Reprinted from the Journal of the American Chemical Society, 1993, *115*.

Convenient Methods for Patterning the Adhesion of Mammalian Cells to Surfaces Using Self-Assembled Monolayers of Alkanethiolates on Gold[1]

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Received February 22, 1993

We describe two convenient and flexible methods for controlling the attachment and spreading of mammalian cells on solid surfaces using patterned, self-assembled monolayers (SAMs). These patterned surfaces were easily formed by the serial adsorption of two or more ω-substituted alkanethiols ($HS(CH_2)_nR$) on gold.[3,4] These methods enable patterns to be formed with dimensions down to 1 μm and permit the examination of controlled adhesion of populations of cells and of individual cells and cellular processes. Previously, several methods have been used to pattern substrates for cell growth in the study of cell adhesion and motility.[5] The ability to form patterns with dimensions comparable to those of cells, exposing well-defined functional groups, will be useful in studying the physical–organic chemistry of cell attachment and growth: partitioning of proteins on adsorption onto surfaces from media; correlation of proteins adsorbed with attachment; influences of local environment on cellular spreading.

We examined adherent cell lines (RBL and P19).[6] These cells were plated onto SAMs patterned into areas in which the exposed groups R promoted (or inhibited) attachment of cells. SAMs terminated with the oligo(ethylene glycol) group (R = $(OCH_2CH_2)_6OH$, EG_6OH) uniformly prevented attachment of cells, as they resist adsorption of proteins;[7] other functional groups promoted attachment of cells to different extents. The high resistance to adhesion provided by the EG_6OH-terminated SAMs allowed us to study differential attachment in both complex (containing fetal bovine serum) and minimal (protein and serum-free) media.

The patterning of the attachment of cells in cultures has been accomplished by patterning the deposition of metals[5] and by patterning alkylsiloxane monolayers by optical,[8,9] UV,[10] and plasma lithography.[11] These methods are useful, but they have limitations: they offer only limited control over surface chemistry[12] and generate regions differentiated primarily by hydrophilicity; they do not provide the control necessary to pattern cells plated

