

DNA FRAGMENTATION IN A MICROFABRICATED MICROFLUIDIC DEVICE

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Abstract

We designed and tested microfluidic devices which allowed fragmentation of genomic λ -phage DNA down to an average size of 1500 bp. DNA samples were pumped through channels with narrow flow constrictions. At a constant volumetric flow rate, fluids containing DNA accelerate when the flow path suddenly narrows. This creates a force field in which the DNA molecules are subjected to stress, causing them to uncoil and fracture. Microfabrication provides a unique opportunity to design and characterize different flow geometries for DNA fragmentation. For instance, the average fragment size decreased by about 50% as the constriction width was halved. The fragmentation process took only a few seconds.

Keywords: DNA fragmentation, microfluidics, hydrodynamic forces

1. Introduction

Random fragmentation of DNA is an important sample preparation step prior to different types of DNA analysis. To obtain short DNA fragments (about 1000 base-pairs (1kb)) from large DNA molecules (up to several hundred kb), DNA can be fragmented using hydrodynamic forces. This technique involves repetitively pumping the sample, consisting of large DNA molecules in an aqueous buffer, through a short and narrow constriction or orifice [1]. Assuming a constant volumetric flow rate, the flow velocity increases rapidly at the constriction inlet. The accelerating flow (elongational flow) subjects DNA molecules to stress so that they uncoil [2]. At very high elongational rates, the DNA strand will break around the midpoint, yielding smaller fragments [3]. The flow rate and the number of recirculations (ten to twenty cycles, with an HPLC pump) through the constriction determine the length of the fragmented product.

We have previously shown that implementation of hydrodynamic DNA fragmentation in chips is advantageous [4]. For instance, microfabricated orifices may be placed in series on one device, eliminating the need to cycle sample through a single orifice and thus simplifying the flow system. Flexible design of fragmentation unit geometry and orifice size is also possible. In this paper, further optimization and characterization of this concept are reported.

2. Experimental

Fragmentation devices were fabricated using a single-mask process. Channel structures were etched into a silicon (Si) wafer by deep reactive-ion-etching (DRIE). A 200-nm oxide layer was then grown thermally to provide chemical resistance. Finally, the Si wafer was anodically bonded to a Pyrex glass wafer with predrilled holes, which served as inlets and outlets. Each wafer included as many as 33 devices.

The wafer was diced so that individual chips could fit into chip holders made in-house. Poly(etheretherketone) (PEEK) tubes were connected to the holders to enable solution delivery to the microchannels in the Si-glass device. The M6 PEEK fittings and ferrules (Upchurch, Bern, Switzerland) were flangeless. The dead volume of the fragmentation setup apart from the Si-glass chip was measured to be about 40 μL ; the dead volume of the Si-glass device itself is calculated to be about 3 μL . The fragmentation of DNA was done by pumping the syringe either manually or with a syringe pump. λ -phage DNA (48 kb) and T2-phage DNA (164 kb) (33 or 67 $\mu\text{g}/\text{mL}$ in 10 mM Tris-HCl/5 mM NaCl/0.1 mM EDTA, pH 7.0) were chosen as samples.

The fragmented DNA was put on dry ice and sent to NorChip AS (Klokkarstua, Norway) by express delivery (DHL), where it was analyzed with the Bioanalyzer 2100 using the DNA 12000 kit, both from Agilent Technologies. The Biosizing software was used for initial data processing and calculation of migration time-size calibration curves using DNA ladders of known length. The data were exported to data analysis software. Peak positions from fragmentation products were obtained by fitting the peaks with a Gaussian function. The average DNA size was obtained by converting the peak positions (in seconds) to DNA size using the migration time-size calibration curve.

3. Results and discussion

With the first generation of devices (Fig. 1A), λ DNA could be reduced from its initial size of 48 kb to small fragments with a size distribution centered around 4 kb [4]. To achieve even smaller fragments and better reproducibility, the device design was improved for better flow dynamics (Fig. 1B) and a build-in filter was etched. With identical orifice size, the new design performed significantly better than the old design (Fig. 1C), and the smallest reproducible fragment size was below 2 kb (see also Fig. 2A). To obtain more insight into the fragmentation mechanism, systematic investigation of the fragmentation parameters was carried out. Experiments showed that the product size decreased with increasing flow rate and decreasing constriction width (Fig. 2A), as reported [1]. Fig. 2A also indicates that at high flow rates the fragmentation depends more on the orifice size than the flow rate. Further experiments showed that several orifices in series produced smaller and more narrowly distributed fragments (Fig. 2B). We also fragmented the approximately three-times-longer T2 DNA. The products were only about 20% longer than the λ DNA (results not shown).

