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Structural Studies of DNA–Cationic Lipid Complexes Confined in Lithographically Patterned Microchannel Arrays[1]

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We have used lithographically patterned microchannel arrays with channel widths ranging from 1 to 20 μm, fabricated using electron beam lithography and reactive ion etching, in structural studies of DNA–cationic lipid complexes in confinement. Various techniques have been developed for loading these DNA–membrane complexes into the microchannels or to form the complexes in situ by sequentially depositing DNA and lipid solutions into the microchannels. Optical microscopy studies indicate that such complex formation is strongly influenced by the periodic channel structure even at channel widths much larger than the persistent length of the DNA molecules. Preliminary x-ray diffraction experiments conducted at Stanford Synchrotron Radiation Laboratory (SSRL) yielded only a weak signal from the lipid bilayers in the complexes. The use of a microfocused x-ray beam produced by the newly developed Bragg–Fresnel optics at a third-generation synchrotron facility may dramatically increase the signal-to-noise ratio and allow observation of orientational as well as positional ordering of DNA molecules induced by the microchannels. Structural control of the DNA–membrane complexes has a broad range of potential applications in gene probe technology and as mesoscopic biomolecular composites.

KEY WORDS: Bragg–Fresnel optics; confinement; DNA complexes; microchannel arrays; x-ray diffraction.

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1. INTRODUCTION

There has been considerable interest in utilizing lithographically defined microstructures as substrates for processing microscopic quantities of materials in the fluid phase. Recent examples include the formation of micron size lipid bilayer patches in areas separated by solid barriers [1], DNA electrophoresis in microscopic maze arrays [2], and as combinatorial gene probes [3]. The use of micropatterning techniques offers two main advantages: (a) the precise control of geometrical parameters (e.g., size and shape) which affect the phase behavior of self-assemblies of macromolecules and (b) the dramatic increase of combinatorial power due to large-scale integration, which is important for fabricating efficient and sensitive bioprobes. As the dimensions of the microstructures decrease, the effects of finite size effects and surface interactions on the orientational and positional order of macromolecules inside the microstructures become more important. Using state-of-the-art microfabrication technology, it is possible to produce confining structures at length scales on the order of common biopolymer (e.g., DNA molecules) persistent lengths (approximately 10 nm), which can lead to new structural and phase behavior.

We present results of optical microscopy and x-ray diffraction studies of the structural properties of DNA in solution and DNA–cationic lipid complexes confined in uniform spacing linear microchannel arrays fabricated on Si and glass substrates. We present optical microscopy data which indicate that the defect structure of linear DNA molecules is strongly influenced by the existence of microchannels even at channel widths much larger than the persistent length of the DNA chains. Structural control of the DNA–membrane complexes has a broad range of potential applications in gene probe technology and as mesoscopic biomolecular composites [4].

2. FABRICATION OF MICROCHANNELS

Microchannels used for studying structures of DNA–lipid complexes were fabricated at the National Nanofabrication Users Network (NNUN) facility at UCSB on borosilicate glass microscope cover slips (nominal thickness, $\sim 170\,\mu$m) and thin ($\sim 100\,\mu$m) (100) Si wafers. The use of thin substrates reduces signal loss due to absorption when the microchannels are employed for structural studies by x-ray diffraction. The periodic line patterns in the microchannels were defined by electron-beam lithography on a hi-lo molecular weight double PMMA (polymethylmethacrylate) resist layer [5]. Pattern transfer onto the substrates was achieved using reactive ion etching (RIE) with a Ni mask formed on the substrates by e-beam evaporation and subsequent resist lift-off [6]. In addition, channel

arrays with Au barriers ($\sim$1500 Å thick) on glass substrates were fabricated using the same lithographic process without etching.

The quality of the microchannels is defined to a large extent by the reactive ion etching process, which removes unmasked substrate material by flooding the surface with highly reactive ion species generated by a gas plasma source in the reaction chamber. Process parameters which directly affect the etch rate, sidewall profile, and roughness of the etched surface include the type of chemistry used, the energy (bias voltage) of the ion species, the temperature, and the gas flow in the reaction chamber. For Si-based microchannels, we used a chlorine-based reactive ion chemistry, which produced a smooth etched surface, faithful pattern transfer, and straight etched sidewall profiles. For microchannels on glass substrates, fluorine-based chemistry was used, which resulted in surfaces that were considerably rougher than Si devices. A scanning electron microscope (SEM) image of a 1-μm spacing channel array on glass is shown in Fig. 1. A typical sample contains multiple $300 \times 300\,\mu$m channel arrays with line spacing ranging from 1 to 20 μm to permit structural studies to be performed as a function of confinement spacing. Arrays with nonperiodic line

