Nano and Microfluidics for Single Molecule Biophysics Applications

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Abstract:

The recently developed abilities to manipulate and visualize single deoxyribonucleic acid (DNA) molecules are making a significant impact on discoveries in cellular and molecular biology. Innovations in micro- and nanofluidics are an important enabler of single-molecule techniques. Two projects were pursued, one to develop microfluidic devices for optical tweezers applications and one to develop porous nanochannel devices for elongation of single DNA molecules. First, microchannel devices were fabricated using polydimethylsiloxane (PDMS) cured on microfabricated silicon templates for future use with optical tweezers in DNA unzipping applications. The ability to form single-molecule DNA-microsphere tethers was demonstrated. Second, porous-wall nanochannels were fabricated using interferometric lithography and silica nanoparticles for use in visualizing fluorescently labeled DNA molecules. The ability to elongate and visualize lambda phage DNA molecules was confirmed in nanochannels on both silicon and quartz substrates. Both of these achievements are an important foundation for future work in analysis of protein-DNA interactions, including a specific goal of analyzing single chromatin fibers isolated from living cells.

Purpose:

With DNA tethers in microchannels, an optical tweezers apparatus can be used to "unzip" the DNA constructs and ultimately to map where nucleosomes occur on chromatin in living cells by comparing force profiles with thermodynamically predictable controls. While very powerful, the current method of Chromatin Immunoprecipitation (ChIP) and real-time polymerase chain reaction (PCR) has problems with sensitivity and other drawbacks. With mapping by single-molecule unzipping, the accuracy would be improved to approximately three base pairs compared to about 100 base pair accuracy with the current method and will reveal correlations on single molecules [1]. Mapping where nucleosomes occur on chromatin in living cells may also be achievable by labeling histones with either gold nanoparticles or quantum dots and elongating them in nanochannels.

Experimental Procedure:

Photolithography techniques were employed for the fabrication of both the microchannels and the porous nanochannels. The microchannels were first designed using AutoCAD and a mask was printed using an inkjet printer (CAD/Art Services Inc, Bandon, OR). SU-8 2025 photoresist (Microchem Corp, Newton, MA) was then spin coated onto a silicon wafer, exposed, and developed. PDMS (Microchem Corp.) was then mixed with a curing agent and poured over the wafer to create the desired patterns. Finally the PDMS was cut from the template and attached to a glass cover-slide using plasma surface activation. Fluid could then be pumped through the channels either by a syringe pump or, more crudely, syringes, in order to create the desired tethers.

Porous nanochannel fabrication involved bottom-up self-assembly and top-down pattern definition. An anti-reflective coating or ARC was spin coated onto a quartz or silicon substrate followed by photoresist. Interferometric lithography was then used to exposed the chip in a periodic fashion, the exposed chip was then developed creating a template around which the silica nanoparticles were applied using several spin coatings (Figure 1). High temperature calcination was then used to remove the photoresist and enhance the structural stability of the nanoparticle assembly by strengthening the binding between adjacent particles [2]. Lambda DNA (New England BioLabs, Ipswich, MA) stained with YOYO-1 (Invitrogen, Carlsbad, CA) was then placed in the channels and visualized.

Results:

We have familiarized two effective methods of enabling chromatin mapping and general single molecule biophysics research. DNA constructs were assembled in PDMS microchannels. The ability to form tethers was confirmed by observation of microspheres undergoing constrained Brownian motion. Lambda DNA stained with YOYO-1 was visualized in the porous nanochannels using both silicon and quartz substrates (Figure 2).

Conclusions and Future Work:

This work has been an effective enabler of numerous biophysics applications. The most immediate work is attaching thinner PDMS segments to the glass cover-slides so that optical tweezing may be effectively performed without too much scattering. With respect to the nanochannels; attaching PDMS and automating the flow process so that single DNA molecules can be elongated and visualized in about thirty seconds would be a huge accomplishment. Labeling nucleosomes in chromatin extracted from yeast cells with gold nanoparticles or quantum dots and elongating them in these nanochannels is also a priority.

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References:

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[2] Deying Xia, Thomas C. Gamble, Edgar A. Mendoza, Steven J. Koch, Xiang He, Gabriel P. Lopez, and S. R. J. Brueck. DNA Transport in Hierarchically-Structured Colloidal-Nanoparticle Porous-Wall Nanochannels (2008).

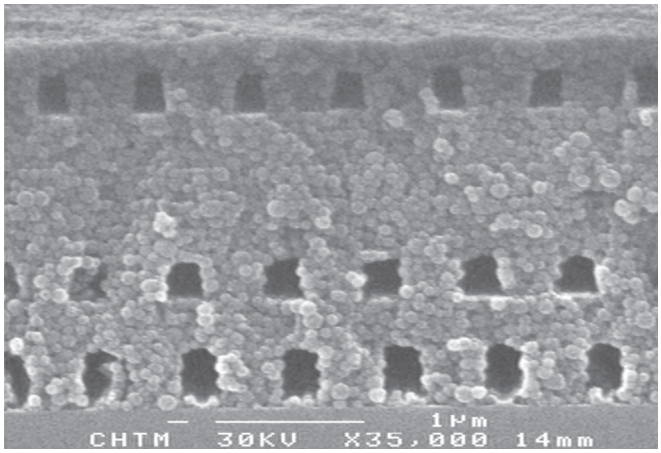

Figure 1: Edge on view of silica nanochannels after calcination demonstrating localized sintering and structural stability (1).

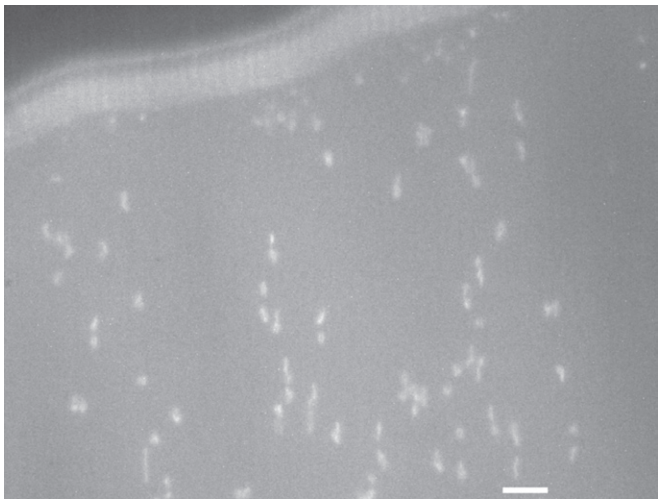

Figure 2: Lambda DNA (bright white streaks) elongated in nanochannels. Scale bar = 5 microns.