Dynamics of Single Polymers in a Stagnation Flow Induced by Electrokinetics

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An electrokinetics-induced stagnation flow was created inside a microscale cross-channel. Compared to hydrodynamic-induced microfluidics, this flow system can be readily assembled and the operation is very simple due to a low pressure drop. Through image analysis, a fairly homogeneous, two-dimensional elongational flow was observed. The initial conformation of DNA molecules and residence time inside the flow field play important roles in determining the extent of DNA stretching. A coarse-grain molecular simulation agrees reasonably well with experimental observations.

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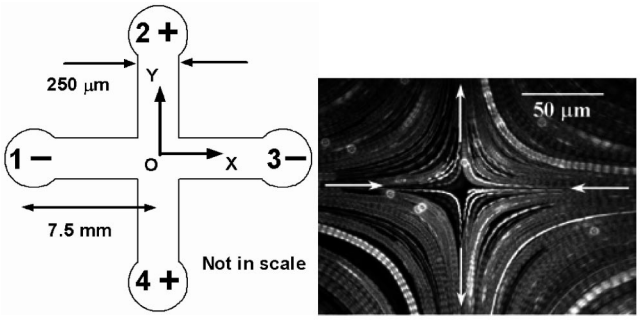
Direct observation and manipulation of single biomolecules and macromolecules can greatly improve our understanding of biomolecular interactions and polymer physics at the molecular scale. In a complex fluid system, the direct visualization of single molecules in the flow field can reveal improved fundamental insights over more traditional ''bulk'' measurements, in which properties are acquired over an ensemble of macromolecules. DNA molecules are the best choice for such studies because of their high molecular weight, monodispersity, and ability to be individually visualized through video-enhanced fluorescence microscopy. Research regarding the coil-stretch transition of single DNA molecules in the elongational flow has been carried out based on either converging geometry [1,2] or cross-slot apparatus [3] using hydrodynamic forces at the microscale. Since the flow is driven by pressure, there is a velocity gradient in the directions perpendicular to the flow (i.e., in both transverse and vertical directions). As a result, measurements need to be taken very close to the centerline in order to minimize the wall effect. Although reducing the depth-to-width ratio of the channel can achieve a broader range of plug flow around the centerline in the transverse direction [2], the velocity gradient still exists in the vertical direction. Recently, a hydrodynamic focusing technique was proposed to generate the elongational flow in a converging geometry [4]. Sandwiched by two flow streams, a stream of DNA solution flows with moving boundaries, resulting in a transverse, pluglike velocity profile. However, there is still a velocity gradient in the vertical direction because the flow is driven by pressure. The fabrication and assembly of flow cells driven by hydrodynamic forces are quite complicated and care needs to be taken for handling, bonding, mounting and connecting tubes because of pressure flow.

In microfluidics, electrokinetic forces (i.e., electrophoresis and electro-osmotic flow) have been widely used for moving fluids or analytes inside the microchannels. The advantages are that the movement of fluids or analytes is uniform with a pluglike velocity profile and the pressure is much lower than in the pressure-driven flow. Furthermore, electrokinetic flows depend less on the channel dimensions. Electric-field-induced DNA stretching has been investigated by several groups [5–11], where the DNA molecules were either tethered on a surface or suspended inside a straight channel filled with ''sieving'' linear polymer molecules to induce DNA stretching. In this work, we utilized electrokinetic forces to generate a fairly homogeneous, two-dimensional (planar) elongational flow pattern inside the microscale cross-channels. Since there is little mechanical pressure, the flow cell can be readily fabricated and the channel dimensions can be easily scaled down. Figure 1(a) shows the schematic of an electrokinetics-induced stagnation flow cell. The substrate used is a polymethyl methacrylate (PMMA) plate micromachined with cross-channels of 250 μm in width and 125 μm in depth. The diameter of the wells is 1.5 mm and the length of each arm is 7.5 mm. A 45 μm-thick PMMA film was laminated onto the surface of the substrate to form closed channels. The PMMA surface possesses a low negative charge density when in contact with aqueous solution. To generate the elongational flow pattern, the microscale cross-channel was first filled with 1x Tris-Borate-EDTA (TBE) buffer solution (pH = 8.3). An electric power of 147 volts was



(a) (b)

FIG. 1. (a) Schematic of the flow cell design. (b) Streamlines of the electrokinetics-induced stagnation flow.

applied to wells 2 and 4 with no voltage in wells 1 and 3. Negatively charged fluorescent polystyrene microspheres (Polysciences, Inc.) with a diameter of 700 nm were used as the tracer at 0.00265% concentration, and the streamlines of the flow pattern are shown in Fig. 1(b) by compounding the videographs. A clear elongational flow pattern with a stagnation point at the center was observed. Under the applied electric field, the overall velocity of particles is the combination of electrophoretic movement of the particles and electro-osmotic flow of the fluid, which can be expressed as follows:

$$\vec{V}_{\text{overall}} = \vec{V}_{EP} + \vec{V}_{EOF}, \quad (1)$$

where V is the velocity and the positive direction is defined from cathode to anode. Note that the direction of particle movement is from wells 1 and 3 to wells 2 and 4. This indicates that the electrophoretic movement of the polystyrene microspheres overcomes the electro-osmotic flow generated by the PMMA surface under the experimental conditions.

