Electronic Supplementary Material (ESI) for Lab on a Chip

Hydrodynamic shearing of DNA in a polymeric microfluidic device

Irina V. Nesterova[b] Mateusz L. Hupert,[a,b] Malgorzata A. Witek,[a,b] and Steven A. Soper*[a,b,c]

*[a] Department of Biomedical Engineering and Department of Chemistry, University of North Carolina, Chapel Hill; [b] Center for Bio-Modular Multi-Scale Systems, Louisiana State University, Baton Rouge, LA 70820, [c] School of Nano-Biotechnology and Chemical Engineering, Ulsan National Institute of Science and Technology, Ulsan, South Korea *E-mail: ssoper@unc.edu*

Electronic Supporting Information (ESI)

Table of Contents

Experimental procedures

Materials. All reagents were obtained from commercial suppliers (e.g. Sigma-Aldrich, Fisher, EM Science etc.) unless otherwise noted. Human DNA was purchased from Promega (Madison, WI) while λDNA was obtained from New England Biolabs (Ipswich, MA). Cyclic Olefin Copolymer (COC, 6013S-04, Tg = 138^{0}C) was obtained from Topas Advanced Polymer, (Florence, KY). Tris/Boric Acid/EDTA buffer (TBE) used for gel electrophoresis and pre-cast/pre-stained 1% agarose gels were purchased from Bio-Rad Laboratories (Hercules, CA). Water utilized for solution preparation was purified in-house using a NanopureInfinity^{TM} water purification system (Thermo Scientific, Waltham, MA). PEEK tubing (0.020''id) used to connect the microfluidic device and fluid delivery/sample collection system was purchased from Upchurch Scientific (Oak Harbor, WA).

Microfluidic device fabrication. The microfluidic device for DNA shearing was fabricated using a sequence of the following steps: 1) preparation of a brass mold insert; 2) hot embossing of the design into COC wafers; 3) device assembly. Detailed description of the entire fabrication process has been published previously by our group.[1] Briefly, the design of the microstructures was prepared using AutoCAD (Autodesk Inc., San Rafael, CA). The microstructures were milled into a brass template (0.25 inch thick alloy 353 engravers brass, McMaster-Carr, Atlanta, GA) using a high precision micromilling machine (KERN MMP 2522, KERN Micro- and Feinwerktechnik GmbH & Co.KG; Germany). Micromilling of the master was performed with 50 to 500 µm diameter solid-carbide milling bits (McMaster-Carr or Quality Tools, Hammond, LA) at 40,000 rpm. Microchannels were then replicated at a temperature of 175^{o}C and a pressure of 8 kg/cm^2 into a 3 mm thick COC substrate using the brass molding tool and a commercial hot-embossing system (HEX 02, Jenoptik Mikrotechnik, Jena, Germany). Individual chips were then cut from the embossed substrate and fluidic access holes were drilled manually at the microchannel termini. After cleaning with Micro 90 solution (Cole-Parmer, Vernon Hills, IL), isopropyl alcohol, and purified water, chips were dried in a convection oven at 60^{0}C for at least 12 h. In the assembly step, microchannels were enclosed with a 500 µm thick COC cover plate by thermal fusion bonding. For this purpose, cleaned and dried COC chips and cover plates were sandwiched between two glass plates and maintained under a pressure of ~1 kg/cm^2 in an oven at a temperature of 130ºC for 23 min. Finally, the capillaries connecting the device to a syringe pump or sample holder were glued to the device using PermaOxy^{TM} epoxy resin (Permatex Inc., Solon, OH).

DNA shearing by the microfluidic device. DNA samples (10 µg/mL in water, 10-5,000 µL) were delivered to the microfluidic device either using a syringe pump (NE-500, New Era Pump System, Wantagh, NY) or by N_2 pressure from a gas tank. The maximum pressure applied was around 1,500 psi. The fluidic system was composed of high pressure tolerant aluminum cassette with a sample holder and connecting capillaries. Typically, when the pressure-driven set up was used, the flow rate through a Type 1 device was ~0.7 mL/min at 1,000-1,200 psi and ~4 mL/min at 200-400 psi through a Type 2 device.

