Published in final edited form as:

Langmuir. 2009 June 16; 25(12): 6992–6996. doi:10.1021/la900166u.

Cell Attachment Behavior on Solid and Fluid Substrates Exhibiting Spatial Patterns of Physical Properties

Ann E. Oliver^{*}, Viviane Ngassam, Phuong Dang, Babak Sanii¹, Huawen Wu, Chanel K. Yee², Yin Yeh, and Atul N. Parikh^{*}

Department of Applied Science, College of Engineering, University of California, One Shields Avenue, Davis, CA 95616 USA

Abstract

The ability to direct proliferation and growth of living cells using chemically and topologically textured surfaces is finding many niche applications, both in fundamental biophysical investigations of cell-surface attachment as well as in developing design principles for many tissue engineering applications. Here we address cellular adhesion behavior on solid patterns of differing wettability (a static substrate) and fluid patterns of membrane topology (a dynamic substrate). We find striking differences in the cellular adhesion characteristics of lipid mono- and bilayers, despite their essentially identical surface chemical and structural character. These differences point to the importance of subtle variations in the physical properties of the lipid mono- and bilayers (e.g., membrane tension and out-of-plane undulations). Furthermore, we find that introducing phosphatidylserine into the patterned lipidic substrates causes a loss of cell-patterning capability. Implications of this finding for the mechanism by which phosphatidylserine promotes cellular adhesion are discussed.

Introduction

The confinement of living cells into spatially defined patterns opens the door to numerous sensing applications and high throughput analysis using parallel or multiplexed arrays. It also enables fundamental studies of how geometry of cellular organization influences motility and biological function, and may also offer useful design rules for tissue engineering. A variety of different approaches have been utilized to achieve such directed cell attachment and growth. Standard protocols typically involve the creation of substrate patterns consisting of islands of an adhesive protein (e.g., laminin or fibronectin) by soft-lithography methods such as micro-contact printing, and coating the surrounding surface with a protein-resistant compound (e.g., a poly(ethylene-glycol)-based polymer)^{1–4}. Alternatively, patterns of physical barriers that prevent cell dispersal, such as using agarose walls, have also proven useful⁵. Because protein (or carbohydrate) layers are deposited onto underlying glass substrates via non-covalent, and often poorly controlled, chemistries, the fidelity and stability of these substrate patterns in serum-containing culture environments continue to hamper their practical applications. Additional approaches have exploited the cell adhesive properties of polyamine (positively charged) silanes over the cell-resistive properties of alkylsilanes (neutral), through the use of photo-resist and/or ablation with ultra-violet radiation^{6–8}. In all of these cases, the substrates are solid and present static spatial patterns of their chemical or electrostatic character.

^{*}Corresponding Authors: Ann E. Oliver, aeoliver@ucdavis.edu; Fax: (530) 752-2444; Atul N. Parikh, anparikh@ucdavis.edu; Fax: (530) 752-2444.

¹Current address: The Molecular Foundry, Lawrence Berkeley National Laboratory, One Cyclotron Rd., MS: 57-2206, Berkeley, CA 94618 USA

²Current address: Amgen, One Amgen Center Drive, B30 E-1-B, Thousand Oaks, CA 91320 USA

In the work reported here, we use spatially patternable solid and fluid substrates to manipulate attachment, intracellular organization, and growth of mammalian cells. The static substrates of patterned wettability both confirm earlier results and also serve as a mechanism for producing the dynamic substrates, which reveal additional insights. Specifically, we employ spatial patterns of laterally fluid lipid mono- and bilayers confined to underlying planar solids. Our results indicate dramatic differences in cell adhesion behavior for mono- and bilayer lipidic substrates. We surmise that these differences arise from previously unappreciated subtle physical properties, including out of plane undulations and lateral tension. Further, this observation exposes an important caveat to the common generalization that all phospholipid surfaces are non-biofouling and resist cell attachment. Finally, we also find that including phosphatidylserine (PS) in the lipidic substrate effectively eliminates the topology-based patterning capability, calling into question commonly held notions for the mechanism by which PS enhances cell attachment to phospholipid bilayers.

Experimental Section

Materials

Lipids were obtained from Avanti Polar Lipids (Alabaster, AL). Octadecyltrichlorosilane (OTS) and toluene were from Sigma/Aldrich (St. Louis, MO). Phosphate buffered saline (PBS) was from USB Corporation (Cleveland, OH). All cell culture products were purchased from Gibco, unless otherwise specified.

