



Short communication

Fabrication of cell pattern on poly(dimethylsiloxane) by vacuum ultraviolet lithography

Jinbo Gan^{a,b}, Hong Chen^{a,b,*}, Feng Zhou^{a,b}, He Huang^{a,b}, Jun Zheng^{a,b}, Wei Song^{a,b},
Lin Yuan^{a,c}, Zhongkui Wu^b

^a State Key Laboratory of Advanced Technology for Materials Synthesis and Processing, Wuhan University of Technology, 122 Luoshi Rd, Wuhan 430070, China

^b School of Materials Science and Engineering, Wuhan University of Technology, 122 Luoshi Rd, Wuhan 430070, China

^c Biomedical Materials and Engineering Center, Wuhan University of Technology, 122 Luoshi Rd, Wuhan 430070, China

ARTICLE INFO

Article history:

Received 5 July 2009

Received in revised form 22 October 2009

Accepted 13 November 2009

Available online 20 November 2009

Keywords:

Cell pattern

Poly(dimethylsiloxane)

Vacuum ultraviolet lithography

Allyl-polyethylene glycol

ABSTRACT

Cell patterning on substrates has played a significant role in the study of basic biology, cell-based biosensor and tissue engineering. In this report, a cell pattern was prepared on poly(dimethylsiloxane) (PDMS) substrate by vacuum ultraviolet (VUV) lithography. After immobilizing allyl-polyethylene glycol (APEG) onto PDMS, a chemical heterogeneous patterned surface was fabricated by VUV (Xe₂ excimer: 172 nm) lithography with copper mesh as a photomask. The UV exposed domains can promote L929 cell adhesion and growth. However, non-exposed regions resist cell attachment because of the repelling property of PEG. Therefore, cell pattern could be achieved without pre-adsorption of cell adhesive species before cell culture.

© 2009 Elsevier B.V. All rights reserved.

1. Introduction

Cell patterning, a method for spatial controlling of cell adhesion and growth on substrates, is important for a wide range of applications, such as basic biological study, cell-based biosensor and tissue engineering [1,2]. The most commonly used substrates are silicon or silica oxide [3], gold [4], glass [5], poly(methyl methacrylate) [6], polystyrene [7], poly(ethylene terephthalate) [8], poly(dimethylsiloxane) (PDMS) [9] and so on. Among these substrates, PDMS is a cheap and flexible material that has been extensively used in biomedical field due to its excellent physical and chemical properties [10,11]. For example, it is transparent and presents significant low autofluorescence (background fluorescence) which makes it suitable for a number of detection schemes, such as UV–vis absorbance and fluorescence. It exhibits well-known biocompatibility due to its biological inertness and nontoxicity. Therefore, cell patterning on PDMS substrate is attractive for its potential applications in biosensor and cell-based devices, cell biology study and the fabrication of implanted devices.

A variety of different techniques have been utilized to prepare cell pattern, such as soft lithography [6], self-assembly monolayer (SAM) [12], electron beam (EB) polymerization using masks (EB-mask method) [13], photolithography [14], vacuum ultraviolet (VUV) lithography [15], etc. Most of these techniques typically involve additional step to introduce cell adhesive species before cell culture, such as fibronectin [6,16], vitronectin [17], laminin [18], collagen [9,19], gelatin [20], RGD-peptides [21,22], etc., which are normally used to direct cells to the designated location. Cell patterns can also be produced without the introduction of cell adhesive species [23–25], to significantly simplify the patterning process [26]. One such a way is to employ the external assistant to guide cells onto substrates with patterned distribution, such as magnetic force [27,28], electric field [29,30] and stencil [31,32]. Another method is to create micron-sized cell adhesive and cell resistant regions on substrates by controlling spatial arrangement of surface chemical properties [33]. As these two regions exhibit different properties for cell adhesion, cell pattern will be formed by directly seeding cells on these substrates [34–39]. This method is more widely used as it provides a beneficial platform to study the fundamental issues of cell biology, such as cell–substrate, cell–media and cell–cell interactions [1,40,41].

In this communication, a cell pattern was fabricated on PDMS substrate via VUV lithography but without introducing cell adhesive species before cell culture. First of all, allyl-polyethylene glycol (APEG) was immobilized onto a PDMS substrate. Then a chemical

* Corresponding author at: School of Materials Science and Engineering, Wuhan University of Technology, 122 Luoshi Rd, Wuhan 430070, China.

Tel.: +86 27 87168305; fax: +86 27 87168305.

