Cell Adhesion

DOI: 10.1002/anie.200603844

A Method for Patterning Multiple Types of Cells by Using Electrochemical Desorption of Self-Assembled Monolayers within Microfluidic Channels**

*Yong Li, Bo Yuan, Hang Ji, Dong Han, Shiqian Chen, Feng Tian, and Xingyu Jiang**

This report describes a method for patterning multiple types of adherent cells on the same substrate by electrochemical desorption of self-assembled monolayers (SAMs) in localized areas defined by a microfluidic system.[1–4] Several groups have previously reported techniques that allow the patterning of two different types of cells. None of these reported techniques, however, could both confine two or more types of cells to specific locations on surfaces without the presence of physical constraints and control the motility of these different types of cells.[1,2,4,5] The technique presented herein will be useful for a number of biological systems, such as in the studies of neuronal development and in the control of tumor growth.[6]

Our method employs a commercially available thiol ($HS(CH_2)_{11}(OCH_2CH_2)_6OH$, abbreviated as "$EG_6$") to form a SAM on the gold surface, which resists adsorption of proteins and adhesion of cells (for convenience, we call this surface the "inert surface").[3] A poly(dimethylsiloxane) (PDMS) stamp with embedded microfluidic channels is used to carry out selective electrochemical desorption of EG_6 from the gold substrate (Figure 1).[7] This procedure allows parts of the inert surface to promote the adsorption of proteins and the adhesion of cells (we call this transformation "activation of the inert surface").[8,9] Each of these individually addressable microchannels can deliver one type of cell to activated regions of the surface, resulting in a pattern of multiple types of cells on the surface. Because an electrochemical reaction can take place only in areas exposed to microfluidic channels, patterned cells are confined to activated regions, which are defined by these channels upon removal of the stamp that carries the fluidic system. As there is no physical barrier between these cells, there is a free exchange of substances between these different types of cells through the liquid medium. This exchange allows the studies of cell–cell interactions when different types of cells are confined to separate locations on the surface. A second step of electrochemical desorption can "turn on" motility of cells and allow them to move under the influence of each other.

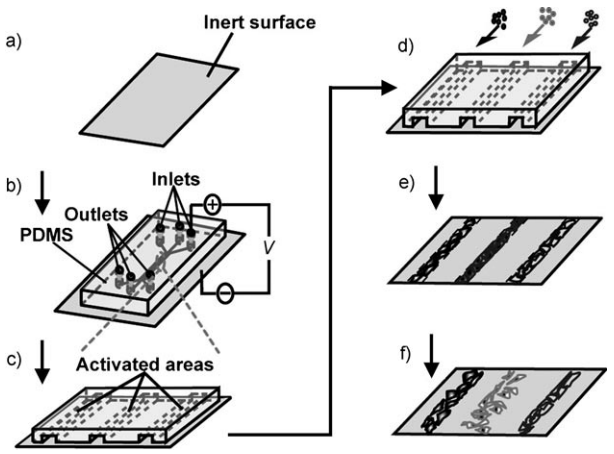

Figure 1. Strategy for patterning different types of cells. a) To obtain "inert" surfaces, we formed SAMs on gold-coated coverslips with EG_6. b) A PDMS stamp with an embedded microfluidic system was brought into contact with the substrate, and the channels were filled with solutions of fibronectin. Application of a cathodic potential on the gold substrates desorbed SAMs inside the channels. c)–f) Magnified views of the main functional locations of the channel system. c) Adsorption of proteins inside the microchannels after electrochemical activation of the surface. d) Adhesion of cells on the floors of the channels. e) After the PDMS stamp was peeled off, a pattern of different types of cells was formed. f) A second step of electrochemical desorption enabled cells that were previously confined in patterns to spread across the previously inert surface.

We illustrate this approach by patterning two types of cells (NIH 3T3 and Hela cells) in stripes. Fabrication of an inert surface is accomplished by coating a gold-covered glass substrate with EG_6. To selectively activate the inert surface, we first coated the inert substrate with a PDMS stamp with embedded microfeatures (see the Supporting Information for its fabrication) to form enclosed microchannels.[10] The features embedded in the PDMS stamp formed the ceilings and vertical walls and the gold substrate formed the floors of the channels; the channels were reversibly sealed. We filled the channels with solutions of the extracellular matrix (ECM) protein fibronectin (100 $\mu g\,mL^{-1}$ in a phosphate-buffered

[*] Y. Li, B. Yuan, Prof. D. Han, Prof. X. Jiang
National Center for NanoScience and Technology
2 ZhongGuanCun North 1st Street, Beijing, 100080 (P.R. China)
Fax: (+86) 10-6265-6765
E-mail: xingyujiang@nanoctr.cn
Prof. H. Ji
School of Phyics, Peking University
Beijing, 100871 (P.R. China)
Prof. S. Chen, Prof. F. Tian
Institute of Medical Equipment, AMMS, P.L.A.
Tianjin, 300161 (P.R. China)

[**] We thank Prof. Lei Jiang and Prof. Chen Wang for their help. Funding for this work was provided by the Chinese Academy of Sciences, the National Science Foundation of China (90406024 and 20605006), and the Ministry of Science and Technology (2005CB724700 and 2006CB705600).

