Biological Applications

Patterning of Dendrimer-Like DNA

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Abstract:

The controlled assembly of hierarchically structured devices from nanoscale building blocks presents a great challenge to the field of nanotechnology. Dendrimer-like DNA (DL-DNA) is a versatile, nanoscale building block with a myriad of potential applications. This study presents a fabrication technique capable of dry etching poly (dimethylsiloxane) (PDMS) stamps with which lambda-DNA (λ-DNA) and DL-DNA can be patterned using molecular combing. 5 μm features were etched into PDMS, and molecular combing was employed to pattern spatial arrangements of λ-DNA. Future studies will explore the application of this process to DL-DNA in order to create multifunctional hierarchical nanostructures of DL-DNA.

Introduction:

The assembly and positioning of nanoscale building blocks in order to fabricate hierarchically structured devices remains a challenge to experimentalists. Nature, however, elegantly uses molecular recognition to fabricate supramolecular and self-assembling complexes of proteins and nucleic acids [1]. Biological molecules such as λ-DNA and DL-DNA are excellent candidates for directing the assembly and arrangement of molecular components. Controlled assembly of DL-DNA is achieved by first synthesizing Y-shaped DNA (Y-DNA), and then ligating individual Y-DNA molecules to themselves. The sequences of the Y-DNA are designed so that the ligations preferentially occur in a tree-like fashion. Higher generations of DL-DNA are constructed using the same strategy.

The objective of this project was to assemble λ-DNA and DL-DNA into patterns by virtue of a patterned PDMS template using molecular combing. In molecular combing of λ-DNA, a PDMS stamp is placed in contact with a small solution of DNA. When the PDMS is peeled off, the liquid-air interface of the traveling meniscus exerts a force on the DNA, stretching it and arranging the DNA into linear configurations. Interestingly, molecular combing can produce uniform arrangements of λ-DNA if the PDMS is properly patterned [2]. The patterned PDMS acts as a template, guiding DNA combing in microscale dimensions. The patterned DNA can then be transferred to glass or mica by placing and holding the PDMS stamp against a glass slide. Hierarchical structures of DNA can be achieved by transferring patterned PDMS multiple times.

Experimental Procedure:

Microwell arrays with a depth and diameter of 5 μm and lattice distance of 15 μm were patterned into PDMS using photolithography and reactive ion etching (REI). PDMS was spun onto silicon wafers to a thickness of 100 μm, and then cured for 90 min at 60°C. To improve the adhesion of the PDMS, silicon wafers were initially treated with air plasma (29 W) at 250 mTorr for 2 min. After curing, SPR 220-7 photoresist was spun onto the PDMS to a thickness of 7 μm and allowed to soft-bake for 30 min at 90°C. After being exposed on an EV620 contact aligner, the substrates were allowed to experience a post-exposure bake at 90°C for 30 min and then developed. Cured PDMS substrates were treated with O_2 plasma (100 W) at 170 mTorr for 60 sec to improve the adhesion of the photoresist. PDMS samples were then dry etched at 43 mTorr for 30 min using a 1:3 ratio of O_2 to CF_4. Etch rates were optimized by varying the pressure and ratio of gases in the REI process. Etch rates were determined by attaching Kapton tape to the PDMS before etching, and then measuring the step height using profilometry. After removing the photoresist with Shipley 1165 remover, PDMS stamps were characterized with SEM and AFM. The depth of the microwell array was measured using optical profilometry.

Molecular combing experiments were performed as previously described [2]. However, an additional force of 250 mN was exerted on some of the samples as the PDMS stamps were peeled off. Fluorescence microscope images were taken during all molecular combing experiments.

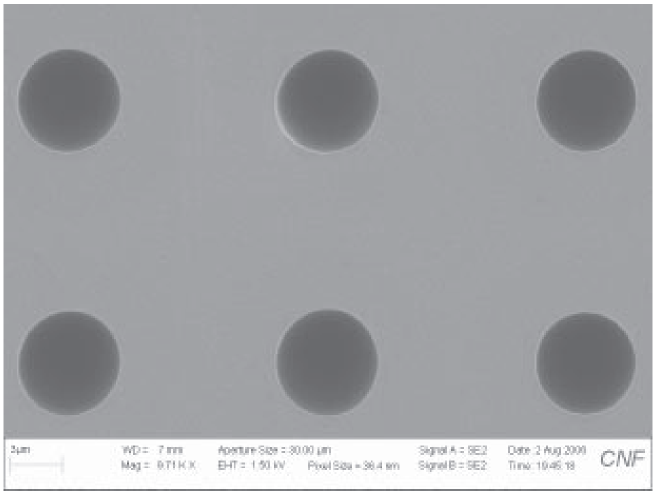

Figure 1: Anisotropically dry-etched PDMS.

Results and Conclusions:

The fluorine-based REI process developed in this study etched PDMS with a stable and directional etch rate (Figure 1). Although dry etching of PDMS has been reported previously [3], to our knowledge this is the first time anyone has demonstrated etching of features as small as 5 μm. This technique complements the standard cast-mold procedure for generating PDMS stamps and will likely be useful in the fabrication of sub-micron PDMS features. A 1:3 mixture of O_2 to CF_4 at 43 mTorr was found to anisotropically dry etch PDMS at a rate of approximately 10 μm per hour. Optical profilometry results revealed well depths of 4.5 μm.

Molecular combing of λ-DNA produced uniform one-dimensional arrangements of DNA. As previously reported [2], linear strands of λ-DNA were observed to attach at the edges of the wells. However, the arrangements did not possess long-range order. It is hypothesized that the treatment of PDMS with O_2 plasma and/or exposure to n-methyl-pyrrolidine within Shipley 1165 remover altered the surface roughness and perhaps even the hydrophobicity of the stamp, compromising the molecular combing process. Unexpectedly, the additional molecular combing force of 250 mN was sufficient to force the DNA into the wells. Unlike λ-DNA, DL-DNA is not a linear molecular; hence, its molecular combing technique must be modified. This study presents the first evidence that our modified molecular combing technique could be employed to pattern uniform arrangements of DL-DNA.

Future Work:

Future studies should explore alternate fabrication techniques capable of producing sub-micron features in PDMS without significantly altering its surface roughness. The application of this molecular combing process to DL-DNA should also be explored in the interest of generating multifunctional hierarchical nanostructures of DL-DNA.

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References:

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[2] Lee et al. PNAS (2005) 102:51; 18321-18325.

[3] Paranjape et al. JVST (2002) 20:3 975-982.

Figure 2: Uniform circles of fluorescent λ-DNA using a modified molecular combing technique.