Sample analysis. Samples were analyzed using gel electrophoresis. Sample containing DNA fragments of 20 kbp and above were separated using Pulsed-Field Gel Electrophoresis (PFGE) using an AutoBase system (Q-Life, Ontario, Canada). The samples were separated with a 0.8% agarose gel, visualized with Ethidium Bromide and sized against the appropriate sizing ladder (i.e. *Molecular Weight Marker for DNA 0.1-200 kb*, (Sigma, St.Louis, MO)). The samples containing fragments below 20 kbp were analyzed by constant field electrophoresis with a 1% agarose gel, visualized with Ethidium Bromide and sized against the appropriate sizing ladder (i.e. *1 Kb DNA Extension Ladder*, Invitrogen, Carlsbad, CA). The separation was performed at 11 V/cm in 1× TBE buffer. After separation, the gels were imaged using a Kodak's Logic Gel imaging system (Eastman Kodak, Rochester, NY). Typically, around 200 ng of DNA was loaded onto 1% gels and around 400 ng onto 0.8 % PFGE gels.

Data processing. The gel images were processed using ImageQuant 5.2 software to obtain intensity distributions vs. migration distance. Then, a procedure similar to the one described by Joneja and Huang,[2] was utilized to access fragment size distributions. First, the fragment length vs. migration distance equation was generated from the DNA ladder using Origin 8 considering a logarithmic relationship between migration distance and fragment length.[3] The R^2 for all of the fits were 0.995 or above. The calibration data were utilized to generate a fragment-length number for each data point in an unknown sample. Then, the intensity distributions were normalized with respect to fragment size to yield fragment number distributions from which average fragment sizes (corresponding to maximum of the distribution) was determined. The 90 % fragment size distribution was determined as containing 90% of the fragments with 5% of the shorter fragments and 5% of longer fragments being cut off.

Evaluation of fragmentation format: multiple constrictions connected in series vs. multiple iterations through a single constriction

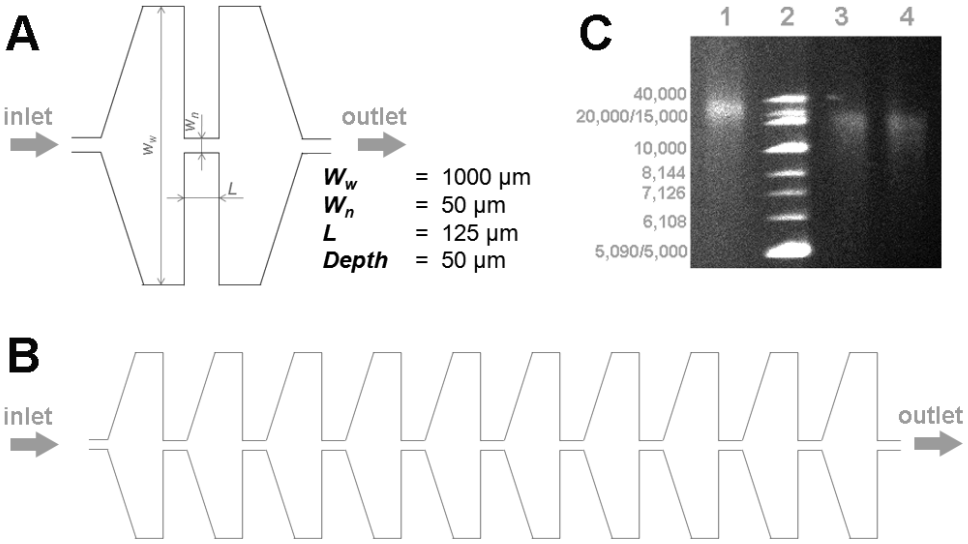

Figure S1. Evaluation of microfluidic device format: multiple iterations through a single constriction (A, identical to dimensions of Type 3 device discussed in the main text) vs. a single iteration via multiple constrictions connected in series (B). (C) Image of a 1% agarose gel (separation conditions: constant field, 11V/cm, 150 min). Lane 1: sample of λ DNA sheared using device depicted in (B); Lane 2: sizing ladder, DNA sizes indicated at left; Lanes 3 and 4: Sample of λDNA sheared using the device depicted in (A) 9 times (lane 3) or 10 times (lane 4). The flow rate was 0.5 mL/min throughout all of these experiments.