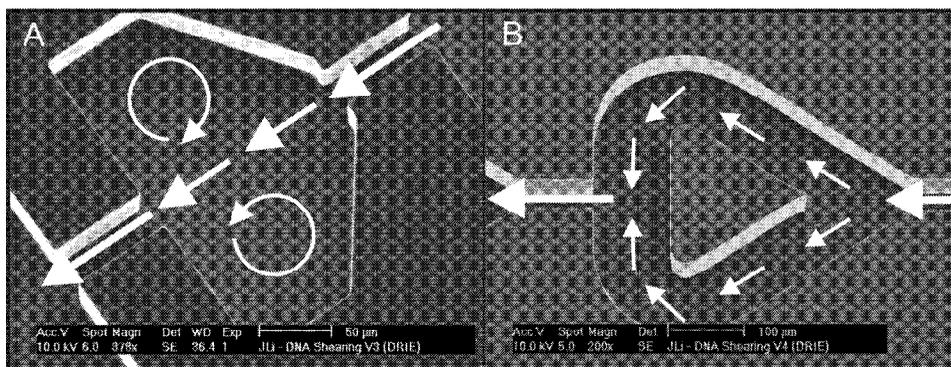


Figure 1. Comparison between two designs. Fig. (A) and (B) are SEM pictures of two DNA fragmentation units made by DRIE in silicon. The new design (B) eliminated the prominent velocity jet (white arrows) in the old design (A), increasing the flow acceleration at the orifice entrance and thus the hydrodynamic force fragmenting the DNA. We compared the two designs by fragmenting λ DNA in devices with ten 15- μm -wide constrictions in series. Fig. (C) shows that at a given flow rate, the new design (Δ) gives significantly smaller fragments than the old design (\blacksquare).

4. Conclusions

Microfluidic systems provide reproducible DNA fragmentation. The results showed that the fragmentation of DNA was not only dependent on the orifice size and flow rate, but also strongly influenced by the flow dynamics due to the geometry of the device. Since our devices could produce fragments smaller than 2 kb, they could be used to fragment DNA prior to many types of DNA analysis, such as subcloning in shotgun sequencing.

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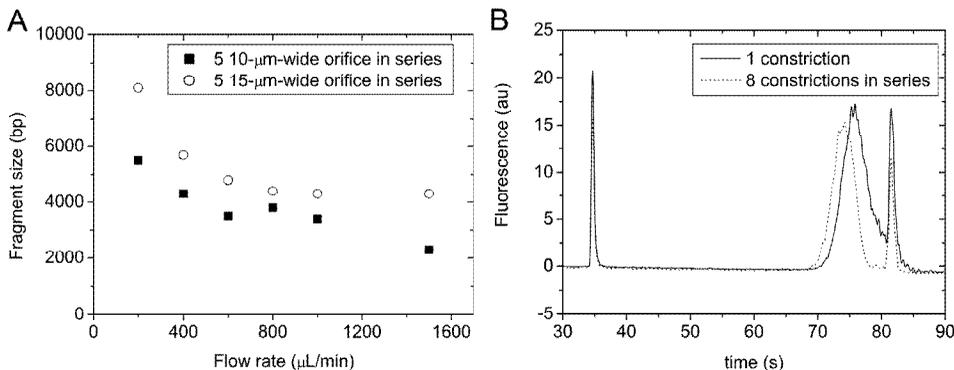


Figure 2. Effect of orifice width, number and flow rate on fragmentation of λ DNA. Fig. (A) shows average fragment size decreased with increasing flow rate and decreasing orifices. Fig. (B) shows microchip gel electrophoresis (Bioanalyzer, Agilent Technologies) data from fragmentation of λ DNA. At the same flow rates, the fragmentation products for eight orifices in series were slightly smaller but also more narrowly distributed. The rightmost and leftmost peaks are internal upper and lower ladder markers of 17000 and 50 bp, respectively. Note that the x-axis is given in time and not bp.

References

1. Oefner, P.J., et al., *Efficient random subcloning of DNA sheared in a recirculating point-sink flow system*, *Nucleic Acids Res.*, 24(20), pp. 3879-86, (1996).
2. Perkins, T.T., D.E. Smith, and S. Chu, *Single polymer dynamics in an elongational flow*, *Science*, 276(5321), pp. 2016-21, (1997).
3. Nguyen, T.Q. and H.H. Kausch, *Mechanochemical degradation in transient elongational flow*, *Advances in Polymer Science*, 100, pp. 73-182, (1992).
4. Lichtenberg, J. et al., *Microfluidic device for nucleic acid fragmentation by shear force*, *Proc. Micro Total Analysis Systems 2002*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 172-175, (2002).