Cell culture

Human retinal pigment epithelium (ARPE-19) cells were obtained from ATCC (CRL-2302; ATCC, Manassas, VA). Cells were cultured at 37 °C in the presence of 5% CO₂ in DMEM/F12 basal medium supplemented with 10% (v/v) fetal bovine serum, 1% (v/v) 200 mM L-glutamine, 1% (v/v) 10,000 units penicillin/streptomycin, 0.1% (v/v) 50 mg/mL gentamicin (Invitrogen, Carlsbad, CA), 0.2% (v/v) normicin (Invivogen, San Diego, CA) and 1.25 g/L cell culture grade NaHCO₃, and was sterilized by filtering through 0.2 µm filter. Cells were grown in 75-cm² cell culture flasks (NUNC #156499) before being sub-cultured onto different substrates for further studies. Total passage number is less than 30.

Substrate preparation

Glass substrates (22 × 22 mm coverslips, Corning, Corning, NY) were cleaned for 3–5 min with piranha etch, a 4:1 mixture of sulfuric acid and hydrogen peroxide heated to 90 °C, to remove organic residues. The substrates were then washed copiously with deionized (18 mΩ-cm) water and dried under a stream of N₂. OTS deposition was conducted as previously described⁹. Briefly, clean coverslips were immersed in 70 mL of a self-assembly solution consisting of 2.5 mM octadecyltrichlorosilane in anhydrous toluene. The substrates were allowed to incubate for approximately 45 min. All silanization reactions were carried out in glass containers under ambient conditions (relative humidity 20–50%). After removal from the self-assembly solution, the film-covered wafers were washed with chloroform and extensively with acetone under ultrasonic conditions to remove all excess reactants. Silanized samples were used within a few days of preparation.

UV photolithography of OTS monolayer

Spatial patterning of OTS-covered substrates was achieved using short wavelength UV radiation⁷. In particular, spatially directed photo-illumination of monolayer samples was achieved using a physical mask and ozone-generating UV lamp¹⁰. The mask, displaying patterns of chrome over quartz substrate, was acquired from Photoscience, Inc. (Torrance, CA). UV radiation was produced using a medium pressure Hg discharge grid lamp (UVP, Inc.,

Upland, CA) in a quartz envelope and maintained in a closed chamber in a chemical hood. The samples were placed in contact with the photomask and positioned approximately 2 – 5 mm from the light source depending on the illumination geometry. The exposure period was approximately 45 – 60 minutes depending on the age of the lamp. After surface modification was complete, the substrates were sterilized by bath sonication in acetone for 5 min, followed by immersion in 70% ethanol for 10–20 min. Solvents were allowed to fully evaporate under sterile conditions in the tissue culture hood.

Plating cells on patterned surfaces

For application of cells to UV/ozone patterned OTS monolayers, the sterile substrates were placed into sterile 35 × 10 mm culture dishes (Corning). Cells ($\sim 7.5 \times 10^4$) were seeded with 3 mL growth medium and cultured under standard conditions. The growth medium was changed after 24 h, and cell growth was monitored by phase contrast microscopy.

For application of cells to monolayer/bilayer patterns, planar lipid films were formed by the vesicle fusion technique¹¹. Two stock solutions, (1) 99 mol % 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) + 1 mol % Texas Red 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt (TR-DHPE); and (2) 5 mol % 1,2-dioleoyl-sn-glycero-3-phosphoserine (DOPS) + 94 mol % POPC + 1 mol % Texas-Red-DHPE, were prepared in chloroform. Solvent was evaporated under a stream of N₂ under sterile conditions, after which the lipid film was placed under vacuum to remove residual chloroform. The lipid was resuspended in deionized water at 2 mg/mL by sonication in a Branson 2510 bath sonicator, and was stored at 4°C until use.

Sterile unilamellar vesicles were prepared by extruding the lipid suspension through 0.1 µm pores, using an Avanti hand held extruder and Nuclepore membrane filters, all of which had been immersed for 30 min in 70% ethanol and allowed to dry in the tissue culture hood. The extruded vesicles were diluted 1:1 with 1X PBS, yielding liposomes at a final concentration of 1 mg/mL in 0.5X PBS.