E-mail address: hongchen@whut.edu.cn (H. Chen).

heterogeneous patterned surface was fabricated by VUV lithography. Since the UV exposed domains and non-exposed regions exhibit different properties for cell adhesion, L929 cells would selectively attach to the UV exposed domains and then cell pattern was formed. This work is an extension of our recent study on the fabrication of a sub-micron hydrophilic microchannel on poly(dimethylsiloxane) (PDMS) by VUV lithography [42].

2. Materials and methods

2.1. Materials

PDMS elastomer base and curing agent (Sylgard 184, Midland, MI) were supplied by Dow Corning. Monoallyl-polyethylene glycol (APEG, Mn = 560) was obtained as a gift from JuTian Chemical Co., Nanjing, China. Calcium fluoride (CaF_2) was purchased from Changchun Goldragonoptics Co., Changchun, China. Copper mesh (used as photomask in this study) was commonly used in transmission electron microscopy. Fibrinogen (Fg, Plasminogen-Depleted, Human Plasma) was purchased from Cal-BioChem (CAS No. 9001-32-5) and Fluorescein isothiocyanate (FITC) was purchased from Amresco.

2.2. Fabrication of patterned surface by vacuum ultraviolet (VUV) lithography

PDMS film was prepared by a standard method according to the production information. After curing and being cut into disks, PDMS surfaces were grafted with APEG by hydrosilylation reaction. Details about the surface modification with APEG have been reported previously [43].

Then a PEG-grafted PDMS (PDMS-PEG) disk and a copper mesh were placed between two pieces of calcium fluoride (CaF_2). In order to make the copper mesh closely contact with PDMS-PEG disk, proper pressure was applied on these two pieces of CaF_2 , and then the PDMS-PEG disk was irradiated with 172 nm vacuum ultraviolet excimer source (Ushio Inc., UEM20-172, Xe_2 excimer, $\lambda = 172$ nm, photo energy: 7.2 eV) for 10 min in atmosphere. Finally, the copper mesh was carefully removed and a patterned PDMS-PEG surface was formed. After ultrasonic rinse in acetone, the patterned surface was characterized by atomic force microscopy (AFM, Nanoscope IV, Digital Instruments, Veeco, US), with tapping mode in air.

2.3. Cell culture and trypan blue staining

L929 cells (purchased from China Center for Type Culture Collection, Wuhan) were routinely cultured in RPMI 1640 medium (Gibco, MD, USA) with 10% fetal bovine serum and incubated at 37 °C in air containing 5% CO_2 . Before seeding, the patterned PDMS-PEG disks were fixed in the wells of a 48-well tissue culture plate. Without pre-adsorption cell adhesive species, 1000 μL L929 cell suspension was dripped onto each patterned surface (cell density: 20,000 cells/ cm^2) and cultured in the incubator (Galaxy S, RS Biotech, UK) for 12 days while cell culture medium was changed every 3 days.

Before trypan blue staining, 860 μL cell culture media was removed carefully. Then 140 μL trypan blue solution (0.4%) was dripped into each well (1:1 with cell culture media). To count the number of viable (unstained) and dead (stained) cells, micrographs of L929 cells were captured by using inverted optical microscope (XDS-1B, Chongqing Optical & Electrical Instrument Co., China) equipped with a CMOS camera (GDS310, Guang Di Optical & Electrical Instrument Co., China). Staining test and optical microscope observation were conducted within 3 min. In order to count the number of L929 cells adhered on the patterns, nine measurements were taken from three samples treated identically.

2.4. Protein adsorption

Fibrinogen (Fg) was labeled with Fluorescein isothiocyanate (FITC) and the labeled protein (Fg-FITC) solution was purified and adjusted to 1.0 mg/mL [44] for the following adsorption experiment. Then the patterned surfaces were transferred into a 96-well tissue culture plate and 250 μL Fg-FITC solution was dripped into each well. After incubated at 25 °C for 3 h, each surface was rinsed by 250 μL phosphate-buffered saline (PBS, pH 7.4) for three times. Protein adsorbed on the patterned surfaces was observed by a laser scanning confocal microscope (TCS-SP2 AOBS, Leica) (excitation 488 nm; emission 500–535 nm).