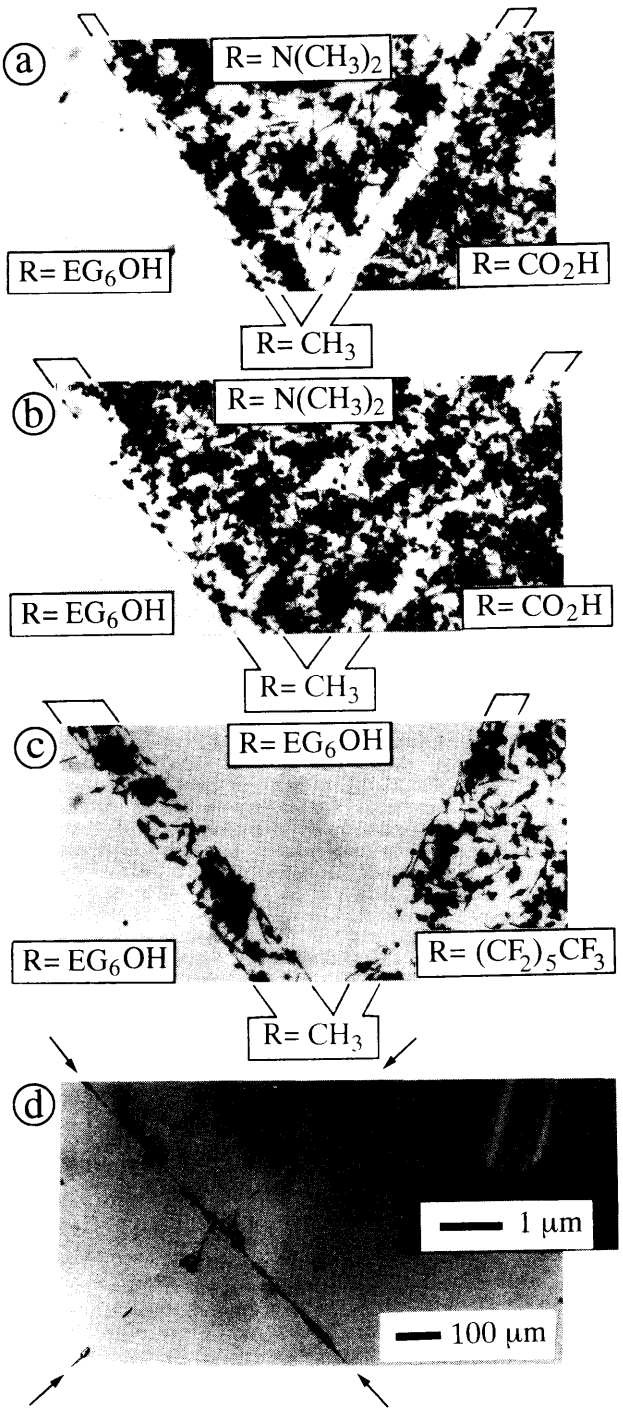

Figure 1. (a–c) Optical micrographs of RBL cells on patterned SAMs formed by the adsorption of $HS(CH_2)_nR$ (R = CH_3, CO_2H, $N(CH_2)_2$, EG_6OH, and $(CF_2)_5CF_3$) on gold.[14] The 100-μm scale bar in d also applies to micrographs a–c. (a) Cells were plated from a suspension containing 10% fetal bovine serum.[13] (b,c) Cells that were plated from a serum-free medium.[13,14] The pattern of SAMs used in a and b are approximately the same. (d) Optical micrographs of differentiated P19 cells attached to a pattern formed by preparing a nonadhesive surface from $HS(CH_2)_{11}EG_6OH$, removing this SAM by forming a groove (arrows) in the gold with a scalpel, and assembling a second monolayer by adsorption of $HS(CH_2)_{15}CO_2H$ within the groove.[17] Inset: scanning electron micrograph of a similar groove.

(1) We acknowledge partial support by the Office of Naval Research and the Defense Advanced Research Projects Agency.
(2) G.P.L. thanks the N.I.H. and the Ford Foundation for providing postdoctoral fellowships.
(3) Bain, C. D.; Troughton, E. B.; Tao, Y.-T.; Evall, J.; Whitesides, G. M.; Nuzzo, R. *J. Am. Chem. Soc.* **1989**, *111*, 321–335.
(4) For reviews, see: Dubois, L. H.; Nuzzo, R. G. *Annu. Rev. Phys. Chem.* **1992**, *43*, 437–463. Whitesides, G. M.; Laibinis, P. E. *Langmuir* **1990**, *6*, 87–96. Ulman, A. *An Introduction to Ultrathin Organic Films from Langmuir-Blodgett to Self-Assembly*; Academic Press Inc.: San Diego, CA, 1991.
(5) Early studies include: Carter, S. B. *Nature* **1965**, *208*, 1183–1187. Carter, S. B. *Nature* **1967**, *213*, 256–260. Maroudas, N. B. *Nature* **1973**, *244*, 353–354. Shay, J. W.; Porter, K. R.; Krueger, T. C. *Exp. Cell Res.* **1977**, *105*, 1–8.
(6) RBL cells are rat basophilic leukemia cells; P19 cells are a mouse embryonic carcinoma line that can be differentiated into neuronal-like cells by the addition of retinoic acid. (Both cell lines can be obtained from the ATCC.)
(7) Prime, K. L.; Whitesides, G. M. *Science* **1991**, *252*, 1164–1167. Prime, K. L.; Whitesides, G. M. *J. Am. Chem. Soc.*, in press.
(8) Kleinfeld, D.; Kahler, K. H.; Hockberger, P. E. *J. Neurosci.* **1988**, *8*, 4098–4120.
(9) Britland, S.; Clark, P.; Connolly, P.; Moores, G. *Exp. Cell Res.* **1992**, *198*, 124–129.
(10) Stenger, D. A.; Georger, J. H.; Dulcey, C. S.; Hickman, J. J.; Rudolph, A. S.; Nielsen, T. B.; McCort, S. M.; Calvert, J. M. *J. Am. Chem. Soc.* **1992**, *114*, 8435–8442.
(11) Vargo, T. G.; Thompson, P. M.; Gerenser, L. J.; Valentini, R. F.; Aebischer, P.; Hook, D. J.; Gardella, J. A. *Langmuir* **1992**, *8*, 130–134.
(12) Wasserman, S. R.; Tao, Y.-T.; Whitesides, G. M. *Langmuir* **1989**, *5*, 1074–1087. Tidswell, I. M.; Rabedeau, T. A.; Pershan, P. S.; Kosowsky, S. D.; Folkers, J. P.; Whitesides, G. M. *J. Chem. Phys.* **1991**, *95*, 2854–2861.

0002-7863/93/1515-5877$04.00/0

from minimal media.[8] SAMs of alkanethiolates on gold provide the basis for more convenient patterning methods. These methods can be lithographic or nonlithographic and provide great flexibility and precision in the specification of the group R that is exposed to the medium.[3,4,7] The capability to limit adsorption of proteins and adhesion of cells provided by R = EG_6OH[7] will be particularly useful in working with minimal, defined media; the combination of nonadsorbing surfaces and media containing no added proteins will permit study of the attachment and growth of cells in the presence of controlled compositions of intentionally added proteins.