Planar lipid films were formed in sterile 35 × 10 mm polystyrene culture (Corning) dishes as follows: a 45 µL droplet of unilamellar vesicles was placed in the center of a culture dish, and the clean, patterned OTS-coated coverslip was placed gently on top. The samples were allowed to incubate in the dark at room temperature for 20 min, and then submerged and rinsed several times in sterile deionized water, and finally with sterile PBS. At no time were the lipid films exposed to air. Fusion of the vesicles with the patterned substrate produces monolayers on the OTS-coated regions and bilayers on the exposed glass surfaces⁹.

Cells were evenly seeded into the culture dishes ($\sim 2 \times 10^5$ per culture dish) containing the modified substrates and incubated under standard culture conditions as described above. After 24 h and again every 48 h thereafter, the growth medium was changed, and cells were observed for up to a week.

Cell staining

Confluent samples of cells growing on different types of patterns were washed three times with PBS. Cell fixation was conducted using fixation medium (CalTag Fix and Perm Reagent A, Invitrogen, Carlsbad, CA) according to manufacturer's instructions. The samples were then washed with PBS, and treated with 0.2% triton X-100 in PBS for 20 min in the dark, followed by a final wash with PBS. The coverslips were each treated with 100 µL staining solution containing 1% bovine serum albumin and 6 units/mL Alexa-Fluor 568 phalloidin (Invitrogen) for 20 min at room temperature. The samples were then washed three times with 0.2% triton X-100 in PBS and three times with PBS. For each sample, one drop of prolong-Gold antifade

reagent with DAPI (Invitrogen) was placed on a clean microscope slide, and a treated coverslip was inverted onto it. Samples were then examined with a Nikon Eclipse TE2000-U inverted epi-fluorescence microscope.

Orientation of nuclei

The distributions of cell-nuclei orientations were determined from fluorescence images by a custom Matlab script. Images were compared to a threshold, and features smaller than $5 \mu\text{m}^2$ were removed. The resulting binary features were visually verified as nuclei, and subsequently fit to ellipses. The distribution of the orientations of the fit ellipses is reported in the manuscript.

Results and Discussion

Cell attachment, growth, and intracellular organization confined by surface wettability

Human retinal pigment epithelial (ARPE-19) cells are damaged during aging, resulting in a condition known as age-related macular degeneration^{12–14}. Micro-arrays of ARPE cells could prove invaluable in the study of the apoptotic disease progression and also evaluation of potential therapies. Since they do not require prior deposition of extra-cellular matrix proteins for adhesion, ARPE cells can adhere to hydrophilic surfaces (e.g., plasma-cleaned polystyrene or glass) (Fig. 1). If the substrate is rendered hydrophobic, such as through deposition of a monolayer of octadecyltrichlorosilane (OTS) on glass¹⁵, however, the cells lose their ability to adhere to the surface and do not survive (data not shown). These results agree well with previous observations of wettability-dependent adsorption of living cells on surfaces^{6–8, 16}, including the elastomeric stamping of OTS on glass¹⁷.

The preference of ARPE-19 cells for hydrophilic surfaces has thus been exploited to confine cell attachment and growth to designated regions directly on glass surfaces, allowing spatial patternability. Uniform monolayers of OTS are deposited on clean glass substrates using the standard solution-phase self-assembly method, followed by deep UV illumination through a photo-mask. The resulting removal of OTS in the unprotected areas yields substrates of alternating hydrophilic and hydrophobic regions of defined geometries⁹. As expected, cells seeded onto such surfaces attach and grow in the hydrophilic glass regions, leaving hydrophobic OTS area virtually cell-free (Fig. 1). Complete control is available over the size, shape, and distribution of the features of cell growth and exclusion. This method provides an advantage over the surface chemistry-based approaches mentioned above in that it does not require the use of photo-resist, or elastomeric stamps and requires the deposition of only a single silane monolayer. In addition, neither clean room conditions nor inert dry argon environments are required for the preparation of each sample. Thus as a simple two-step process, it represents a moderate improvement in the efficiency of cell patterning for cells that are competent to adhere to clean silica, such as the ARPE-19 cell line. A similar process of UV ablation of adhesive laminin or poly-lysine surfaces^{18, 19} would not be appropriate for this cell type, because ablation of the adhesive surface would simply reveal glass surfaces, which are also permissive to cell attachment.

