Characterization of DNA Flow through Microchannels

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ABSTRACT

Advances in silicon fabrication technology enable the reproduction of laboratory apparatus, and theoretically biochemical processes, on a microscale. The implementation of this technology, however, necessitates additional study as fluid dynamics change at this scale and, consequently, the behavior of biological molecules within these devices may also change. To investigate the extent this occurs, epifluorescence microscopy was used to observe the flow of DNA molecules through microchannels. The molecular conformation of the DNA under flow is extremely sensitive to 1) the initial conformation of the DNA as it enters the flow, 2) the position of the DNA molecule in the channel, and 3) the time at which the image was taken due to the vorticity-induced tumbling of the molecule. From these experiments, a non-monotonic relationship appears to exist between flow rate and DNA length. Further characterization of this relationship is underway.

Keywords: MEMS, hydrodynamics, DNA

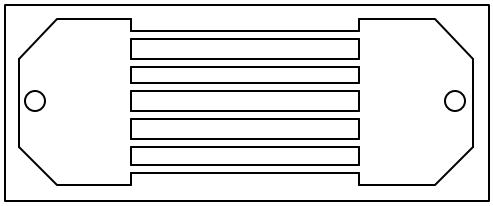
INTRODUCTION

The ability to conduct biochemical reactions on a chip that are traditionally executed in a laboratory can significantly impact both research and medicine; this technology increases the efficiency of experimental analyses whether in mapping a genome or in diagnosing and treating disease. Directing the flow of DNA, proteins, or cells through silicon micro-channels, however, harbors multiple concerns. Use of the technique requires that the behavior of the molecule in the MEMS device is analogous to its behavior in a test tube. Specifically, several key relationships need defining including the effect of the velocity fields on the structural integrity of the molecule; the impact of non-Newtonian and viscoelastic properties of the fluid on the velocity fields; and the surface interactions between the silicon channel and the molecule.

Elucidating the mechanisms that govern biological fluid flow is not trivial. Classical theories require revision when applied to microfluidics. In this low Reynolds number regime, effects that are traditionally ignored gain importance due to the enormous surface to volume ratios in the device and due to the similarity in scale between the macromolecule and the MEMS channel; fluid flow is laminar, viscous forces dominate over inertial forces, and surface tension becomes a factor.[1] Consideration of the behavior of the DNA molecules adds complexity to this problem. The DNA molecules are viscoelastic; a stressed polymer will exert a counter force on its solvent, resulting in viscosity enhancement and turbulent drag reduction.[2] Furthermore, the DNA chains independently and continually change shape due to Brownian motion. Therefore, the shape of the DNA backbone varies with time.[3] Because this behavior holds for all DNA chains in a flow field, treating the molecules as a continuum may not be valid. Thus, by subjecting DNA molecules to a uniform velocity gradient in a microchannel, we hope to lend insight into these phenomena.

MATERIALS AND METHODS

Below is a schematic of the device used in our experiments; it is not drawn to scale. Figures 1a and 1b show SEM photographs of the device channels. Beginning with n-type single surface polished silicon wafers, the silicon wafers were wet oxidized to yield a surface oxide layer 1 μm thick. The first lithography step defined the channels and the fluid reservoirs. A plasma etch was used to remove the sacrificial oxide layer covering these structures. The second lithography step defined the through-holes in the reservoirs. The photoresist applied for lithography served as a mask for the device during a subsequent reactive ion etch of the through-holes. Removal of the photoresist then permitted the reactive ion etch of the channels and fluid reservoirs; the oxide layer remaining from the plasma etch protected areas of the device not etched. Finally, the surface oxide layer was removed with HF, and the individual devices were separated with a diamond saw. The device dimensions are 28 mm by 10 mm. Reservoirs extend from the edge of channels 8 mm long, 100 μm wide, and 120 μm deep. The through-holes are 1 mm in diameter.



