Creating Addressable Aqueous Microcompartments above Solid Supported Phospholipid Bilayers Using Lithographically Patterned Poly(dimethylsiloxane) Molds

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Herein we report the use of microcontact displacement (μCD) to generate addressable arrays of aqueous solutions above fluid lipid bilayers by bringing a patterned PDMS mold into conformal contact with a phospholipid membrane on a solid supported substrate. Epifluorescence microscopy established that the bilayer material was displaced in regions where contact between the mold and substrate was made. Photobleaching experiments confirmed that the bilayer sectors remained individually fluid but completely separated. The microcompartments created by μCD could be individually injected with aqueous solutions that remained sealed from their neighbors. This procedure was then exploited for screening a small library of aqueous-phase molecules for their ability to inhibit binding between surface-bound ligands and soluble protein receptors.

There is great impetus for creating combinatorial libraries of solid supported fluid lipid membranes because these systems retain many of the properties of native cell surfaces. Presenting ligand arrays on fluid bilayers could be exploited for the development of biosensors and screening assays, as well as for use in understanding fundamental processes of ligand–receptor interactions. Recently we showed that supported arrays of fluid phospholipid membranes can be formed on planar solid supports with unique chemical constituents at each address by using a combination of photolithographic patterning and microcapillary injection. This technique creates a patterned array of chemically distinct bilayers, each of which is in contact with the identical aqueous solution. Perhaps an even more desirable goal would be to address aqueous solutions above an array of planar supported bilayers. In combination with surface-specific detection, this strategy would enable the rapid screening of a library of soluble molecules for their efficacy in inhibiting ligand–receptor interactions in a fluid membrane environment that is similar to in vivo conditions. In this paper, we describe a novel soft lithographic technique for partitioning and addressing aqueous solutions above supported phospholipid membranes which may be exploited as a multivalent screening assay. This highly flexible methodology affords the ability to create a large number of aqueous compartments consisting of various chemistries, pH values, and ionic strengths, as well as ligand and inhibitor concentrations above patterned arrays of lipid membranes on a single planar support.

In the early 1990s, Kumar and Whitesides introduced micro-contact printing (μCP) as a strategy for patterning arrays of self-assembled monolayers by employing a poly(dimethylsiloxane) (PDMS) elastomer coated with alkanethiol and stamping the materials onto a gold substrate. Biebuyck and co-workers extended the use of elastomers to generate microfluidic networks by bringing lithographically patterned PDMS molds, which had been rendered hydrophilic by oxidation, into contact with solid substrates such as Au, glass, or silica/ PDMS formed the walls and roof of the sealed microcompartments while the floor consisted of the substrate. By derivatizing the substrate surface and flowing in the appropriate biological or chemical components, elaborate thin films of varying composition could be formed on the surface with micrometer-level resolution. Here we show that bringing lithographically patterned PDMS molds into contact with planar supported lipid bilayers in aqueous solution leads to highly selective microcontact displacement (μCD) of the phospholipid membrane from the substrate. Leaving the mold in place creates sealed and addressable aqueous compartments above patterned membrane arrays.

To functionalize the substrate, a continuous fluid egg phosphatidylcholine bilayer containing 1 mol % Texas Red DHPE is formed on a planar borosilicate support using the vesicle fusion method. A lithographically patterned PDMS mold containing a grid pattern of 60-μm-thick raised lines is gently placed in contact with the supported bilayer and the system is observed under an epifluorescence microscope (Figure 1). As can be seen strikingly

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from the fluorescence images, the bilayer is displaced from the solid support where the mold makes conformal contact with the surface. For the images shown, the process takes place over a period of ~90 min, although it could be made to occur in less than 5 min by applying additional pressure. Quantitative fluorescence measurements based on line profile data indicated that multiple bilayers are not formed during the process. Instead, the majority of the membrane material displaced from the surface forms free vesicles in the aqueous solution. Two additional fates of bilayer material also have been observed. When the PDMS mold is not flat enough to make conformal contact with the entire support, some material becomes trapped between the PDMS surface and the borosilicate support. Furthermore, a small amount of material sometimes is observed to congregate where the bilayer meets the elastomer walls. This latter material can be easily rinsed away upon removal of the PDMS mold. Interestingly, μCD occurs in the presence of both hydrophobic and hydrophilic (freshly oxygen plasma treated) PDMS molds. Apparently, the interaction between the PDMS and the underlying glass surface is sufficiently strong to force bilayer displacement under a variety of PDMS surface chemistries. The partitioned bilayers remain fluid on the surface with a diffusion constant of ~4 × 10⁻⁸ cm²/s as determined from the fluorescence recovery after photobleaching (FRAP) technique.¹¹,¹²

