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Research News

Micropatterning Fluid Membranes**

By Nick Ulman,* Jay T. Groves, and Steven G. Boxer*

1. Introduction

Biological research and diagnostics and semiconductor integrated circuit manufacturing are similar in that both are concerned with objects on the nanometer to micrometer scale. In semiconductor manufacturing, automated microlithography is the key technology for defining patterns on Si wafers. Feature sizes as small as 0.25 μm are state-of-the-art in high volume production while 10 nm linewidths have been reported in research.

It is only in the last few years that microlithography has been applied to biological research and technology. An interesting example is light-directed chemical synthesis.[1] In this technique a photolithographic mask selectively exposes and photochemically deprotects portions of a solid substrate. Chemistry is then used to build up a diverse set of chemical compounds, usually polymers created one unit per lithographic cycle. Binary masking yields 2^n compounds in n chemical steps. Chips with thousands of different oligonucleotides positioned in regular arrays have been created by this technique and used for genetic screening.

An important goal in applying microlithography to biological systems will be to find an appropriate interface between microstructures, including integrated circuits, and living cells. Such an interface will facilitate communication with, and control of, cells in a precise, engineered fashion. There are already instruments that monitor some cellular responses such as the acidification of a cell's immediate environment.[2]

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[**] Supported in part by the Joint Services Electronics Program (G. S. Kino), NSF Biophysics Program, NSF (H. M. McConnell), and an NSF-sponsored User Grant at the Stanford Nanofabrication Facility. We thank H. M. McConnell, G. S. Kino and D. M. Bloom for advice and encouragement.

We have recently investigated methods for controlling synthetic cell membranes.[3,4] We are able to make planar, fluid membranes that are divided into arrays of small boxes. This is done by photolithographically patterning the substrate upon which the membrane is assembled. Planar, fluid, patterned membranes are compatible with both living cells and silicon wafers and form a unique interface between the two.

The chemical nature of a synthetic membrane can be tailored to achieve highly specific and selective interactions that occur between cells. The supported synthetic membrane can play the role of a living cell for some interactions.[5,6] Therefore supported membranes offer a particularly good interface to living organisms.

2. Micropatterning Fluid Membranes

Supported membranes are self-assembled, two-dimensional, fluid systems. The bilayer membrane consists of two opposed leaflets of phospholipid molecules and is the basic structure central to all living cell membranes.[7] Supported bilayers were originally developed for research on the interactions between living cells.[5,6] They can be formed by spontaneous fusion of lipid bilayer vesicles with an appropriate hydrophilic surface such as oxidized Si.[8,9] Forces on the membrane include electrostatic, hydration, and long range van der Waals interactions. An energetic minimum traps the membrane near the surface, and the membrane is typically separated from the substrate by a 10 Å thick layer of water.[10–12] A supported membrane mimics a living membrane in many ways, including maintaining lateral fluidity (Fig. 1, schematic diagram).

Fluidity in a supported membrane is long range. Both leaflets of the bilayer diffuse freely over the entire surface of the substrate. Lateral fluidity of supported membranes is a key feature that distinguishes them from other surfaces. Fluidity provides the membrane with a variety of unique properties, but it also means that membrane components are continually mixing. A micropatterned fluid membrane is a surface that preserves many of the properties of cell surfaces while gaining precise control over diffusive mixing and flow of membrane components.

We have created micropatterned fluid membranes by designing a substrate that imparts a pattern to the self-assembling membrane above it.[3] Patterns of varying surface electrostatic and chemical properties (e.g., photoresist,

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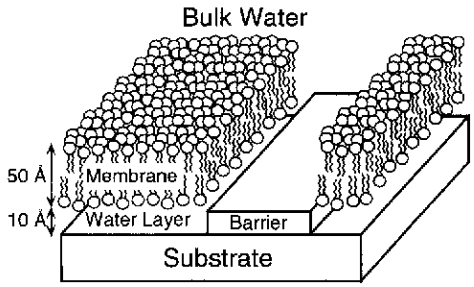

Fig. 1. Schematic diagram of a supported bilayer membrane partitioned by a microfabricated barrier. The size of the membrane is exaggerated to illustrate its structure. Actual membranes are typically 50 Å thick and are separated from the surface by a ~10 Å layer of water. The functionality of a barrier depends on the surface properties of the material, not its topography. Reprinted with permission from *Science* **1997**, *275*, 651–653. Copyright 1997 American Association for the Advancement of Science.

gold, or aluminum oxide) are made on solid planar substrates such as Si or quartz wafers. When a membrane is assembled on such a surface by vesicle fusion it is fluid over some areas and immobilized or absent in other areas, as directed by the patterned support. We have made arrays of square patterns that support fluid membranes separated by lines of membrane-immobilizing surfaces that serve as barriers to lateral diffusion. An array of approximately 2500 200 μm × 200 μm square fluid membranes has been created on a 1 cm^2 substrate area. The smallest membranes observed so far are 5 μm × 5 μm with 1 μm dividers; there are about 2.8 million of these on a 1 cm^2 substrate (Fig. 2A).