Initial experiments focus on the conformation of the DNA molecule as it is pumped through the device channels. Driven by a syringe pump (Sage Instruments model M362), fluid enters and exits the device via the through-holes; vinyl tubing secured by epoxy connects the pump to the device.

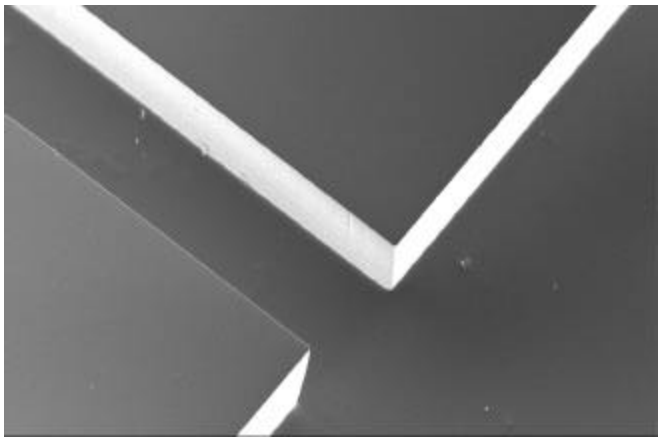


Figure 1a: SEM of device channels, 79X mag.

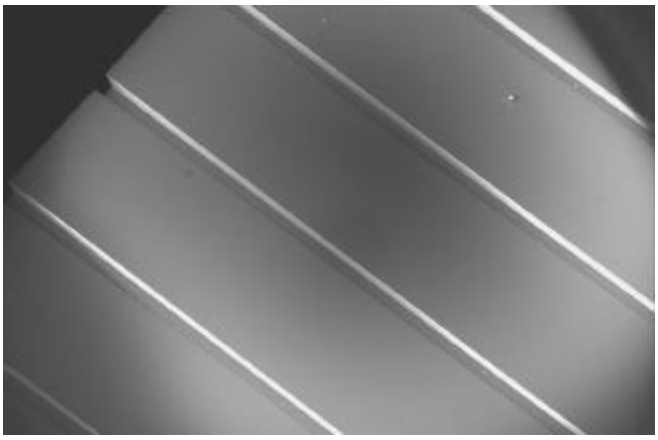


Figure 1b: SEM of device channels, 12X mag.

Finally, a glass coverslip bonded to the surface of the device encloses the fluidic path. To minimize shearing effects induced by pumping, the residence time of the DNA in the reservoirs exceeds by several fold the relaxation time of the DNA. Using equations for Newtonian flow as reference, pressure drops across the channel range from 870 Pa to 2.6×10^4 Pa and Reynolds numbers from 2.17 to 64.9 for flow rates between 1 ml/hr and 30 ml/hr.

Epifluorescence microscopy enables visualization of the DNA as it flows through the channels. In these experiments, λ-phage DNA (Sigma) is fluorescently labeled with a dimeric cyanine nucleic acid stain YOYO1 (Molecular Probes) at a ratio of 5:1 DNA base pairs per dye molecule. The concentration of the DNA in the buffer solution is 0.05 μg/ml, and the length of the fully elongated DNA is 22 μm. The buffer solution consists of 10 mM tris-HCl, 2 mM EDTA, 10 mM NaCl, and 4% B-mercaptoethanol (Fisher).

RESULTS

Figure 2 shows examples of DNA molecules at a variety of flow rates. These single images represent the conformation of the molecules during steady flow. The shear stress induced by the velocity gradient seemingly results in the elongation of the DNA molecules.

The coupled effects of the flow field and surface interactions between the DNA and the walls of the channel can be observed in studies such as those illustrated in Figure 3. Under laminar flow conditions, the shear rate is highest at the channel walls and lowest at the channel centerline. Hence, on average, one expects to observe relatively stretched conformations near the walls and coiled conformations near the centerline. Figure 3a captures DNA flowing through the middle of the channel at 3 ml/hr. Figure 3b illustrates the same flow rate, except the imaging area extends from the wall to the middle of the channel. The extension of the DNA at the wall versus the middle of the channel differs; the DNA is coiled more at the wall. The more highly coiled state near the wall is surprising and may be due to either non-Newtonian effects on the velocity field or surface interactions between the wall and the DNA.