3. Results and discussion

3.1. Characterization of the patterned PDMS-PEG surface

The topography and chemical properties of the patterned PDMS-PEG surface were characterized by AFM analysis. As clearly shown in the height image (Fig. 1(A)), a patterned surface was prepared and the surface layer of about 145 nm was ablated (Fig. 1(A), inset). It is well known that brightness of AFM phase image is relevant to the chemical property and/or rigidity of the substrate's surface [45]. Thus, different brightness between the UV exposed domains and non-exposed regions in phase image (Fig. 1(B)) indicate that there were some chemical composition changes and/or rigidity changes in the UV exposed domains. Based on the height and phase images, we may speculate that when exposed to the high energy VUV excimer source, surface grafted PEG layer was ablated; however, it still remained on the non-exposed regions. Meanwhile, width of the UV exposed domains (square) was estimated to be 50.2 μm (BC) and width of the non-exposed regions (stripe) was about 26.7 μm (AB), respectively. Therefore, a chemical heterogeneous patterned surface was achieved on PDMS-PEG by VUV lithography with copper mesh as a photomask.

3.2. Cell patterning and trypan blue staining

In this study, L929 cells were used as model cells to perform cell patterning. After cultured on patterned PDMS-PEG and patterned PDMS surfaces for 3 days, cell living status was observed by trypan blue staining. On the patterned PDMS-PEG surface, L929 cells selectively adhered to the UV exposed domains; most of them closely attached to the substrate. In contrast, few cells adhered or grew poorly in the non-exposed regions (Fig. 2(A)). This result showed a uniform cell pattern in accord with the patterned substrate.

In order to count L929 cells occupying on each UV exposed domain, Image J was taken for cell counting and results were shown in Fig. 2(C). On 50.2 $\mu\text{m} \times 50.2 \mu\text{m}$ patterns, the UV exposed domains containing three to six L929 cells occupied nearly 88% of these domains. Meanwhile, it was also observed that on the patterned PDMS-PEG surfaces, nearly all the L929 cells were located on the UV exposed domains. Although most of them could not spread with the shape of normally cultured L929 cells, trypan blue staining test (Fig. 2(A)) showed that only a few of the cells were dead (indicated by arrows) and cell viability was 97.0% (Fig. 2(D)). It can be speculated that these domains (50.2 $\mu\text{m} \times 50.2 \mu\text{m}$) were suitable for three or four L929 cells to spread and grow. When more cells attached to the UV exposed domains, they could not spread well due to the limited growth area (most of cells were round (Fig. 2(A))).

In addition, it was also noted that vertical depth of the UV exposed domain (145.8 nm) has little influence on cell patterning. Here, native PDMS disks without surface grafting of PEG were chosen to conduct VUV lithography and L929 cell culture experiments under the same condition. According to the above observation,

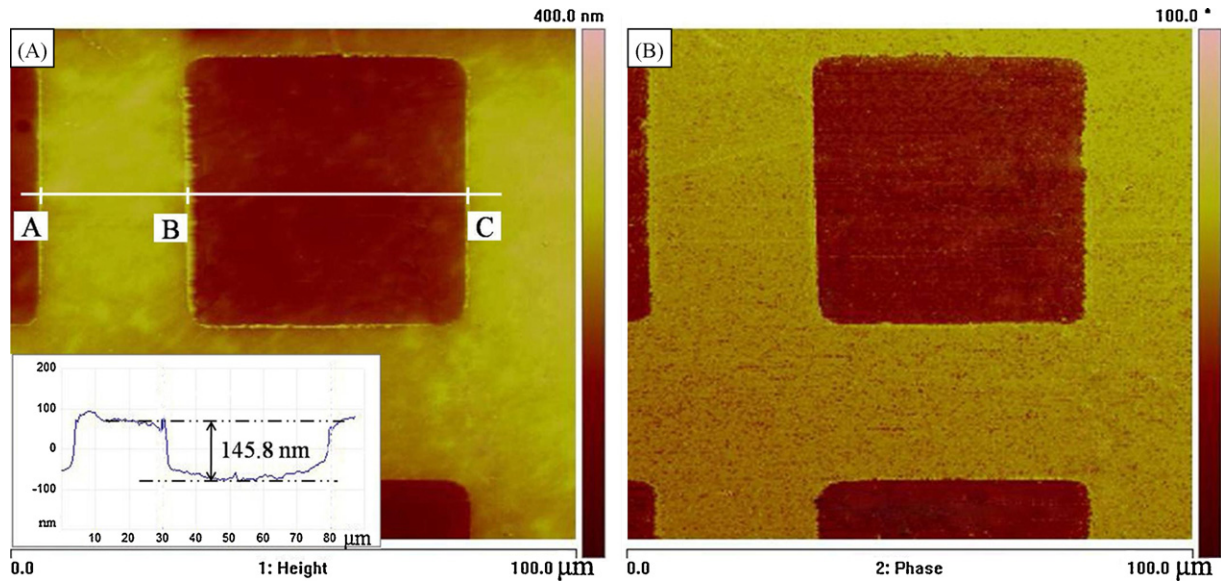


Fig. 1. AFM images of patterned PDMS-PEG surfaces: (A) height image and section analysis (inset); (B) phase image.

although the surface layer of native PDMS was ablated and a patterned surface was formed (data not shown), both the UV exposed domain and the non-exposed region were pure PDMS substrate. Thus L929 cells could adhere to both regions and grew well despite of the physical topology (Fig. 2(B)). Most of them spread well with the fusiform morphology (Fig. 2(B)) and cell viability was 95.4% (Fig. 2(D)). These results demonstrate that it's the chemical het-

erogeneity rather than physical topology that brings about cell patterning.

