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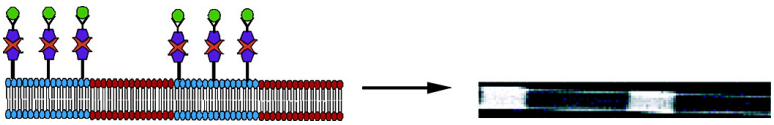

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In Situ Fabrication of Three-Dimensional Chemical Patterns in Fused Silica Separation Capillaries with Polymerized Phospholipids

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Immobilization of biologically active molecules in chemically specific, spatially defined patterns presents a number of opportunities for chemical analysis, such as expanding chemical sensing, high-throughput identification of pharmacological and physiological binding events, and capture of biologically relevant ligands from complex matrices. Immobilized domains of antibodies, enzymes, and other important biologically active compounds have been generated in microfabricated chips, in porous polymer monoliths, and on colloidal particles.[1−4] The creation of chemical patterns for microfluidic applications most commonly involves assembly of microflow channels over a preassembled chemical pattern which produces channels that are functionalized on only one side of the channel. A promising approach for the fabrication of biomolecular patterns within fluidic channels using in situ photoirradiation has been used to covalently tether enzymes within glass/PDMS channels[5] and polymer monoliths in fused silica capillaries.[3]

Formation of chemical patterns in supported phospholipid bilayers (SPB) provides an attractive alternative,[6,7] though in situ generation of three-dimensional patterns in enclosed volumes has been more challenging. Incorporation of SPBs into sensing and separation devices is intriguing since SPBs can support the function and integrity of many membrane-bound proteins and molecules facilitating the investigation of ligand−membrane protein interactions.[2,6,8] Further, SPBs minimize nonspecific adsorption of soluble proteins ubiquitous in biological samples.

The ability of lipid bilayers to self-assemble on a variety of substrates facilitates the deposition of SPBs on curved surfaces[4] and/or inside enclosed micron-sized channels.[9] Though the lateral mobility of lipids and tethered molecules in SPBs[6,8,10] may be advantageous for sensing applications, long-term maintenance of SPB pattern boundaries requires that physical barriers be prepared prior to bilayer assembly. Boundaries are primarily constructed using photolithographic and soft lithographic approaches that are not amenable to enclosed or highly curved surfaces, thus presenting the primary obstacle to in situ formation of SPB patterns on the interior walls of microchannels.[11]

Here, we report a new approach to form lipid-based chemical patterns inside fused silica separation capillaries using a combination of lipid bilayer self-assembly and UV photopolymerization of the reactive phospholipid, bis-SorbPC [1,2′-bis[10-(2′,4′-hexadienoyloxy)decanoyl]-*sn*-glycero-3-phosphocholine].[12] Fused silica capillaries are used as a model for microfabricated fluidic channels. The process for patterning capillaries is depicted in Figure 1. To chemically pattern the inside of the capillary, the walls are first coated with photoreactive phospholipids to generate a lipid bilayer via vesicle fusion.[8,9] After removing excess lipid vesicles from the capillary, the lipids are irradiated with UV light through a variable feature size photomask (extended experimental details can be found in the Supporting Information). Unpolymerized lipids are removed from the capillary through a combination of surfactant rinses and air purges since only the UV photopolymerized SPB is stable to these conditions. Removal of unpolymerized lipids exposes bare regions of fused silica onto which lipid bilayers containing different chemically functionalized lipids can be assembled. Using this approach, we have produced chemically patterned capillaries with inner diameters ranging from 10 to 150 μm.

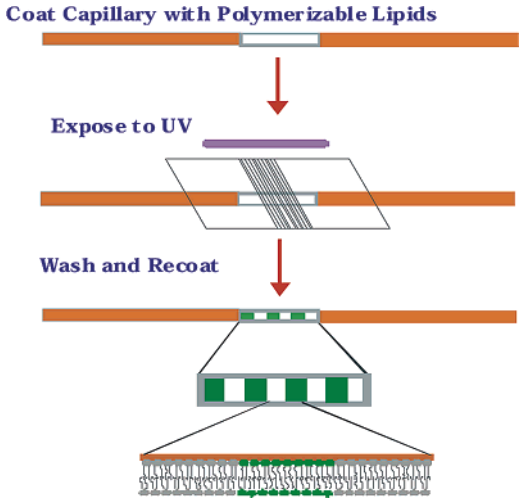

Figure 1. Schematic of polymer lipid patterning process.

A representative fluorescence image of UV-polymerized bis-SorbPC patterns formed in situ in completely enclosed channels is shown in Figure 2A. Following UV polymerization, unpolymerized bis-SorbPC residing in the nonirradiated regions of the capillary is removed, resulting in a bar-code-like pattern of polymerized poly-lipid domains that appear bright in the image after development with FM 1-43, a fluorogenic membrane stain. The formation of defined poly-lipid domains using bis-SorbPC is highly reproducible (>90%), and the observed chemical and environmental stability of the poly-lipid regions is consistent with previous reports on planar surfaces.[12a]

Following production of poly(bis-SorbPC) domains, the bare capillary regions can be chemically and biochemically functionalized using readily available phospholipid headgroup chemistries (Figure 2B−F). In Figure 2B, vesicles containing a mixture of 90% dioleylphosphatidylcholine (DOPC) and 10% rhodamine dioeyl phosphatidylethanolamine (Rh-DOPE) were fused into the void regions. Here, the bright regions represent the deposition of Rh-DOPE-doped fluid lipid domains confined within the bis-SorbPC polymer boundaries formed in situ.