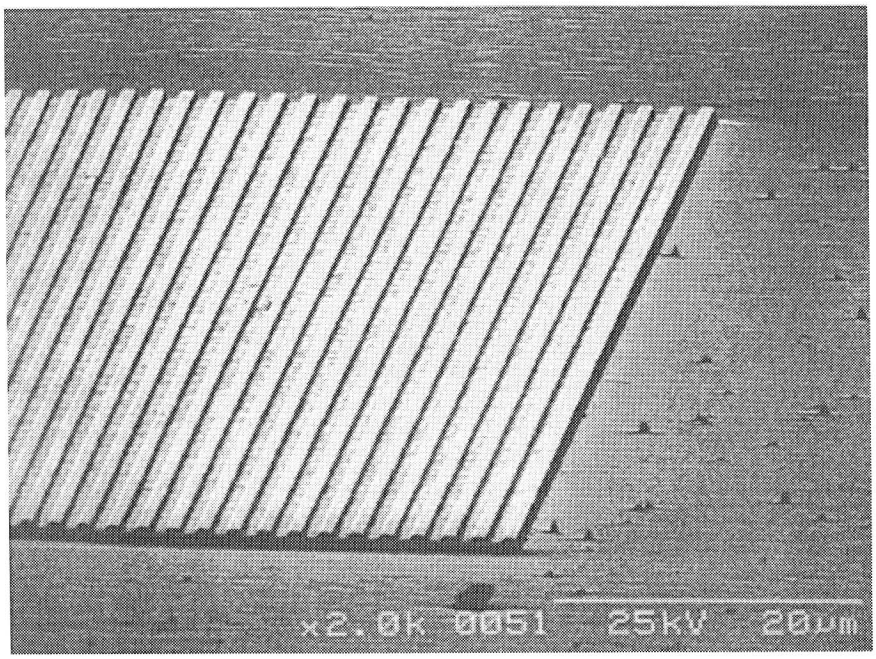

Fig. 1. A scanning electron microscope (SEM) image of a 1 μm spacing microchannel array on a glass substrate fabricated using e-beam lithography and Fluorine-based reactive ion etching.

spacing, notably in the form of linear Fresnel zones, were fabricated as microfocusing x-ray optics but could equally well be used for confining materials [4].

In the RIE process, the mask integrity ultimately determines the depth of microchannels which can be achieved. The etcher which we used for this work lacked the ability to produce energetic reactive ions. Consequently, the low etch rate associated with the fluorine-based etching process limits channel depth to $\sim 1.5\,\mu$m on glass substrates (the use of chlorine-based etching with Si substrates can yield $\sim 3\,\mu$m thickness). Prolonged etching tends to aggravate the "microloading effect," by locally depleting the reactive species in the narrow channels, leading to shallower depths in densely spaced microchannel arrays and deviation from the desired rectangular channel profiles. Currently we are investigating the use of energetically enhanced etching processes to produce deeper channels ($>5\,\mu$m) and more straight-channel side walls.

3. DNA–MEMBRANE COMPLEXES CONFINED IN MICROCHANNEL ARRAYS

It has recently been shown that DNA and cationic liposome mixtures spontaneously self-assemble into a multilamellar structure where a periodic, one-dimensional (1D) lattice of parallel DNA chains is confined between stacked lipid sheets [4]. The DNA lattice in such DNA–cationic lipid complexes are polycrystalline, with no positional or orientational correlation between the DNA chains across adjacent layers. It may be possible to use microlithographic channel arrays to induce such correlations, due to confinement effects and surface interactions. In this preliminary study employing optical microscopy and x-ray diffraction, we used lithographically fabricated $300 \times 300\,\mu$m arrays of various microchannel widths (1 to 20 μm) on 170-μm-thick glass plates (typical channel depth is about 1.5 μm) to confine the DNA–membrane complexes. Potential applications of such biomolecular composites include the development of molecular sieves with controlled pore sizes (~ 1 to 10 nm), nanolithography, and novel electrophoretic media.

One of the key aspects of this study is the loading of fluids and gels into the microchannels. We have developed a broad range of techniques for loading DNA–cationic lipid complexes into microchannels. Such techniques can be classified into two categories: (a) *ex situ* complex formation, where preformed complexes are introduced into the microchannels, and (b) *in situ* complex formation, where the constitutive components of the complexes are deposited sequentially into the channels, so that these components form

the complex within the confined geometry of the microchannels. Examples of both methods are illustrated below.