3.3. Protein adsorption

In this report, a cell pattern was prepared on the chemical heterogeneous patterned PDMS-PEG surface without pre-adsorption

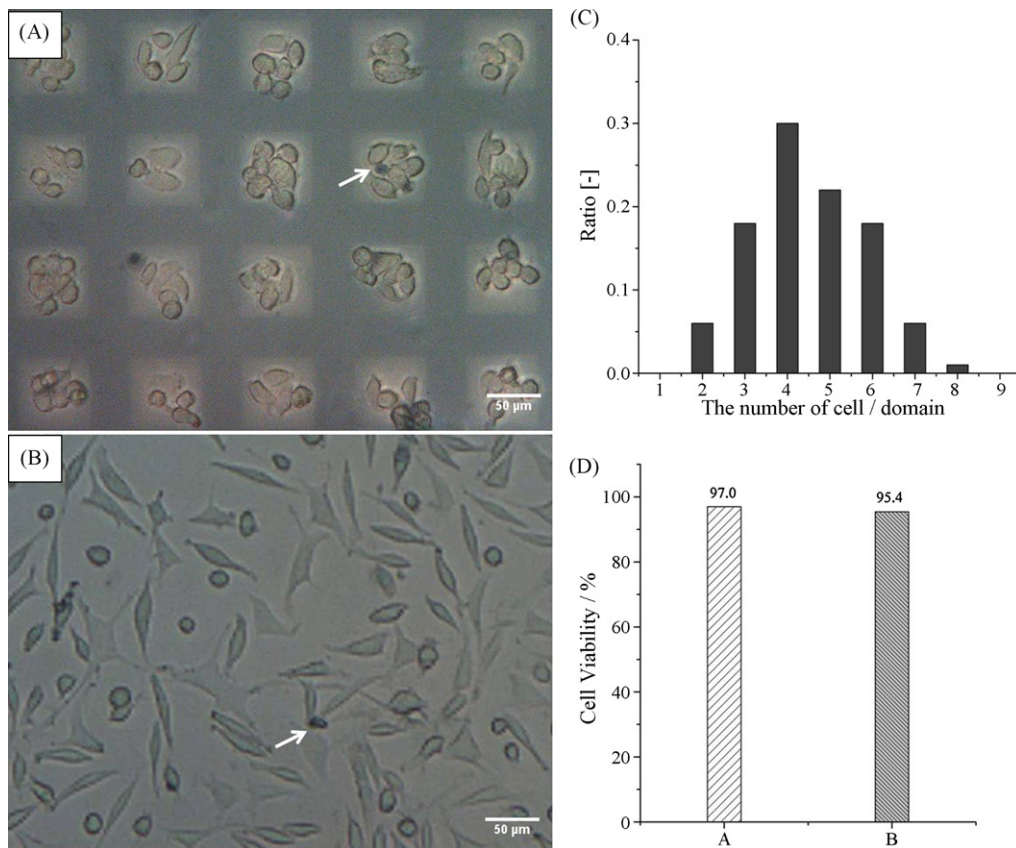


Fig. 2. L929 cells cultured on different surfaces for 3 days: the representative optical micrographs of trypan blue staining test on (A) patterned PDMS-PEG surface; (B) patterned PDMS surface; dead cells (stained) indicated by arrows. (C) Histogram for the number of adherent cells per domain with the patterned domain sizes as shown in (A); cells were counted and averaged over 300 domains. (D) Histogram for the cell viability of (A) and (B); cells were counted over 1200. Scale bar: 50 μm.