Such static substrates also afford remarkable control over the intracellular organization²⁰. ARPE-19 cells were directed to attach and grow in features of different shapes, specifically stripes or squares, or on unpatterned glass substrates. For the cells growing in stripes, both the actin cytoskeleton and nuclei are prominently aligned parallel to the axis of the stripe (Fig. 1e), whereas for cells growing in squares (data not shown) or on unpatterned glass substrates (Fig. 1d), the actin cytoskeleton remains more dispersed and does not show any noticeable effect of aligning with the substrate pattern of wettability. As shown in Fig. 1f, a quantitative analysis reveals that an overwhelming proportion (~90%) of the cells growing on the stripe-shaped feature exhibit nuclear orientations within the range $\pm 20^\circ$ from the long axis of the stripe.

Nuclei of cells growing on unpatterned glass or on square features, in contrast, are distributed evenly over the entire 180° spectrum. These results indicate that the nuclei (and by extension other intracellular organelles) are also highly regulated in their organization and morphology by the physical chemical nature of the growth surface, confirming the work of Charest et al. (2006)²¹.

Because intracellular organization influences many key signaling functions, such observations suggest that use of patterns of confinement geometries may provide a simple strategy for a high through-put functional analysis of how morphological organization of sub-cellular components influences signaling. Further, such information might enable the design of arrays of differently stimulated living cells from a single parent stock. Recent work in our laboratory is aimed at such studies in the context of cellular apoptosis.

Lipidic substrate morphology affects cellular attachment and growth

Despite the high level of attention paid to the topic of cellular attachment over many years^{22–27}, several issues remain poorly understood. For instance, it has long been known that phospholipid bilayers provide an unsuitable substrate for the attachment and growth of adherent cell types^{28, 29}. While the details of the mechanisms by which lipidic surfaces resist cell- and protein-adsorption remain to be elucidated, it has been variously suggested that surface electrostatics (net electrical neutrality of the head-group), head-group hydration (strongly bound water), and lateral fluidity (lack of a firm anchor for the cell's focal adhesion)^{28, 30, 31} may play important roles.

The static substrates described above provide a foundation for addressing these types of questions. When phospholipid vesicles are fused to a substrate with alternating hydrophilic and hydrophobic surfaces, lipid bilayers form on the hydrophilic areas while monolayers form over the hydrophobic regions⁹ (Fig. 2a). If cells are introduced to such laterally fluid substrates, their growth on the bilayer surfaces is poor as expected. However, a surprising level of growth was seen on the monolayer regions. In fact, although the cells' preference for the monolayer substrate over the bilayer substrate was not absolute, it allowed a novel method for patterning the cells (Figs. 2b and 2c). Such ability will be cell-type dependent, however, as earlier work showed macrophages to require a specific lipid hapten-antibody interaction to achieve cellular attachment to phospholipid monolayers³².

Head-group electrostatics and hydration are similar for the monolayers and bilayers since they are composed of the same lipid. Further, lateral mobility for lipids in both types of films are comparable^{9, 33} and fall in the range of published values for supported phospholipid bilayers ($0.5 - 5 \mu\text{m}^2/\text{s}$)³⁴. Thus, our results provide a strong indication that these factors alone cannot fully explain the resistance of bilayers to cellular attachment and growth.

One plausible explanation may be found in the differences between the out-of-plane mobility and corresponding membrane tensions of lipid mono- and bilayers. Since planar bilayers are not fixed to the surface, but rather “float” over a thin water layer of a few angstroms^{35, 36}, the entire bilayer assembly can undulate in the Z-direction. Such undulatory motion can be expected to be highly restricted for lipid monolayers, because of their tight adherence to the supporting silane monolayer, which is in turn covalently bound to the silica substrate. This difference in flexibility was evidenced recently by the reactions of each type of lipid film to the deposition of glass beads on the surface³⁷. Phospholipid bilayers were able to partially wrap the exterior surface of the beads, due to their out-of-plane mobility, whereas the monolayers were not capable of this Z-axis mobility and remained essentially planar³⁷. In addition, prior work has shown rigidity of lipidic substrate to be important for cell attachment. Lipidic substrates composed of DPPC or DSPC (gel phase at 37 °C) were found to be permissive to fibroblast adhesion, whereas substrates composed of DOPC or egg PC (fluid at 37 °C) were

not adhesive³⁸. The effect was shown to be physical rather than chemical, as egg PC could be converted to an adhesive substrate by crosslinking with osmium tetroxide (solid at 37 °C, as evidenced by electron spin resonance)³⁸. Since, in the current study, the phospholipids exposed to the cell membranes were identical, the primary difference between monolayers (permissive to cell attachment) and bilayers (resistive to cell attachment) was this out-of-plane flexibility. We therefore propose that a full explanation for the resistance of lipid bilayers to cell attachment and growth should include the aspect of membrane dynamics normal to the plane of the bilayer.