Micropatterned fluid membranes are an interesting substrate for lithography. As a demonstration we used light to photobleach lipids that are tagged with a fluorescent dye, Texas Red DHPE. One percent by mole of the lipids in the membrane are fluorescently labeled and make the membrane appear orange in fluorescence micrographs. When the Texas Red is bleached it ceases fluorescing and the membrane appears black. In Figure 2B we show that individual boxes in an array can be bleached. There is no diffusion across the dividers between the boxes. The membrane was bleached by a circular spot of light, but diffusive mixing causes the entire bounded region to be affected.

Lithography on a fluid substrate means that exposure time and open area on the lithographic mask are interchangeable parameters. The substrate divides the membrane into fluid pixels. If the exposure is long enough, all the molecules will spend some time under illumination even if the illuminated area is less than the area of the membrane pixel. For shorter exposure times, or smaller mask openings, only a fraction of the pixel is exposed. This is shown in Figure 2C, where the exposure time is varied in 30 s increments.

3. Applications

Since lithography can be performed on the fluid substrate, we expect that micropatterned membranes would also be a suitable platform for light-directed chemical synthesis. Proteins can be incorporated in or tethered to supported membranes. With appropriate photochemistry it should be possible to use light to direct which proteins are associated with particular pixels in an array of membranes.

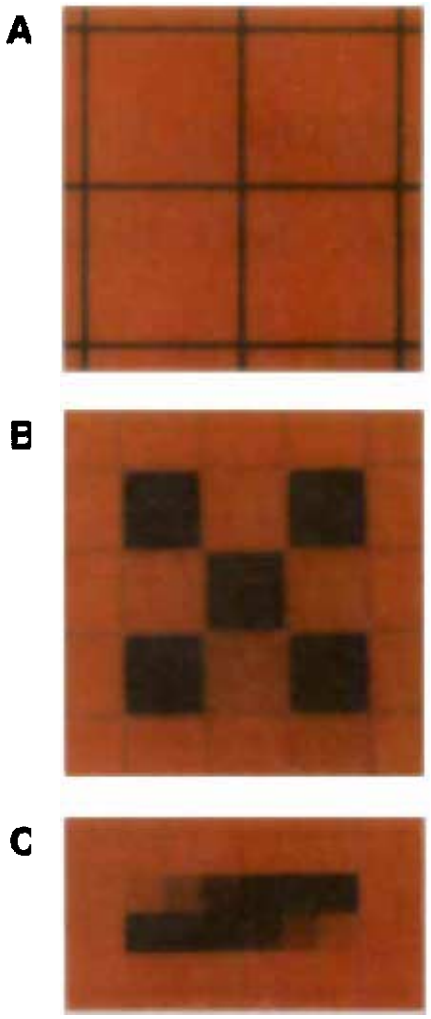

Fig. 2. A) Fluorescence photograph of four, 200 μm × 200 μm, corrals of fluid, supported bilayer on an oxidized Si wafer patterned with photoresist. The photoresist barriers appear dark compared to the bright fluorescence from Texas Red DHPE lipid probe incorporated in the membrane. B) Array of 100 μm × 100 μm corrals in which certain individual corrals were photobleached dark. Diffusive mixing of molecules in each corral causes the circular bleach spot to spread, filling the entire square region, but barriers prevent mixing between separate corrals. C) Array of 50 μm × 50 μm corrals in which certain corrals were photobleached for varying exposure times. Similar patterns may be created by masking variable fractional areas in different corrals during a single exposure. Reprinted with permission from *Science* **1997**, *275*, 651–653. Copyright 1997 American Association for the Advancement of Science.

Membranes are promising candidates for coating surfaces in microfluidic systems that handle cells. Membranes can be assembled on any shape surface as long as the surface electrostatic properties are properly prepared. Membrane surfaces reduce or eliminate non-specific binding between many proteins and solid substrates. They can also elicit specific binding interactions with cells.

A topic of great interest to defense organizations is developing technology for advanced sensors for biological

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warfare. These applications require an interface between the biomolecule that first interacts with the munition molecule and electronics for processing and data manipulation. The supported membrane is a candidate for this application.

4. Conclusion

Lithography is a mature technology that currently allows one to create structures on the submicrometer scale. Advanced techniques such as electron beam lithography and scanning probe lithography[13] extend this to the single nanometer scale and beyond. Microcontact printing permits patterning of curved surfaces.[14,15] These tools can be used for manipulating cells and their constituents.

It is important to focus effort on the cell membrane. As many as 70 % of all drug targets under consideration by the pharmaceutical industry are membrane proteins. And since all intercellular communication must occur across a membrane, the membrane will be an integral part of the interface between cells and Si electronics.

We are investigating micropatterning fluid bilayers as a first step on the road to forming a specific interface to living cells. Initial results indicate that lithography applied to supported lipid bilayer membranes is a rich area for research.

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