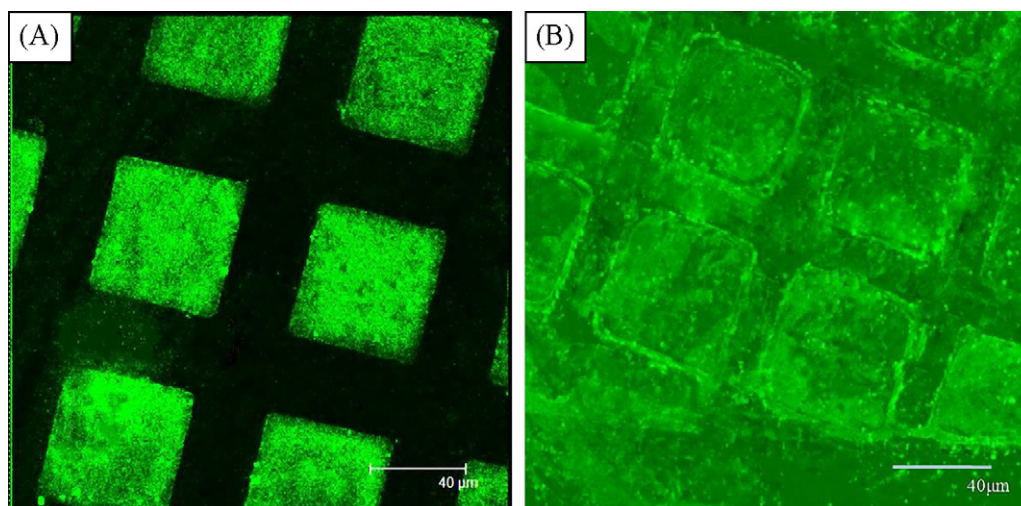


Fig. 3. Laser scanning confocal microscope photographs of Fg-FITC adsorbed on (A) patterned PDMS-PEG surface; (B) patterned PDMS surfaces. Scale bar: 40 μm .

of cell adhesive species before cell culture. It's generally believed that protein adsorption onto a surface is the initial stage of cell adhesion [46]. Therefore, in cell culture medium, non-specific protein adsorption to UV exposed domains may be the major reason for cell patterning.

In order to confirm our assumption, fibrinogen (Fg), a commonly used model protein to examine non-specific protein adsorption, was chosen to conduct protein adsorption on the patterned PDMS and PDMS-PEG surfaces. After incubated in Fg-FITC solution (1.0 mg/mL) at 25 °C for 3 h, laser scanning confocal microscope was used to provide qualitative information about the spatial distribution of protein on these surfaces and the results were shown in Fig. 3. As the green fluorescence intensity is proportional to the amount of absorbed Fg, it can be found that a considerable amount of Fg was adsorbed in the UV exposed domains while little Fg was adsorbed in non-exposed regions (Fig. 3(A)) on the PDMS-PEG surface, leading to a clear and uniform protein pattern on this surface. However, on the patterned PDMS surface, a large amount of Fg was adsorbed on both the UV exposed domains and the non-exposed regions (Fig. 3(B)). These results corresponded with the distribution of L929 cells cultured on the patterned surfaces (Fig. 2).

The above results are easy to understand. On a patterned PDMS-PEG surface, surface grafted PEG layer on UV exposed domains was ablated by high energy VUV excimer source and PDMS substrate was exposed; however, grafted PEG layer still remained on non-exposed regions. Because of the repelling property of PEG to non-specific protein adsorption [47], almost all the proteins in cell culture medium including cell adhesive species (e.g., extracellular matrix (ECM) proteins) would be adsorbed on UV exposed domains rather than non-exposed regions. Consequently, these proteins promoted and also confined cell adhesion and growth to the UV exposed domains (Fig. 2(A)). In contrast, few cells adhered or spread in the non-exposed regions (Fig. 2(A)) as few proteins were adsorbed in these regions. On a patterned PDMS surface, however, substrates of both the UV exposed domains and non-exposed regions were PDMS, which can bring about strong non-specific protein adsorption [48]. Thus proteins in cell culture medium would be adsorbed onto both regions, followed by L929 cells spreading and growing on the whole surface (Fig. 2(B)).

Sugiura et al. [20] have fabricated cell pattern on PDMS substrate by photo-induced graft polymerization of poly(ethylene glycol). However, pre-adsorption of gelatin was required and cell pattern was not uniform as some of the domains pre-adsorbed with gelatin were not occupied by HepG2 cells. In our study reported here, a convenient method has been provided to prepare uniform cell

pattern on PDMS without pre-adsorption of cell adhesive species before cell culture. PEG is a commonly used anti-fouling species to block protein adsorption and we have successfully grafted PEG onto PDMS by covalent immobilization [43]. Therefore, on the patterned PDMS-PEG surface, few proteins or L929 cells were located in the non-exposed regions where grafted PEG still remained. Consequently, well-defined cell pattern was achieved and it can be maintained for 12 days (cell culture medium was changed every 3 days) as covalently immobilized PEG was stable under the cell culture condition. Furthermore, this method can be utilized to confine single cell on the patterned domain and to pattern co-culture of heterotypic cells by controlling the surface property of PDMS and adjusting the feature size of the photomask. As evaluation and analysis of cell interaction in single cell level on patterned surfaces is important in basic cell study, this method would be of potential application in cell-based biosensor, basic cell biology and tissue engineering fields.