As a drop of concentrated DNA solution dries on a surface, a thin layer of DNA molecules is left behind. Such a receding drop of DNA solution can be used to deposit DNA into microchannels. This can be seen in Fig. 2, which is an optical micrograph of a dried sample (5 mg · ml^{-1} solution of λ-phage DNA) under crossed polarizers. Assemblies of DNA molecules are known to organize into various birefringent liquid crystalline phases, each with a characteristic set of well-defined defect structures. From Fig. 2, it can be seen that the existence of the microchannels strongly perturbs the native defect structure of the DNA and introduces a spatial modulation equal to that of the microchannels, which are 5 μm wide. The defect structure appears to be similar to those generated by undulations in the hexagonal phase. More detailed analysis is currently underway.

It is possible to use micropipette techniques to inject DNA and lipid solutions into the individual channels sequentially, while monitoring the process in real time using an optical microscope (see Fig. 3). A micropipette with a tip size of ~ 1 μm was employed to inject sequentially DNA (λ-phage DNA), then lipid [50–50% mixture of the neutral lipid DOPC (dioleoyl-phosphatidylcholine) and the cationic lipid DOTAP (dioleoyl-trimethylammonium propane)] solutions into a 300×300 μm array of 10 μm channels. The in situ formation of the complex can be discerned in two

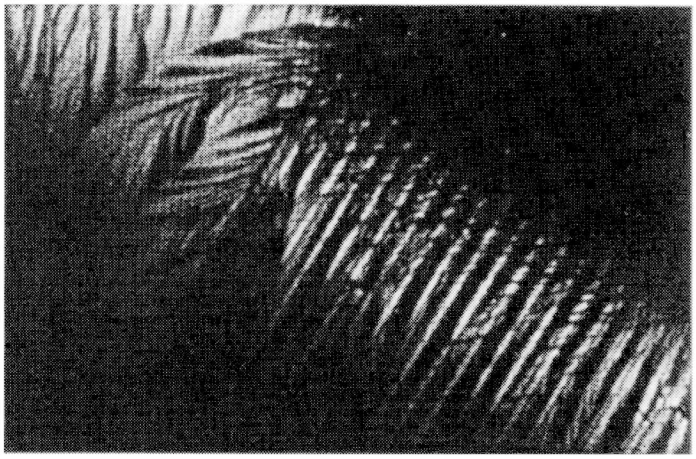

Fig. 2. An optical micrograph of a dried sample (5 mg · ml^{-1} solution of λ-phage DNA) under crossed polarizers. The native DNA defect structure can be seen at the upper left region of the photograph, which is outside of the microchannel array. The array induces a spatial modulation in the DNA defect structure.

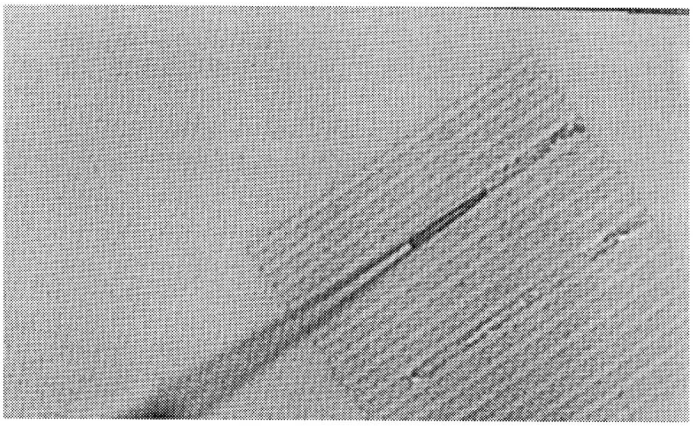

Fig. 3. A micropipette with a tip size of ~ 1 μm was employed to inject sequentially DNA, then lipid solutions into a 300×300 μm array of 10 μm channels. The *in situ* formation of the complex can be discerned in two filled channels.