An alternative explanation for the preference of cells for the monolayer regions could be that defects in the monolayer allow protein deposition to the underlying substrate. Indeed, in many cases, serum or extracellular matrix proteins enable cellular attachment to a surface²³. Thus, it is interesting to note that although both the lipid monolayers and bilayers of the patterned surface have been shown to be resistant to serum protein binding or displacement⁹, a narrow region exists between these two types of films, to which BSA can bind⁹. The possibility exists, therefore, that protein binding in this “moat” region might initiate cell attachment, following which the cells might attach over the entire surface of the monolayer. It is important to note, however, that the same surfaces and proteins are available to the cells seeded on the patterned OTS substrates lacking lipidic films. Since, in the absence of lipidic films, the OTS regions resisted cell attachment, it would seem unlikely that adherence to the underlying OTS could represent the mechanism by which the cells are able to attach to the monolayer regions. Even if defects smaller than the resolution of the light microscope were present, which might allow the selective deposition of adhesive proteins (e.g., laminin, fibronectin) over other serum proteins (e.g., BSA), such defects would be likely to be found in both types of lipidic substrates at roughly the same probability. Finally, the possibility that protein adsorption might aid attachment does not diminish the utility of the method, which represents a novel process for attaching cells to a membrane mimetic surface.

Phosphatidylserine causes loss of cell patterning capability over lipidic substrates

A second point, which may be clarified through the use of alternating patterns of lipid mono- and bilayers, is the role of phosphatidylserine (PS) in cellular attachment to lipidic surfaces. PS, which is negatively charged, has been shown to enhance the attachment and growth of adherent cell types to phospholipid bilayers³⁰. Because identically-charged phosphatidylglycerol (PG) did not confer the same advantage, it has been argued that rather than simply supplying charge, the presence of PS might allow the formation of defects, through which cells could penetrate through the fluid bilayer and attach to the underlying substrate³⁰.

In the present study, phosphatidylcholine (PC) vesicles containing 5% PS were fused to patterned OTS substrates, producing alternating regions of lipid mono- and bilayers, as described above (Fig. 3a). Cells presented to such substrates attached and grew over both the monolayer and bilayer regions (Figs. 3b and 3c) in a remarkably uniform manner, indicating a dramatic loss of the topology-based patterning capability. While it could be argued that the cells might attach to the monolayer as described in the previous section and to the bilayer through the putative PS-induced defects, the observed uniformity in the cellular growth (e.g., density and morphology) reflects the cells' total disregard for the properties of the underlying substrate or its pattern. This in turn points to the presence of a single mechanism of attachment over both the mono- and bilayer surfaces. These data suggest that PS may have some additional role besides allowing cells to contact the underlying substrate.

PS is involved in *in vivo* cell attachment as well. For instance, certain disease states result in the appearance of PS in the outer leaflet of erythrocytes, which causes them to adhere to the endothelium^{39–41}. In addition, PSR, the receptor that recognizes PS in the outer leaflet of apoptotic bodies and allows their removal^{42, 43}, has been identified in fibroblasts, epithelial, and HeLa cells⁴², all of which are used regularly for adherent cell culture. The current data

suggest that it is necessary, therefore, to consider this type of specific binding in any explanation of PS-enhanced attachment to planar phospholipid bilayers.

Conclusions

We have employed spatially patternable solid (static) and fluid (dynamic) substrates to manipulate attachment, intracellular organization, and growth of mammalian cells. Our results demonstrate that cells discriminate lipid mono- and bilayer morphologies supported on solid surfaces despite the similarities in the surface chemical character and lateral fluidities of the two lipid morphologies. Furthermore, their spatial patterns offer a convenient means to spatially direct proliferation and growth of mammalian epithelial cells. Based on these results, we hypothesize that the differences in Z-axis mobility (including out-of-plane undulations and lateral tension) between mono- and bilayer configurations contribute to the resistance of phospholipid bilayers to cellular attachment and growth. Additional experiments employing lipid configurations of systematically varied undulations (such as by modulating bilayer-substrate adhesion strength and by using corrugated substrates) are needed to fully decipher the origin of these differences. Furthermore, our observation of loss of cell patternability over lipidic surfaces containing PS suggests that the PS-mediated enhancement of cellular attachment to lipid bilayers is not fully explained by existing notions of defect-nucleation. This raises the possibility that specific interactions, such as with the PSR, must also be considered.