4. Conclusions

In summary, a simple and convenient method has been reported for cell patterning on PDMS substrate without pre-adsorption of cell adhesive species before cell culture. The chemical heterogeneous patterned PDMS-PEG surface was prepared by VUV lithography. For cell patterning, L929 cells selectively adhered and grew in the UV exposed domains while few cells were attached to the non-exposed regions. Fluorescent images of Fg-FITC adsorbed on these patterned surfaces were in accord with the distribution of L929 cells cultured on these surfaces. By tailoring the surface property of PDMS and adjusting the feature size of photomask, this method would pave another way for the research and application of basic cell biology and cell-based devices.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (90606013, 20634030, 50973087), the Ministry of Education of China (107080, NCET0606055), and the Ministry of Science and Technology of China (2008CB617510).

References

- [1] F.L. Yap, T. Zhang, Protein and cell micropatterning and its integration with micro/nanoparticles assembly, *Biosens. Bioelectron.* 22 (2007) 775–788.

- [2] D. Falconnet, G. Csucs, H.M. Grandin, M. Textor, Surface engineering approaches to micropatterned surfaces for cell-based assays, *Biomaterials* 27 (2006) 3044–3063.
- [3] M. Veis, M. Zhang, Effect of silicon oxidation on long-term cell selectivity of cell-patterned Au/SiO₂ platforms, *J. Am. Chem. Soc.* 128 (2006) 1197–1203.
- [4] H.W. Ma, J.H. Hyun, P. Stiller, A. Chilkoti, Non-fouling oligo(ethylene glycol)-functionalized polymer brushes synthesized by surface-initiated ATRP, *Adv. Mater.* 16 (2004) 338–341.
- [5] A. Magnani, A. Priamo, D. Pasqui, R. Barbucci, Cell behaviour on chemically microstructured surfaces, *Mater. Sci. Eng. C* 23 (2003) 315–328.
- [6] H.W. Ma, Z. Zhang, T.P. Beebe Jr., A. Chilkoti, Fabrication of biofunctionalized quasi 3-dimensional microstructures of a nonfouling comb polymer by soft lithography, *Adv. Funct. Mater.* 15 (2005) 529–540.
- [7] S. Iwanaga, Y. Akiyama, A. Kikuchi, M. Yamato, K. Sakai, T. Okano, Fabrication of a cell array on ultrathin hydrophilic polymer gels utilizing electron beam irradiation and UV excimer laser ablation, *Biomaterials* 26 (2005) 5395–5404.
- [8] A. Duncan, F. Rouais, S. Lazare, L. Bordenave, C.H. Baquey, Effect of laser modified surface microtopochemistry on endothelial cell growth, *Colloids Surf. B: Biointerfaces* 54 (2007) 150–159.
- [9] S. Hou, K. Yang, M. Qin, X.Z. Feng, L. Guan, Y. Yang, C. Wang, Patterning of cells on functionalized poly(dimethylsiloxane) surface prepared by hydrophobin and collagen modification, *Biosens. Bioelectron.* 24 (2008) 912–916.
- [10] J.C. McDonald, G.M. Whitesides, Poly(dimethylsiloxane) as a material for fabricating microfluidic devices, *Acc. Chem. Res.* 35 (2002) 491–499.
- [11] A. Piruska, I. Nikcevic, S.H. Lee, C.H. Ahn, W.R. Heineman, P.A. Limbach, C.J. Seliskar, The autofluorescence of plastic materials and chips measured under laser irradiation, *Lab Chip* 5 (2005) 1348–1354.
- [12] S. Zhang, L. Yan, M. Altman, M. Lasse, H. Nugent, F. Frankel, D.A. Lauffenburger, G.M. Whitesides, A. Rich, Biological surface engineering: a simple system for cell pattern formation, *Biomaterials* 20 (1999) 1213–1220.
- [13] Y. Tsuda, A. Kikuchi, M. Yamato, A. Nakao, Y. Sakurai, T. Okano, The use of patterned dual thermoresponsive surfaces for collective recovery as co-cultured cell sheets, *Biomaterials* 26 (2005) 1885–1893.