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

saline solution) and applied a cathodic potential ($\approx$1.2 V versus a stainless-steel electrode, the anode, for 30 s) on the gold substrate. The solution of fibronectin provided both the electrolyte necessary for electrochemistry and the ECM protein, which adsorbed onto the surface to aid in the adhesion of cells once the electrochemistry-mediated activation of the surface was completed. We placed the anode in a droplet of solution that led to the microchannel, and used the gold surface as the cathode. The electrochemical potential desorbed only the SAMs exposed to the fluids in the microchannels. Desorption of EG_6 in the channels activated the surface for adsorption of proteins and adhesion of cells. After adsorption of fibronectin for 2 h in these activated areas, we delivered NIH 3T3 and Hela cells in a culture medium to the surface through the separate channels. Cells were allowed to settle on and adhere to the surface for 40 min.

After the attachment of cells, we peeled the PDMS stamp off the surface of the substrate. Because electrochemical desorption of SAMs took place only in the microchannels, the rest of the surface on the gold substrate was still inert and prevented adhesion and invasion of cells. The inert regions on the surface confined two or more types of cells to separate locations without the presence of physical barriers (such as a PDMS stamp).[1] We generated a pattern of three stripes of cells: the middle stripe consisted of only NIH 3T3 cells and the two stripes on either side only contained Hela cells (Figure 2). The cells remained in their patterns of confinement for at least three days. These stripes of different types of cells allowed studies of the interactions of cells through exchange of soluble molecules in the liquid medium without physical contact between the different cells.

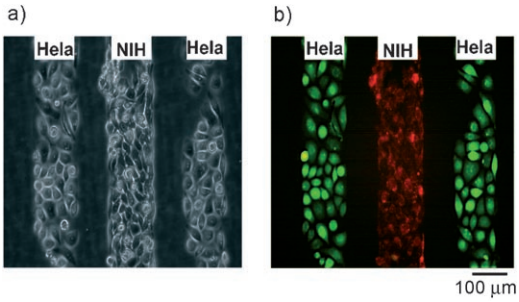

Figure 2. Phase-contrast (a) and fluorescence (b) micrographs of two types of cells patterned on a substrate modified with EG_6 by using selective desorption of SAMs. a) NIH 3T3 cells adhered only on the middle stripe and Hela cells only on the two side stripes. b) The two kinds of cells were labeled with different fluorescent dyes (Hela with celltrace calcein green AM, and NIH 3T3 with celltrace calcein red-orange AM).

To study the motile behavior of multiple cells initially constrained to their patterns, we carried out a second step of electrochemical desorption on the gold-coated substrate. The cells were patterned in stripes in culture media so that the cells could be released from their confinements.[9] After the cathodic voltage desorbed the remaining SAMs, cells confined in patterns moved and spread across previously inert areas (Figure 3). NIH 3T3 cells covered more distance than

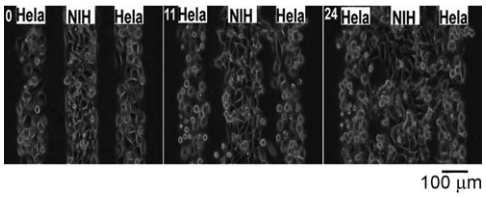

Figure 3. We confined NIH 3T3 cells in the middle stripe and Hela cells in the two side stripes. Application of a cathodic potential of approximately 1.2 V for 30 s on the substrates allowed all cells to move freely on the surface. Time-lapse micrographs were recorded after the application of the voltage pulse. The numbers indicate the time (in hours) after application of the pulse.

Hela cells. Experiments of this kind allow biologists to screen how one type of cells can influence the motility of another by secreted, soluble molecules, as well as through direct physical interactions after the different types of cells come into contact.

Several methods for patterning multiple types of cells have been explored before.[1–4,11] The major disadvantage of all these methods, compared with the method reported herein, is that they do not allow two types of cells to be confined to spatially separate regions with a well-defined geometry while still allowing free exchange of soluble materials between these different types of cells. For example, Whitesides and co-workers have developed a powerful technique that uses three-dimensional microfluidic systems to make complex patterns for deposition of different types of cells.[1] This technique allows the patterning of two types of cells without lifting the stamp containing the microfluidic system and as such, the two populations of cells cannot interact with each other; once the stamp is removed, however, the cells move freely and are no longer confined to their patterns. Furthermore, it requires complex fabrication and sophisticated physical manipulation. Mrksich and co-workers developed a noninvasive technique to pattern the attachment of two different types of cells that uses microcontact printing of thiols to pattern one population of cells and then electrochemical modulation of the surface to promote the adhesion of a second population of cells to attach onto previously inert regions.[2] This technique requires relatively simple physical manipulation and controls the composition of the surface molecularly; however, it requires a complex chemical synthesis.[2] A few other research groups reported other techniques that can pattern two types of cells.[4,11] These methods are all limited in their ability to pattern different types of cells in confined areas. Our method, in contrast, is simple to implement and can confine different types of cells to specific areas while at the same time allowing studies of their mutual interactions through the liquid medium surrounding them. In this method, the minimal separation between different types of cells is about 20 μm; at smaller distances, the cells tend to spread across discontinuous patterns to form mixed populations of cells.