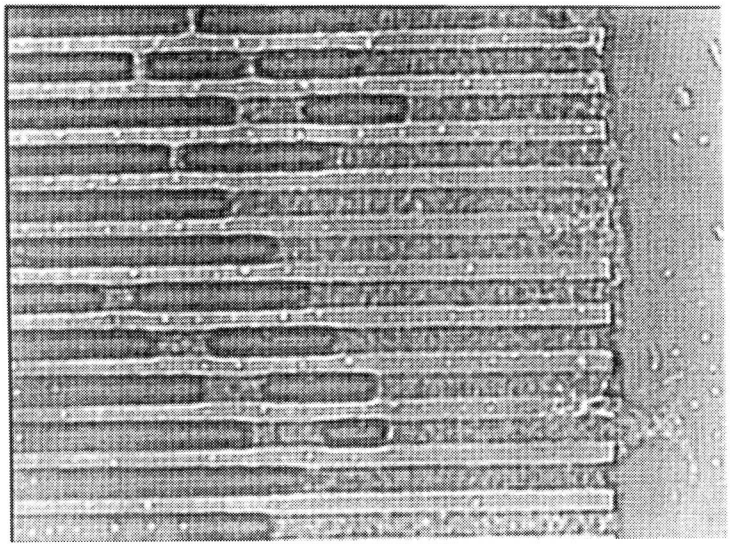

Fig. 4. An ethanol/chloroform (10/90%) solution of the DNA-cationic lipid complexes (lipid-to-DNA ratio of 5.5) was used to fill this 10 μm channel array. The complexes are clearly confined between the walls of the channels.

filled channels. The fiber bundles of complexes in each of the channels are weakly birefringent, which is consistent with previous observations of unconfined complexes.

We have attempted to use a solution of the DNA–cationic lipid complex for microchannel loading. The 10-μm spacing channel array shown in Fig. 4 was filled using an ethanol/chloroform (10/90%) solution of the complexes (calf thymus DNA, 50–50% DOPE/DOTAP mixture) at a lipid-to-DNA ratio of 5.5. Figure 4 suggests that the complexes are confined between the walls of the channels. Examples of confinement in smaller channels have also been achieved, and further refinements are in progress. An unambiguous characterization of complexes in channels, however, requires the use of x-rays.

4. X-RAY CHARACTERIZATION

X-ray diffraction measurements on DNA–membrane complexes in microchannels were conducted at beamline 7-2 of Stanford Synchrotron Radiation Laboratory (SSRL). The complexes were prepared on an array of 5 μm channels by sequential deposition of DNA and lipid. DNA was initially deposited using the receding drop method (1 $mg \cdot ml^{-1}$ solution of λ-phage DNA), followed by lipid deposition (25 $mg \cdot ml^{-1}$ of a 50–50% DOPE/DOTAP mixture). Preferential complex formation along the direction of the channels was observed *in situ* with an optical microscope.

The glass microchannel sample was transferred to a dry helium cell for the synchrotron x-ray diffraction experiment set up in normal incidence transmission geometry. The sample was oriented so that the microchannels were normal to the scattering plane. The incident beam, monochromatized to 8.0 keV, was defined with slits so that the beam size at the sample position was 200×200 μm, to avoid illumination of materials outside the channel array area while allowing integration over many channels.

Figure 5 shows the x-ray diffraction pattern collected from the sample after approximately 8 h of data acquisition. The observed peak corresponds to the expected position of the first-order diffraction intensity of the multilamellar lipid sheets with intercalated DNA chains. This indicates that at least some of the lipid sheets are oriented perpendicular to the sample surface. The low measured intensities did not allow an analysis of DNA ordering, as the DNA diffraction peak is usually much weaker than that for the lipid.

The use of microfocusing x-ray optics with comparable spatial resolution (~ 1 μm or less) to the channel spacing and enhanced brightness (flux/area) at the sample position can provide important advantages for the structural characterization of spatially confined materials such as the DNA–membrane complexes inside the microchannel arrays. We are currently developing Bragg–Fresnel lenses (BFLs) [6] which are capable

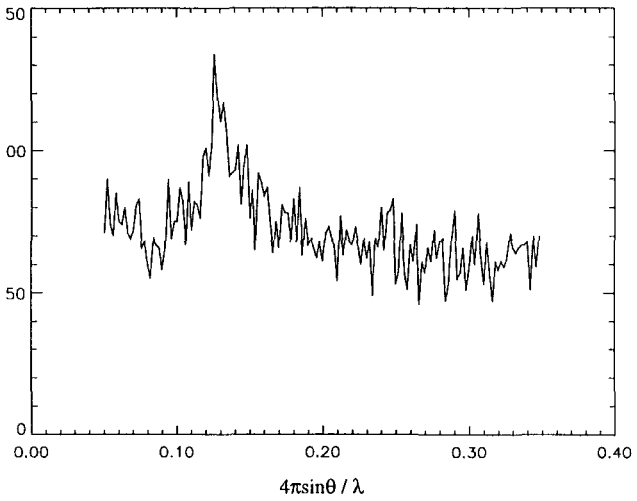

Fig. 5. X-ray diffraction pattern of DNA–lipid complexes formed *in situ* in microchannels. Data collected at SSRL beamline 7-2. The first-order peak from lipid sheets is visible, but the higher-order peak and the DNA ordering peak [4] are absent.