The goal of this experiment was to establish the optimal shearing format by comparing multiple iterations through a single constriction to single iterations through multiple constrictions connected in series. The design of the device utilized in these experiments is depicted in Figure S1. Our observations can be summarized into two major conclusions:

1. The device with constrictions connected "in series" caused substantial pressure build up that made Type 1 (10 µm orifice) and Type 2 (30 µm orifice) devices extremely difficult to operate. Similar observations have been reported earlier.[4] The device with constrictions identical to Type 3 (50 µm orifice) was capable to withstand the experimental conditions and the resulting samples were evaluated.
2. The average fragment size for 10 consecutive iterations through a single constriction (Figure 1A) was 13.6 kbp; fragments size for a single iteration through 10 constrictions connected in series (Figure 1B) was 18.1 kbp and generated a much broader distribution compared to 10 iterations through a single constriction.

While the first problem mentioned above can potentially be resolved via changes in the device fabrication approach (e.g. use of solvent assisted bonding may result in much higher pressure tolerances for COC devices),[5] the second issue is much more complicated. We believe that the observed differences in fragment size from the single constriction device versus the device with a series of constrictions stems from two sources:

1. *DNA chain relaxation lag*. The reported relaxation time for DNA molecules in aqueous conditions for elongational flow is 0.04 s,[6] which is longer than the time it took a molecule to cross one constriction (0.0003 s at a flow rate of 0.5 mL/min for the Type 3 device). This means that for the "in series" device, a DNA molecule is partially or completely stretched by the time it reaches the next constriction element. In accordance with earlier reports,[7] elongated molecules tend to break at their midpoints. Our results as well as other reports[8] indicated that molecules not pre-stretched break more than once after a single constriction. As a result, having molecules pre-stretched in all constrictions that may be encountered for the "in series" device would result in only a single breakage thereby producing overall larger fragment sizes.

2. *Altered flow characteristics after first constriction*. The fluid mechanics of elongational flows through small orifices in microfluidic systems (unlike macro systems) has been investigated rather scarcely even though some reports (for pure laminar conditions) has appeared recently.[9-11] We applied the computational approach established in reference 11 to our system to predict pressure drops after the 1st constriction in a Type 1 device at a flow rate of 1 mL/min. The pressure drop was found to be ~80 psi, a substantial drop for a single constriction indicating that the flow conditions beyond hte 1st constriction might be significantly altered. Unfortunately, it is difficult to predict the exact pressure drop through a micro-orifice analytically[10] due to numerous factors such as knowing the exact sharpness of orifice boundaries,[12] surface roughness,[13] exact orifice geometry, and orifice length-to-diameter ratio.[11] However, it has been experimentally demonstrated that even for systems with low Reynolds numbers (Re<100), the pressure downstream of a constriction decreases to a level far lower than the predicted value.[11] Our devices operate at much larger Re's indicating the flow is at a laminar/turbulent transition stage (especially considering a non-tapered entrance to the orifice) causing higher contributions of friction to the shearing. Re in the orifice was calculated to be 850 for the Type 1 (10 μm) device at a working flow rate of 1 mL/min, 1930 for a Type 2 (30 μm) device at a working flow rate of 4 mL/min and 1870 for a Type 3 (50 μm) device at a working flow rate of 5mL/min. Also, substantial flow instabilities are possible for Re exceeding 1000 in microfluidic systems.[13] The instabilities may include formation of cavitation[14, 15] and/or flow detachment.[12] This would indicate that in addition to pressure loss downstream from the 1st constriction, the potential flow instabilities may lead to even more pronounced alterations in the elongational strain fields resulting in decreased efficiency of the shearing process.

However, only accurate theoretical modeling of the flow followed by experimental conformation will provide us with a definite answer. Simulations and experimental conformation of the flow instabilities generated around single or series constrictions at high volume flow rates is a subject for a future research report.