Acknowledgments

The authors thank Adrian Brozell for technical assistance in the production of the quartz/nickel photomask. This paper integrates work performed under a grant from Biomolecular Materials program, the U.S. Department of Energy Office of Science (Grant # DE-FG02-04ER46173), as well as the National Institutes of Health's Roadmap for Medical Research (Grant # PHS 2 PN2 EY016570B).

References

1. Chen CS, Ostuni E, Whitesides GM, Ingber DE. *Methods in Molecular Biology* 2000;139:209–219. [PubMed: 10840789]
2. Nelson CM, Raghavan S, Tan JL, Chen CS. *Langmuir* 2003;19:1493–1499.
3. Raghavan S, Chen CS. *Advanced Materials* 2004;16:1303–1313.
4. Whitesides GM, Ostuni E, Takayama S, Jiang XY, Ingber DE. *Annu. Rev. Biomed. Eng* 2001;3:335–373. [PubMed: 11447067]
5. Nelson CM, Chen CS. *Febs Letters* 2002;514:238–242. [PubMed: 11943158]
6. Corey JM, Wheeler BC, Brewer GJ. *Ieee Transactions on Biomedical Engineering* 1996;43:944–955. [PubMed: 9214810]
7. Dulcey CS, Georger JH, Krauthamer V, Stenger DA, Fare TL, Calvert JM. *Science* 1991;252:551–554. [PubMed: 2020853]
8. Kleinfeld D, Kahler KH, Hockberger PE. *Journal of Neuroscience* 1988;8:4098–4120. [PubMed: 3054009]
9. Howland MC, Sapuri-Butti AR, Dixit SS, Dattelbaum AM, Shreve AP, Parikh AN. *Journal of the American Chemical Society* 2005;127:6752–6765. [PubMed: 15869298]
10. Vig JR. *Journal of Vacuum Science & Technology a-Vacuum Surfaces and Films* 1985;3:1027–1034.
11. Parikh AN, Groves JT. *Mrs Bulletin* 2006;31:507–512.
12. Dunaief JL, Dentshev T, Ying GS, Milam AH. *Archives of Ophthalmology* 2002;120:1435–1442. [PubMed: 12427055]
13. Hinton DR, He SK, Lopez PF. *Archives of Ophthalmology* 1998;116:203–209. [PubMed: 9488273]
14. Reme CE, Grimm C, Hafezi F, Wenzel A, Williams TP. *News in Physiological Sciences* 2000;15:120–125. [PubMed: 11390893]
15. Parikh AN, Allara DL, Azouz IB, Rondelez F. *Journal of Physical Chemistry* 1994;98:7577–7590.

