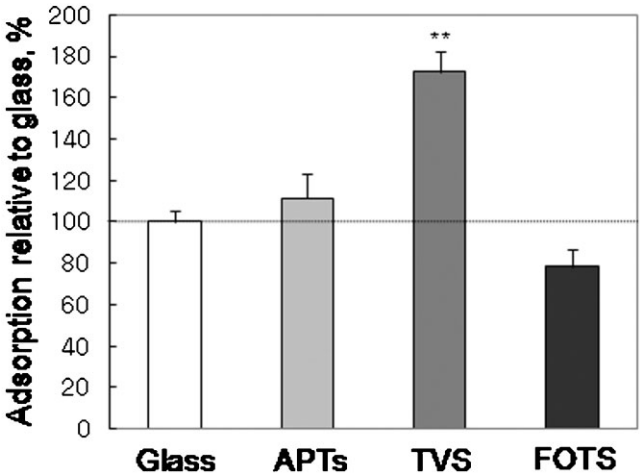

Figure 7. Protein adsorption analysis of APTs-, TVS-, and FOTS-based SAM micropatterned substrates. Protein adsorption was measured by analyzing the fluorescent intensity of FITC-BSA, relative to a glass substrate. The TVS-based substrate showed greater protein adsorption, whereas proteins were not adhered well to the FOTS-based substrate. The protein adhesion on TVS-based micropatterns was compared with a glass substrate ($^{**}P < 0.01$).

Conclusions

A surface micropatterned array system using photolithography and liquid/vapor SAM deposition technique was developed. The APTs-, TVS-, and FOTS-based SAM micropatterned array systems enabled the control of the hydrophilic and hydrophobic properties. The hydrophilic and hydrophobic surface properties played an important role in controlling the cell adhesion and patterning. The cells did not adhere well to hydrophobic TVS and FOTS substrates, whereas the cells were more adhered to hydrophilic APTs substrates. Thus, the cells were selectively patterned on glass substrates within hydrophobic TVS- and FOTS-based micropatterns. Furthermore, the protein adsorption to APTs-, TVS-, and FOTS-based SAM micropatterned substrates indicated that proteins were more adhered to TVS surfaces. We demonstrated that the surface functional terminal group enabled the control of protein adsorption. Therefore, this SAM-based surface micropatterning technique could be a potentially powerful tool to pattern cells and biomolecules.

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