In conclusion, we used electrochemically constrained microchannels to pattern multiple types of cells on the same substrate. This technique has the capability to pattern different types of cells with precisely controlled distances while

allowing the free exchange of soluble molecules; it also allows these cells to move under the influence of each other. The manipulation and microfabrication required in this approach are straightforward, and we therefore believe that it can be extensively applicable for studying fundamental biomedical problems based on cell–cell interactions. Furthermore, spatially selective electrochemical transformations may find uses in microfabrication and surface engineering.[3,12]

Received: September 19, 2006
Published online: December 21, 2006

Keywords: cell adhesion · electrochemistry · microfluidics · monolayers

[1] D. T. Chiu, N. L. Jeon, S. Huang, R. S. Kane, C. J. Wargo, I. S. Choi, D. E. Ingber, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 2408 – 2413.
[2] M. N. Yousaf, B. T. Houseman, M. Mrksich, *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 5992 – 5996.
[3] a) G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang, D. E. Ingber, *Annu. Rev. Biomed. Eng.* **2001**, *3*, 335 – 373; b) X. Jiang, J. N. Lee, G. M. Whitesides, *Scaffolding in Tissue Engineering* (Ed.: P. Ma), CRS Taylor & Francis Group, Ann Arbor, **2006**, pp. 199 – 215.
[4] a) A. Folch, M. Toner, *Biotechnol. Prog.* **1998**, *14*, 388 – 392; b) S. N. Bhatia, M. L. Yarmush, M. Toner, *J. Biomed. Mater. Res.* **1997**, *34*, 189 – 199; c) A. Tourovskaia, T. Barber, B. T. Wickes, D. Hirdes, B. Grin, D. G. Castner, K. E. Healy, A. Folch, *Langmuir* **2003**, *19*, 4754 – 4764; d) A. Khademhosseini, K. Y. Suh, J. M. Yang, G. Eng, J. Yeh, S. Levenberg, R. Langer, *Biomaterials* **2004**, *25*, 3583 – 3592; e) C. C. Co, Y. C. Wang, C. C. Ho, *J. Am. Chem. Soc.* **2005**, *127*, 1598 – 1599.
[5] M. Yamato, C. Konno, M. Utsumi, A. Kikuchi, T. Okano, *Biomaterials* **2002**, *23*, 561 – 567.
[6] a) H. Chen, Z. He, A. Bagri, M. Tessier-Lavigne, *Neuron* **1998**, *21*, 1283 – 1290; b) A. Orimo, P. B. Gupta, D. C. Sgroi, F. Arenzana-Seisdedos, T. Delaunay, R. Naeem, V. J. Carey, A. L. Richardson, R. A. Weinberg, *Cell* **2005**, *121*, 335 – 348.
[7] E. Delamarche, A. Bernard, H. Schmid, B. Michel, H. Biebuyck, *Science* **1997**, *276*, 779 – 781.
[8] a) M. Mrksich, G. M. Whitesides, *Trends Biotechnol.* **1995**, *13*, 228 – 235; b) C. S. Chen, M. Mrksich, S. Huang, G. M. Whitesides, D. E. Ingber, *Science* **1997**, *276*, 1425 – 1428.
[9] X. Jiang, R. Ferrigno, M. Mrksich, G. M. Whitesides, *J. Am. Chem. Soc.* **2003**, *125*, 2366 – 2367.
[10] M. Mrksich, L. E. Dike, J. Tien, D. E. Ingber, G. M. Whitesides, *Exp. Cell Res.* **1997**, *235*, 305 – 313.
[11] a) H. Kaji, M. Kanada, D. Oyamatsu, T. Matsue, M. Nishizawa, *Langmuir* **2004**, *20*, 16 – 19; b) H. Kaji, K. Tsukidate, T. Matsue, M. Nishizawa, *J. Am. Chem. Soc.* **2004**, *126*, 15026 – 15027; c) C. Zhao, I. Witte, G. Wittstock, *Angew. Chem.* **2006**, *118*, 5595 – 5597; *Angew. Chem. Int. Ed.* **2006**, *45*, 5469 – 5471.
[12] a) A. M. Taylor, M. Blurton-Jones, S. W. Rhee, D. H. Cribbs, C. W. Cotman, N. L. Jeon, *Nat. Methods* **2005**, *2*, 599 – 605; b) X. Y. Zhu, K. L. Mills, P. R. Peters, J. H. Bahng, E. H. Liu, J. Shim, K. Naruse, M. E. Csete, M. D. Thouless, S. Takayama, *Nat. Mater.* **2005**, *4*, 403 – 406; c) M. Cabodi, N. W. Choi, J. P. Gleghorn, C. S. D. Lee, L. J. Bonassar, A. D. Stroock, *J. Am. Chem. Soc.* **2005**, *127*, 13788 – 13789.