- [14] J.Y. Lee, S.S. Shah, C.C. Zimmer, G. Liu, A. Revzin, Use of photolithography to encode cell adhesive domains into protein microarrays, *Langmuir* 24 (2008) 2232–2239.
- [15] S. Asakura, A. Hozumi, T. Yamaguchi, A. Fuwa, A simple lithographic method employing 172 nm vacuum ultraviolet light to prepare positive- and negative-tone poly(methyl methacrylate) patterns, *Thin Solid Films* 500 (2006) 237–240.
- [16] M. Yamato, C. Konno, S. Koike, T. Shimizu, A. Kikuchi, K. Makino, T. Okano, Nanofabrication for micropatterned cell arrays by combining electron beam irradiated polymer-grafting and localized laser ablations, *J. Biomed. Mater. Res. A* 67 (2003) 1065–1071.
- [17] C.D. McFarland, C.H. Thomas, C.D. Filippis, J.G. Steele, K.E. Healy, Protein adsorption and cell attachment to patterned surfaces, *J. Biomed. Mater. Res.* 49 (2000) 200–210.
- [18] P. Krsko, T.E. McCann, T.T. Thach, T.L. Laabs, H.M. Geller, M.R. Libera, Length-scale mediated adhesion and directed growth of neural cells by surface-patterned poly(ethylene glycol) hydrogels, *Biomaterials* 30 (2009) 721–729.
- [19] A. Revzin, P. Rajagopalan, A.W. Tilles, F. Berthiaume, M.L. Yarmush, M. Toner, Designing a hepatocellular microenvironment with protein microarraying and poly(ethylene glycol) photolithography, *Langmuir* 20 (2004) 2999–3005.
- [20] S. Sugiura, J. Edahiro, K. Sumaru, T. Kanamori, Surface modification of polydimethylsiloxane with photo-grafted poly(ethylene glycol) for micropatterned protein adsorption and cell adhesion, *Colloids Surf. B: Biointerfaces* 63 (2008) 301–305.
- [21] H. Hatakeyama, A. Kikuchi, M. Yamato, T. Okano, Patterned biofunctional designs of thermoresponsive surfaces for spatiotemporally controlled cell adhesion, growth, and thermally induced detachment, *Biomaterials* 25 (2007) 3632–3643.
- [22] J. Hyun, H. Ma, P. Banerjee, J. Cole, K. Gonsalves, A. Chilkoti, Micropatterns of a cell-adhesive peptide on an amphiphilic comb polymer film, *Langmuir* 18 (2002) 2975–2979.
- [23] V. Dhir, A. Natarajan, M. Stancescu, A. Chunder, N. Bhargava, M. Das, L. Zhai, P. Molnar, Patterning of diverse mammalian cell types in serum free medium with photoablation, *Biotechnol. Prog.* 25 (2009) 594–603.
- [24] J. You, J.S. Heo, J. Lee, H.S. Kim, H.O. Kim, E. Kim, A fluorescent polymer for patterning of mesenchymal stem cells, *Macromolecules* 42 (2009) 3326–3332.
- [25] I. Beaulieu, M. Geissler, J. Mauzeroll, Oxygen plasma treatment of polystyrene and zeonor: substrates for adhesion of patterned cells, *Langmuir* 25 (2009) 7169–7176.
- [26] J.P. Frimat, H. Menne, A. Michels, S. Kittel, R. Kettler, S. Borgmann, J. Franzke, J. West, Plasma stencilling methods for cell patterning, *Anal. Bioanal. Chem.* 395 (2009) 601–609.
- [27] K. Ino, M. Okochi, H. Honda, Application of magnetic force-based cell patterning for controlling cell–cell interactions in angiogenesis, *Biotechnol. Bioeng.* 102 (2009) 882–890.
- [28] G. Frasca, F. Gazeau, C. Wilhelm, Formation of a three-dimensional multicellular assembly using magnetic patterning, *Langmuir* 25 (2009) 2348–2354.
- [29] M. Suzuki, T. Yasukawa, H. Shiku, T. Matsue, Negative dielectrophoretic patterning with different cell types, *Biosens. Bioelectron.* 24 (2008) 1043–1047.
- [30] A. Sebastian, A.M. Buckle, G.H. Markx, Tissue engineering with electric fields: immobilization of mammalian cells in multilayer aggregates using dielectrophoresis, *Biotechnol. Bioeng.* 98 (2007) 694–700.
- [31] A. Folch, B.H. Jo, O. Hurtado, D.J. Beebe, M. Toner, Microfabricated elastomeric stencils for micropatterning cell cultures, *J. Biomed. Mater. Res.* 52 (2000) 346–353.