Figure 3c suggests that channel geometry may affect the molecular conformation of the DNA. The DNA as it emerges from the through-hole at 3 ml/hr is coiled. The ten-fold reduction in the device geometry from the through-hole to the channel may also account for the elongation of the DNA. Finally, at flow rates capable of inducing DNA

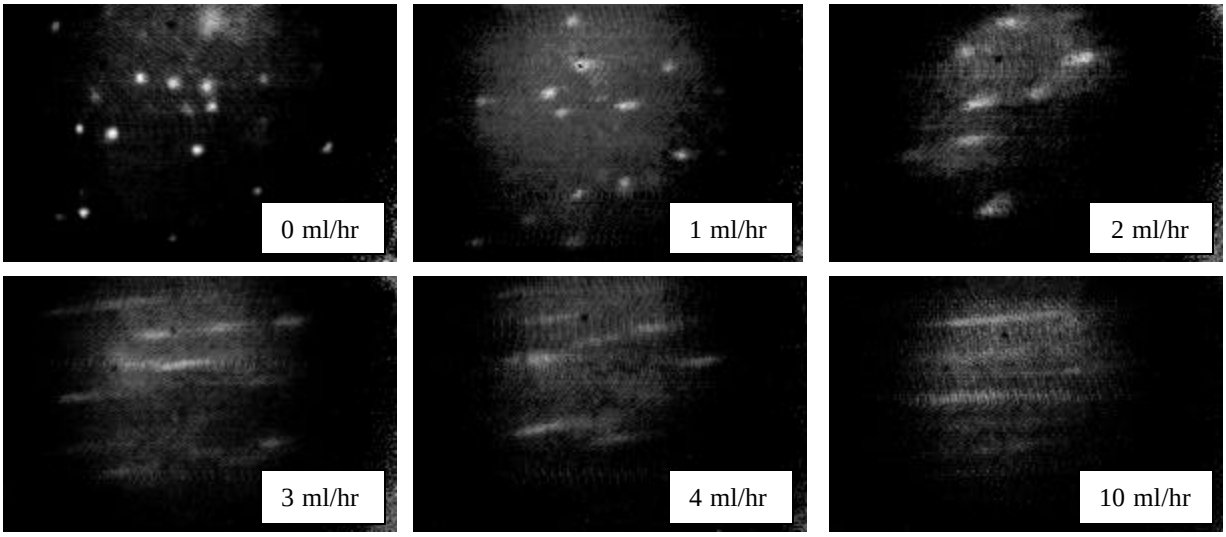


Figure 2: Stretching of DNA with Increasing Velocity

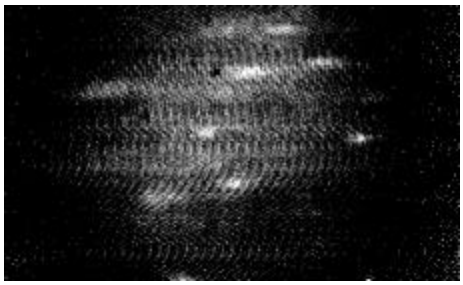


Fig. 3a: DNA flow through middle of channel

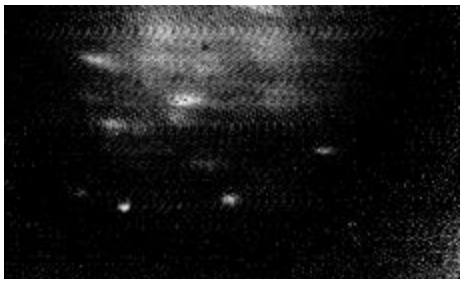


Fig. 3b: DNA flow from wall to middle of channel

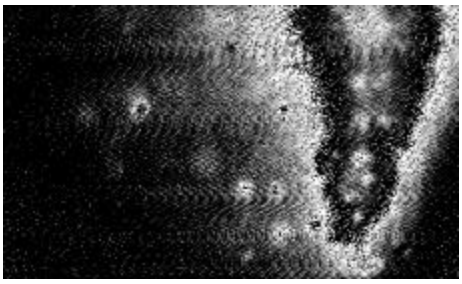


Fig. 3c: DNA flow at entry of channel

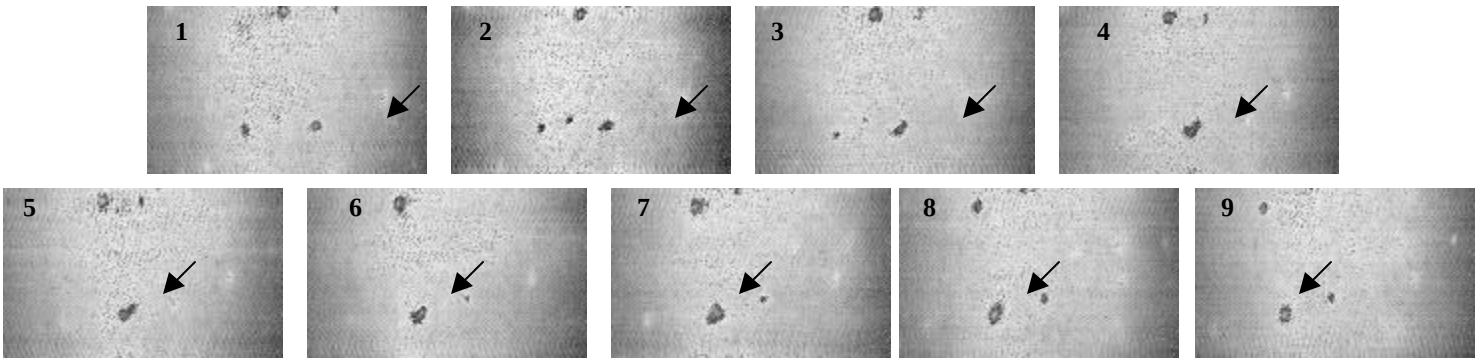


Figure 4: DNA Flow, 3 ml/hr, 0.1 sec/frame

extension, the DNA exhibits vorticity as seen in Figure 4. The rotation of the DNA molecule as it flows through the channel is problematic at increased DNA concentrations when the separation between molecules decreases and the molecular chains are susceptible to entanglement and possibly shearing.

DISCUSSION

These single images suggest that the conformation of the DNA molecule under flow is not uniform, but rather depends on several factors. The conformation of the DNA molecule constantly changes as a result of Brownian motion and vorticity in the flow field. Consequently, its position as it enters the flow field determines both its immediate shape and its resultant stability. Certain starting conformations of the DNA in the flow will result in greater stretching, and hence a higher probability of chain scission and entanglement with other chains.

Secondly, as seen in Figure 3, the position of the DNA molecule in the cross-section of the channel may influence its conformation. Either surface interactions between the DNA and the silicon or perhaps the vorticity-induced tumbling of the DNA molecule against the wall hinder its flow and induce a more coiled conformation relative to molecules in the middle of the channel. Third, device geometry may also play a role. Finally, it is important to remember that the conformation of the molecule constantly changes and, therefore, the position of the molecule as recorded in these single images reflects this time dependence. Thus, these single images show a non-monotonic relationship between flow rate and DNA length: the relationship between flow rate and the "average" DNA length (averaged over many starting conformations and over the tumbling of the chain) is under investigation.

In conclusion, understanding the behavior of the molecule in the MEMS device is critical for establishing experimental parameters capable of yielding consistent and predictable results. The non-Newtonian and viscoelastic properties of DNA can significantly alter the dynamics of the flow field even at low concentrations. Furthermore, these effects will be more pronounced at increased DNA concentrations and in more complicated geometries.

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