An important consequence of the partitioning procedure is the formation of individually sealed aqueous compartments above each of the supported lipid bilayers. These compartments can be separately addressed by patterning small holes in the portion of the elastomer that serves as the roof above the confined aqueous compartments. For this purpose, we made circular holes that were 100 μm in diameter.¹³ Pulled microcapillary tips mounted on an XYZ translation stage are used to transfer solutions into the holes under the microscope.⁴ Figure 2a shows the epifluorescence image of supported lipid bilayer patches containing 1 mol %Texas Red-labeled lipids that are patterned by contact with a PDMS mold. Injection of fluorescein dye into the upper right-hand box clearly demonstrates that the aqueous compartment above this bilayer is sealed from its neighbors (Figure 2b).

It is straightforward to demonstrate the utility of this partitioning procedure for screening libraries of small soluble molecules for their ability to inhibit binding between surface-bound ligands and aqueous-phase receptors. To do this, we formed a supported phospholipid bilayer containing 2 mol %biotinylated lipids and placed it in contact with the patterned PDMS surface. Biotin is a small molecule that binds the soluble protein streptavidin with high affinity and has been well characterized with planar supported lipid bilayer systems.¹⁴ Eight-nanoliter droplets of 1 mM N-2,4-DNP-glycine, 1 mM bovine serum albumin, and 1 mM biotin PEO-

![Figure 1](Image 1)

**Figure 1.** A time series of fluorescence images of a surface supported phospholipid bilayer in contact with a lithographically patterned PDMS mold. The membrane was formed by the fusion of small unilamellar egg phosphatidylcholine vesicles to a borosilicate support in a pH 7.2 sodium phosphate buffer solution containing 100 mM NaCl. The images were taken at (a) 2, (b) 28, and (c) 90 min after the mold was placed in contact with the membrane surface.

![Figure 2](Image 2)

**Figure 2.** Epifluorescence images taken (a) after microcontact displacement with a PDMS mold and (b) subsequent injection of a 5-(and-6)-carboxyfluorescein dye into the upper right-hand compartment. Narrow tunnels were used to lead up to the observed patches to facilitate simultaneous injection and observation in our upright microscope configuration (Nikon E800).

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[15] The final concentrations of these species are attenuated by a factor of ~20 through dilution as the aqueous container volumes were roughly 150 nL.
[16] Some streptavidin was observed to adsorb to the walls of the PDMS mold. This process could be suppressed by preadsorbing bovine serum albumin as suggested in ref 9.
amine were added to the aqueous compartments in boxes 1–3, respectively. Compartment 4 was left unaltered as a final control. Upon addition of 8-nL droplets of a 10 μM concentration of fluorescently labeled streptavidin to all four of the compartments, it could be seen that the fluorescence from the aqueous phase16 above each of the bilayers looked identical (Figure 3a). Using the evanescent wave generated from the 594-nm line of a HeNe laser to excite only the surface-bound, Texas Red-labeled streptavidin17 reveals that a high concentration of streptavidin is bound in boxes 1, 2, and 4 while the surface of box 3 remains protein free (Figure 3b). This experiment demonstrates the ability to deliver soluble analytes to these arrays multiple times and to evaluate their ability to prevent surface absorption events (Figure 4). Indeed, this methodology should be generally applicable to investigating the efficacy of candidate drugs to inhibit multivalent binding of ligands and receptors18,19 in a fluid environment.

Energies now shift to delivering solutions in a massively parallel fashion to bilayers displaying recognition components and examining surface absorption phenomena in varying solution environments. We are currently pursuing these studies using weaker receptor–ligand pairs with the expressed goal of measuring binding constants and inhibitor efficacy as a function of the surface and inhibitor chemistries. Results will be reported in due course.

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