Figure 1a shows the pattern of attachment of cells plated from media containing fetal bovine serum (10%) on a patterned SAM comprised of alkanethiolates with R = CH_3, CO_2H, $N(CH_3)_2$, and EG_6OH,[13,14] Little cell attachment occurred on hydrophobic areas of the monolayer (R = CH_3) and on the areas exposing EG_6OH groups. Cells attached to the more hydrophilic surfaces. When cells were plated from serum-free medium (Figure 1b,c), they attached to *both* hydrophobic and hydrophilic areas. EG_6-OH-terminated monolayers successfully prevented cell attachment in both media. In serum-free medium,[15] the cells attached to the different types of adherent areas at similar densities;[16] this result confirms the findings of Kleinfeld *et al.* obtained using alkylsiloxane surfaces.[8]

Using ellipsometry,[7] we detected no protein adsorption on EG_6-OH-terminated monolayers when they were exposed to the growth medium (10% serum) for a length of time corresponding to the attachment period (2 h). By comparison, SAMs with R = CH_3, CO_2H, $N(CH_2)_2$, and $(CF_2)_5CF_3$ all adsorbed a layer of protein 15–25 Å thick.

Figure 1d demonstrates a second method of forming patterns that control the attachment and spreading of individual cells (in this example, differentiated P19 cells) and their neural processes.[6] A groove was micromachined into the surface of a gold film supporting a nonadhesive, EG_6OH-terminated SAM, using a scalpel blade.[17] A second, carboxyl-terminated SAM was formed in the groove by exposing the fresh gold surface to a solution of $HS(CH_2)_{15}CO_2H$. Plating of P19 cells from media containing 10% fetal bovine serum onto this system resulted in good localization of cellular processes in the grooved regions.

Our studies demonstrate that patterned SAMs of alkanethiolates on gold are convenient substrates for studies of the interactions of cells with solids, synthetic surfaces. These studies indicate that SAMs will be useful in manipulating the attachment of ensembles of cells and the spreading of individual cells and in studying the influence of adsorption of proteins onto surfaces on attachment of cells to those surfaces.

(13) Cells were seeded at a density of 10^5 cells/mL in Minimal Essential Media (GIBCO, BRL) in the presence or absence of 10% heat-inactivated fetal bovine serum (GIBCO, BRL). Unattached cells were removed after 2 h by rinsing the samples 3 times with media. After the 2-h attachment period, the attached cells were provided with media containing 10% heat-inactivated fetal bovine serum. After 2 days of growth, cells were fixed with 6% glutaraldehyde in buffer, dehydrated in cold methanol (4 °C), dried, and photographed.

(14) We used a micropen containing $HS(CH_2)_{15}CH_3$ to draw lines directly on the gold substrate. SAMs derived from $HS(CH_2)_{15}CO_2H$, $HS(CH_2)_{11}N(CH_3)_2$ and $HS(CH_2)_{11}EG_6OH$ were formed by pinning drops of ethanolic solutions of these thiols (1 mM) between lines formed from $HS(CH_2)_{15}CH_3$. SAMs were formed from $HS(CH_2)_2(CF_2)_5CF_3$ by exposure of the bare gold to the vapor of the thiol for 2 min. See: Lopez, G. P.; Biebuyck, H. A.; Frisbie, D.; Whitesides, G. M. *Science* **1993**, *260*, 647–649. Lopez, G. P.; Biebuyck, H. A.; Whitesides, G. M. *Langmuir*, in press.

(15) No proteins were intentionally added to the growth media in the experiments that used serum-free media. This does not preclude the presence of proteins in the vicinity of cells as they attach. Proteins may be entrained along with cells as they are added to the plating media. There is also the possibility that the cells may excrete proteins into the plating media.

(16) A separate experiment demonstrated the ability of a specific protein to promote cell attachment to hydrophobic SAMs and to equalize attachment to hydrophilic and hydrophobic regions. When patterned SAMs were exposed to a solution of laminin (1 mg/mL, 12 h) and then used for cell attachment (in serum-free media), cells attached to both hydrophilic and hydrophobic areas (although not to EG_6OH-terminated monolayers): Singhvi, R. S.; Wang, D. I. C.; Ingber, D.; López, G. P.; Kumar, A.; Whitesides, G. M., unpublished data. These results, together with the data presented here and by Kleinfeld *et al*, suggest that the preferential fractionation of cell-adhesion proteins onto hydrophilic substrata occurs when these surfaces are exposed to growth media containing serum, while hydrophobic regions are covered with adsorbed proteins that do not stimulate cell attachment.

(17) Abbott, N. L.; Folkers, J. P.; Whitesides, G. M. *Science* **1992**, *257*, 1380–1382.