Control experiments: effect of sample delivery/collection system components and high pressure on DNA shearing

The goal of this experiment was to demonstrate that hydrodynamic DNA shearing is a result of the microfluidic device solely and that other parts of the system did not contribute significantly to target DNA size changes. An intact DNA molecule was allowed to passed through the entire system (including the capillaries and connectors) except for the microfluidic device at the maximum flow rate of 5 mL/min, was collected and analyzed using PFGE (Fig. S2a). The results indicated no DNA shearing was produced by the sample delivery/collection system in the absence of the microfluidic device. Also, potential effects of subjecting λDNA to high pressure was evaluated. The DNA in solution was pressurized in the sample chamber at 1,500 psi for 10 min. No effect of pressure on DNA fragment size was detected (Fig. S2b).

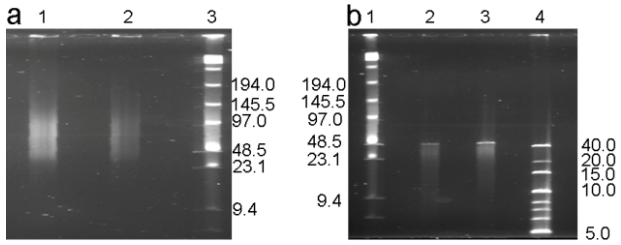

Figure S2. Images of PFGE gels of (a) intact human DNA (lane 1), human DNA filtered through the entire sample delivery/collection system except for the microfluidic device (lane 2); sizing ladder (lane 3, fragment size assignments are on the right). (b) intact λDNA (lane 2); λDNA was subjected to a pressure of 1,500 psi for 10 min in a sample cell with high gas-pressure (lane 3), sizing ladders (lanes 1 and 4, fragment size assignments are on the right (for lane 4) and left (for lane 1)).

References

1. M. L. Hupert, W. J. Guy, S. D. Llopis, H. Shadpour, S. Rani, D. E. Nikitopoulos and S. A. Soper, *Microfluid. Nanofluid.*, 2007, **3**, 1-11.
2. A. Joneja and X. H. Huang, *Biotechniques*, 2009, **46**, 553-556.
3. C. P. Ordahl, T. R. Johnson and A. I. Caplan, *Nucleic Acids Res.*, 1976, **3**, 2985-2999.
4. L. Shui, M. Jin, J. G. Bomer, E. T. Carlen and A. van den Berg, presented in part at the *21st Micromechanics and Micro Systems Europe Workshop*, Enschede, Netherlands, 2010, pp.124-127.
5. D. A. Mair, M. Rolandi, M. Snauko, R. Noroski, F. Svec and J. M. J. Frechet, *Anal. Chem.*, 2007, **79**, 5097-5102.
6. P. J. Shrewsbury, S. J. Muller and D. Liepmann, *Biomed. Microdevices*, 2001, **3**, 225-238.
7. R. D. Bowman and N. Davidson, *Biopolymers*, 1972, **11**, 2601-2624.
8. Y. R. Thorstenson, S. P. Hunicke-Smith, P. J. Oefner and R. W. Davis, *Genome Research*, 1998, **8**, 848-855.
9. T. Hasegawa, M. Suganuma and H. Watanabe, *Phys. Fluids*, 1997, **9**, 1-3.
10. W. Y. Lee, M. Wong and Y. Zohar, *J. Microelectromech. Syst.*, 2002, **11**, 236-244.
11. T. A. Jankowski, E. N. Schmierer, F. C. Prenger and S. P. Ashworth, *J. Fluids Eng.-Trans. ASME*, 2008, **130**, 7.
12. K. Ramamurthi and K. Nandakumar, *Flow Meas. Instrum.*, 1999, **10**, 133-143.

13. F. Kusmanto, E. L. Jacobsen and B. A. Finlayson, *Phys. Fluids*, 2004, **16**, 4129-4134.
14. C. Mishra and Y. Peles, *J. Microelectromech. Syst.*, 2005, **14**, 987-999.
15. C. Mishra and Y. Peles, *Phys. Fluids*, 2005, **17**, #113602.