of focusing x-ray into submicron spots, for direct x-ray imaging and microdiffraction applications in confined systems. Due to the energy-independent focusing capabilities of BFLs, high-energy x-rays could be used in diffraction studies utilizing the microchannels, leading to reduced absorption by the substrates. Linear and circular BFLs have been fabricated, using the same processes employed for making microchannel arrays, on Si and III–V compound semiconductor (GaAs, InP, and GaAs/AlGaAs/GaAs heterostructure) substrates (Fig. 6). Preliminary characterization of BFLs at SSRL produced ~ 5 μm focused x-ray beams [7]. Submicron focused beams can be produced by using BFLs at a third-generation synchrotron facility, enabling diffraction measurements to be performed on DNA–membrane complexes confined in single channels with sufficient intensities.

5. CONCLUSIONS

Microchannel arrays with line spacing between 1 to 20 μm were fabricated on glass and Si substrates for structural studies of confined complex fluids. Methods were developed to deposit DNA–cationic lipid complexes in microchannel arrays. Optical microscopy and x-ray diffraction studies were conducted on these complexes in the microchannels. The formation of complexes is influenced by the spatial modulation of the microchannels.

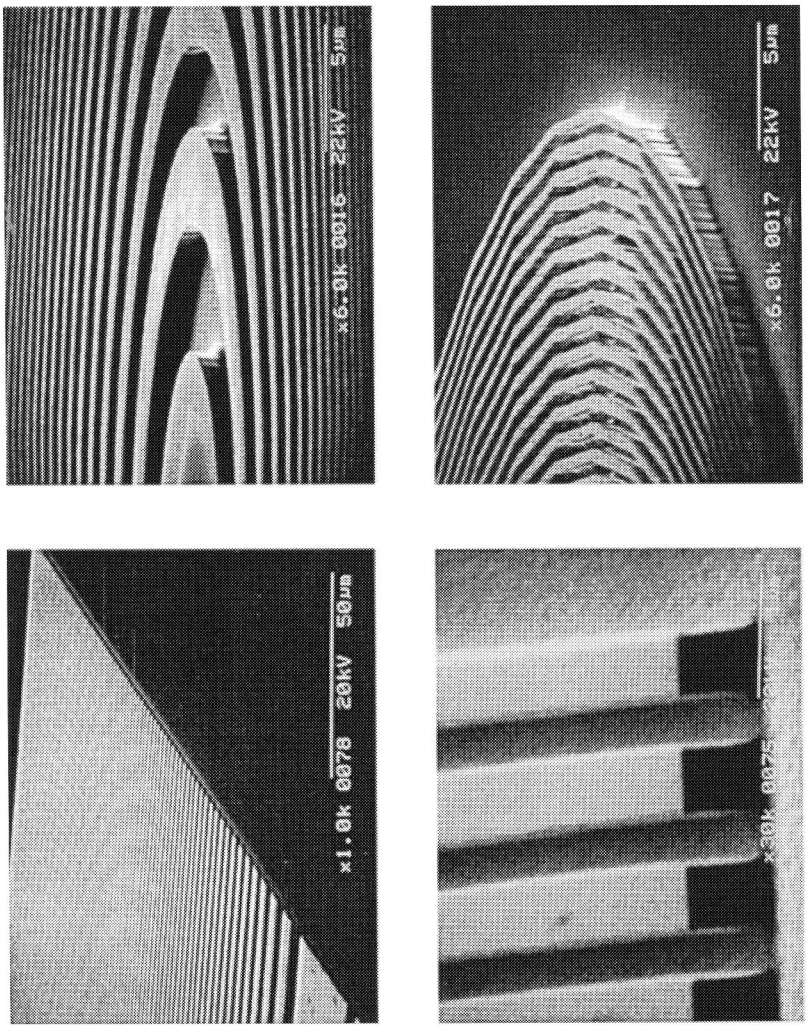

Fig. 6. SEM images of linear and circular BFLs fabricated on (111) GaAs substrates, showing the inner and outer areas under different magnifications. Both BFLs have an innermost zone radius of 12.2 μm, an outermost zone width of 0.5 μm, and 150 zones.

Experiments are under way to employ BFL focused microbeams for studying biomaterials such as the DNA–lipid complexes in the confined channel arrays, which should result in a dramatic increase in the signal-to-noise ratio.

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