- [32] E. Ostuni, R. Kane, C.S. Chen, D.E. Ingber, G.M. Whitesides, Patterning mammalian cells using elastomeric membranes, *Langmuir* 16 (2000) 7811–7819.
- [33] H.W. Chien, T.Y. Chang, W.B. Tsai, Spatial control of cellular adhesion using photo-crosslinked micropatterned polyelectrolyte multilayer films, *Biomaterials* 30 (2009) 2209–2218.
- [34] K. Jang, K. Sato, K. Mawatari, T. Konno, K. Ishihara, T. Kitamori, Surface modification by 2-methacryloyloxyethyl phosphorylcholine coupled to a photolabile linker for cell micropatterning, *Biomaterials* 30 (2009) 1413–1420.
- [35] Y.K. Kim, S.R. Ryoo, S.J. Kwack, D.H. Min, Mass spectrometry assisted lithography for the patterning of cell adhesion ligands on self-assembled monolayers, *Angew. Chem. Int. Ed.* 48 (2009) 3507–3511.
- [36] A. Kira, K. Okano, Y. Hosokawa, A. Naito, K. Fuwa, J. Yuyama, H. Masuhara, Micropatterning of perfluoroalkyl self-assembled monolayers for arraying proteins and cells on chips, *Appl. Surf. Sci.* 255 (2009) 7647–7651.
- [37] M.E. Buck, A.S. Breitbart, S.K. Belgrade, H.E. Blackwell, D.M. Lynn, Chemical modification of reactive multilayered films fabricated from poly(2-alkenyl azlactone)s: design of surfaces that prevent or promote mammalian cell adhesion and bacterial biofilm growth, *Biomacromolecules* 10 (2009) 1564–1574.
- [38] L.H. Jin, B.Y. Yang, L. Zhang, P.L. Lin, C. Cui, J. Tang, Patterning of HeLa cells on a microfabricated Au-coated ITO substrate, *Langmuir* 25 (2009) 5380–5383.
- [39] A.E. Oliver, V. Ngassam, P. Dang, B. Sanii, H.W. Wu, C.K. Yee, Y. Yeh, A.N. Parikh, Cell attachment behavior on solid and fluid substrates exhibiting spatial patterns of physical properties, *Langmuir* 25 (2009) 6992–6996.
- [40] J. Nakanishi, T. Takarada, K. Yamaguchi, M. Maeda, Recent advances in cell micropatterning techniques for bioanalytical and biomedical sciences, *Anal. Sci.* 24 (2008) 67–72.
- [41] K. Leong, A.K. Boardman, H. Ma, A.K.Y. Jen, Single-cell patterning and adhesion on chemically engineered poly(dimethylsiloxane) surface, *Langmuir* 25 (2009) 4615–4620.
- [42] Z. Wu, H. Yan, H. Chen, H. Huang, One-stage fabrication of sub-micron hydrophilic microchannels on PDMS, *Appl. Surf. Sci.* 255 (2009) 4702–4704.
- [43] H. Chen, L. Wang, Y. Zhang, D. Li, W.G. McClung, M.A. Brook, H. Sheardown, J.L. Brash, Fibrinolytic poly(dimethyl siloxane) surfaces, *Macromol. Biosci.* 8 (2008) 863–870.
- [44] H. Chen, W. Song, F. Zhou, Z. Wu, H. Huang, J. Zhang, Q. Lin, B. Yang, The effect of surface microtopography of poly(dimethylsiloxane) on protein adsorption, platelet and cell adhesion, *Colloids Surf. B: Biointerfaces* 71 (2009) 275–281.
- [45] H. Wang, A.B. Djurisic, W.K. Chan, M.H. Xie, Factors affecting phase and height contrast of diblock copolymer PS-b-PEO thin films in dynamic force mode atomic force microscopy, *Appl. Surf. Sci.* 252 (2005) 1092–1100.
- [46] E. Ostuni, R.G. Chapman, M.N. Liang, G. Meluleni, G. Pier, D.E. Ingber, G.M. Whitesides, Self-assembled monolayers that resist the adsorption of proteins and the adhesion of bacterial and mammalian cells, *Langmuir* 17 (2001) 6336–6343.
- [47] H. Chen, L. Yuan, W. Song, Z. Wu, D. Li, Biocompatible polymer materials: role of protein–surface interactions, *Prog. Polym. Sci.* 33 (2008) 1059–1087.
- [48] V. Linder, E. Verpoorte, W. Thormann, N.F. de Rooij, M. Sigrist, Surface biopassivation of replicated poly(dimethylsiloxane) microfluidic channels and application to heterogeneous immunoreaction with on-chip fluorescence detection, *Anal. Chem.* 73 (2001) 4181–4189.