16. Matsuzawa M, Potember RS, Stenger DA, Krauthamer V. *Journal of Neuroscience Methods* 1993;50:253–260. [PubMed: 8107505]
17. Lu LC, Kam L, Hasenbein M, Nyalakonda K, Bizios R, Gopferich A, Young JF, Mikos AG. *Biomaterials* 1999;20:2351–2361. [PubMed: 10614941]
18. Hammarback JA, Palm SL, Furcht LT, Letourneau PC. *Journal of Neuroscience Research* 1985;13:213–220. [PubMed: 3973933]
19. Corey JM, Wheeler BC, Brewer GJ. *Journal of Neuroscience Research* 1991;30:300–307. [PubMed: 1798054]
20. Spargo BJ, Testoff MA, Nielsen TB, Stenger DA, Hickman JJ, Rudolph AS. *Proceedings of the National Academy of Sciences of the United States of America* 1994;91:11070–11074. [PubMed: 7972011]
21. Charest JL, Eliason MT, Garcia AJ, King WP. *Biomaterials* 2006;27:2487–2494. [PubMed: 16325902]
22. Burridge K, Fath K, Kelly T, Nuckolls G, Turner C. *Annual Review of Cell Biology* 1988;4:487–525.
23. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. *Science* 1997;276:1425–1428. [PubMed: 9162012]
24. Hynes RO. *Cell* 1992;69:11–25. [PubMed: 1555235]
25. McClary KB, Ugarova T, Grainger DW. *Journal of Biomedical Materials Research* 2000;50:428–439. [PubMed: 10737886]
26. Wilson CJ, Clegg RE, Leavesley DI, Percy MJ. *Tissue Engineering* 2005;11:1–18. [PubMed: 15738657]
27. Yamada KM. *Annual Review of Biochemistry* 1983;52:761–799.
28. Andersson AS, Glasmaster K, Sutherland D, Lidberg U, Kasemo B. *Journal of Biomedical Materials Research Part A* 2003;64A:622–629. [PubMed: 12601773]
29. Margolis LB, Dyatlovitskaya EV, Bergelson LD. *Experimental Cell Research* 1978;111:454–457. [PubMed: 580085]
30. Groves JT, Mahal LK, Bertozzi CR. *Langmuir* 2001;17:5129–5133.
31. Kam L, Boxer SG. *Journal of Biomedical Materials Research* 2001;55:487–495. [PubMed: 11288076]
32. Hafeman DG, Vontscharner V, McConnell HM. *Proceedings of the National Academy of Sciences of the United States of America-Biological Sciences* 1981;78:552–4556.
33. Sanii B, Parikh AN. *Soft Matter* 2007;3:974–977.
34. Lee GM, Jacobson K. Lateral mobility of lipids in membranes. In *Cell Lipids* 1994;Vol. 40:111–142.
35. Johnson SJ, Bayerl TM, McDermott DC, Adam GW, Rennie AR, Thomas RK, Sackmann E. *Biophysical Journal* 1991;59:289–294. [PubMed: 2009353]
36. Koenig BW, Kruger S, Orts WJ, Majkrzak CF, Berk NF, Silverton JV, Gawrisch K. *Langmuir* 1996;12:1343–1350.
37. Dixit SS, Szmodis A, Parikh AN. *Chemphyschem* 2006;7:1678–1681. [PubMed: 16847842]
38. Margolis LB. *Biochim. Biophys. Acta* 1984;779:161–189. [PubMed: 6375722]
39. Choe H-R, Schlegel RA, Rubin E, Williamson P, Westerman MP. *British Journal of Haematology* 1986;63:761–773. [PubMed: 3730297]
40. Closse C, Dachary-Prigent J, Boisseau MR. *British Journal of Haematology* 1999;107:300–302. [PubMed: 10583215]
41. Wali RK, Jaffe S, Kumar D, Kalra VK. *Diabetes* 1988;37:104–111. [PubMed: 3335275]
42. Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RAB, Henson PM. *Nature* 2000;405:85–90. [PubMed: 10811223]
43. Li MO, Sarkisian MR, Mehal WZ, Rakic P, Flavell RA. *Science* 2003;302:1560–1563. [PubMed: 14645847]

Figure 1.

Phase contrast micrographs of ARPE-19 cells growing on glass/OTS substrates patterned as described in the text: (a) $250 \times 250 \mu\text{m}$ glass squares; (b) $10 \times 10 \mu\text{m}$ glass squares; (c) $100 \mu\text{m}$ wide glass stripes. Also shown are fluorescence micrographs of ARPE-19 cells growing on (d) unpatterned glass; (e) a $100 \mu\text{m}$ wide glass stripe. The actin cytoskeleton appears red due to staining with phalloidinalexa-568, and the nuclei are stained blue with DAPI. Existing fluorescence was enhanced by brightness/contrast adjustments in Photoshop. (f) The distribution of nuclear tilt angles with respect to the vertical axis of the image, as taken from 60 – 100 nuclei from three fields of view for each type of substrate. Scale bars in (a–c) represent $100 \mu\text{m}$, and those in (d, e) represent $50 \mu\text{m}$.

Figure 2. ARPE-19 cells growing on a pattern of phospholipid (POPC + 1% Texas Red-DHPE) bilayers (bright $250 \times 250 \mu\text{m}$ squares) surrounded by a monolayer grid (dim regions) as imaged by fluorescence microscopy (a); phase contrast microscopy (b); or both together (c).

Figure 3. ARPE-19 cells growing on a pattern of phospholipid (94% POPC + 5% DOPS + 1% Texas Red-DHPE) bilayers (bright $100 \times 100 \mu\text{m}$ squares) surrounded by a monolayer grid (dim regions) as imaged by fluorescence microscopy (a); phase contrast microscopy (b); or both together (c).