The fluorescence image in Figure 2C demonstrates the capacity to chemically pattern specific binding events. Here, we assembled a mixture of biotin-functionalized lipids (10% biotin-DOPE/90% DOPC) into the void regions of the pattern. Upon addition of fluorescein-labeled streptavidin, alternating bright (biotin containing) and dark (poly(bis-SorbPC)) regions are observed. When patterns are created using 0% biotin-DOPE, the alternating pattern was not

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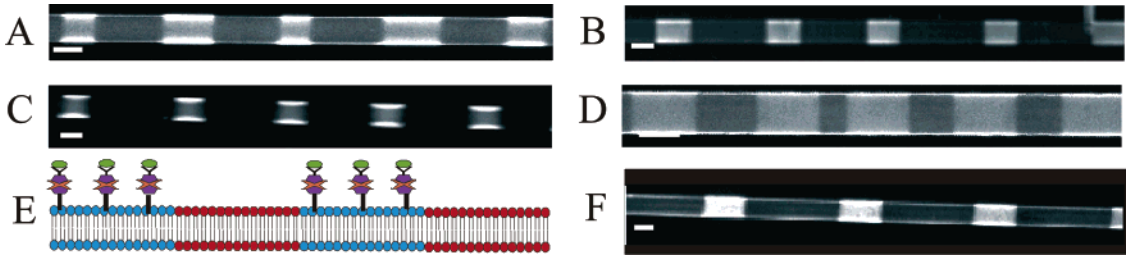

Figure 2. Functional chemical patterning in fused silica capillaries using UV polymerized bis-SorbPC. (A) Fluorescence image of bis-SorbPC chemical pattern formed in situ in a 75 μm i.d. capillary developed using FM 1-43, a fluorogenic membrane stain. (B) Fluorescence image of a two-component chemical pattern formed in situ in 50 μm i.d. capillary using rhodamine-terminated DOPE (bright) assembled into regions devoid of bis-SorbPC. (C) Fluorescence image of 50 μm i.d. capillary containing alternating regions of biotinylated DOPE (bright regions) and bis-SorbPC (dark regions) following introduction of FITC-streptavidin. (D) Fluorescence image of 100 μm i.d. capillary containing alternating regions of polymerized bis-SorbPC (dark regions) and DOGS−NTA (bright regions) following introduction of 6xHis-EGFP. (E) Schematic of immunocomplex formed in bis-SorbPC-patterned capillaries. Biotin-functionalized patterns were formed as in C, followed by addition of biotinylated anti-6xHis. (F) Fluorescence image of 50 μm i.d. capillary with alternating regions of immunocaptured 6xHis-EGFP (bright regions) and bis-SorbPC (dark regions). Scale bars represent (A) 75 μm; (B) 50 μm; (C) 50 μm; (D) 100 μm; and (F) 50 μm.

present and negligible fluorescence was observed. Thus, these data also demonstrate the marked reduction of nonspecific binding provided by the poly-SPB.

Figure 2D demonstrates the generation of chemical patterns using Ni^{2+}-charged DOGS−NTA (1,2-dioleoyl-*sn*-glycero-3-{*N*-(5-amino-1-carboxypentyl)iminodiacetic acid}) to introduce the capacity to bind and/or capture 6xHis-tagged proteins and peptides, a commonly used tool for purifying recombinant proteins and peptides. We have used this strategy to tether enhanced green fluorescent protein (EGFP) into spatially localized domains, demonstrating the ability to produce biofunctional patterns that are readily extended to a variety of recombinant proteins and enzymes. Figure 2D shows the specific binding of 6xHis-EGFP to 10% DOGS−NTA/90% DOPC domains. When the same capillary is exposed to EGFP lacking the 6xHis-tag, no fluorescence is observed (data not shown).

Finally, we have investigated the utility of this method for creating chemical patterns that can be used to monitor physiological and pharmacological binding events, as well as to perform low volume immunoassays. A key requirement of biofunctionalized patterns prepared on solid surfaces is the preservation of the binding activity of the immobilized molecules. The immunoreactive sandwich structure depicted in Figure 2E was created in fluid lipid domains formed in nonpolymerized regions of a bis-SorbPC patterned capillary. Biotinylated anti-6xHis antibody was introduced into the capillary using biotin-lipid/streptavidin patterning. Injection of 6xHis-EGFP introduces antigen binding, thereby generating the bright domains observed in Figure 2F.

In summary, we have developed a process to prepare micron scale, chemically and biochemically functionalized patterns inside three-dimensional, completely enclosed fluidic channels, in situ. Pattern formation is made possible through the use of self-assembled lipid bilayers that can be UV photopolymerized to generate cross-linked polymer phospholipid membranes and provide a matrix for incorporating functional proteins within the chemical pattern. The availability of a large variety of headgroup functionalized and UV polymerizable phospholipids makes it possible to create regions of varying chemical functionality within the self-assembled lipid membrane and provides the basis for multifunctionalization of solid support surfaces without the need for microfabricated barriers or lift-off stamps. Further, the ability to functionalize polymerizable lipid bilayers through the addition of dopant lipids[12] will allow preparation of more chemically complex patterns through serial repetition of polymerization steps, of a size that is limited primarily by the photolithographic resolution.

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Supporting Information Available: Detailed experimental protocols. This material is available free of charge via the Internet at http://pubs.acs.org.

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