To visualize the coil-stretch transition of single DNA molecules in the electrokinetics-induced stagnation flow, λ-DNA (New England Biolabs, 48 K base-pairs) was used and labeled with fluorescent dye (YOYO-1) at a dye-base pair ratio of 1:5, followed by diluting in a pH = 8 buffer solution consisting of 10 mM tris-HCl, 2 mM EDTA and 10 mM NaCl at a final concentration of about $10^{-4}C^*$ (C^* is the concentration at which the macromolecules completely fill the space without overlapping), i.e., $\sim$0.03 μg/ml [1]. An oxygen scavenger, beta-mercaptoethanol, was added in the solution at 4% weight/weight (w/w) to prevent DNA from photobleaching. Incubation was conducted in the dark at room temperature for a minimum of 2 hours. 18% (w/w) glucose and 40% (w/w) sucrose were then added in the solution to increase the bulk viscosity to 30 cp. The DNA solution was delivered into the cross-channels and the flow cell was mounted onto an epifluorescence microscope equipped with a $100x/1.3$ NA oil immersion objective lens.

Figure 2 shows the measured velocity distribution of DNA molecules in the transverse (x) and vertical (z) directions [12]. At $y = 67$ μm (inside the intersection), the velocity of DNA molecules was nearly the same in the transverse direction up to $x = 40$ μm, then gradually increased afterwards. In a pure elongational flow field, the velocity profile should satisfy $(u, v) = (\dot{\varepsilon}x, -\dot{\varepsilon}y)$, and the velocity magnitude V is given as

$$V = \dot{\varepsilon} \times \sqrt{x^2 + y^2}, \quad (2)$$

where x and y are the distance away from the stagnation point and $\dot{\varepsilon}$ is the elongational rate. Velocities of DNA molecules at y positions near the centerline other than 67 and 225 μm were also measured. A straight line was obtained when plotting the DNA velocity vs y position.

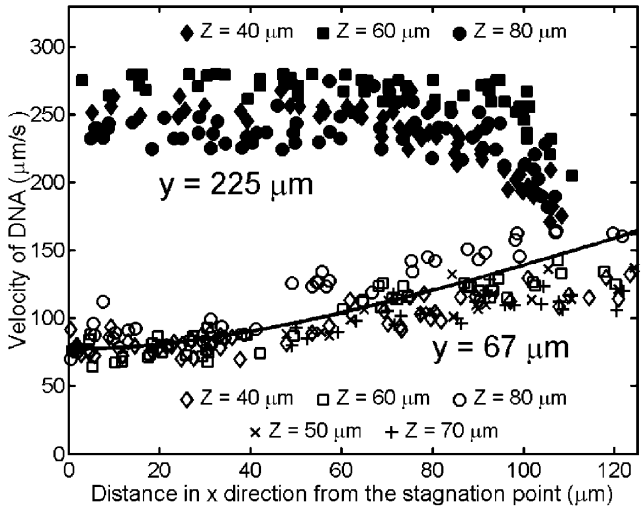


FIG. 2. Velocity distribution of DNA molecules at various locations in the transverse (x) and vertical (z) directions. $Z = 0$ μm is at the bottom of the channel.

From the slope, the elongational rate was found to be 1.26 sec^{-1}. The velocity distribution can then be calculated using Eq. (2), which is represented by the solid line in Fig. 2. It shows strong agreement with experimental measurements up to around $x = 90$ μm, indicating that a nearly pure elongational flow field can be generated by electrokinetic forces. At $y = 225$ μm (outside the intersection), a nearly constant velocity of DNA molecules was observed in the transverse direction up to $x = 100$ μm, then gradually decreased afterwards. Since DNA molecules have left the elongational flow field in the intersection area and are inside the straight channel, a more pluglike velocity profile is expected. However, the observed velocity of the DNA molecules near the channel wall decreased, which might result from the increase of viscous drag due to the hydrodynamic interactions between the DNA molecules and the solid wall [13]. In the vertical direction, the velocities of DNA molecules at selected x and y positions with z locations ranging from 20 to 80 μm from the bottom of the channel are fairly uniform. These results show that a fairly homogeneous, two-dimensional (planar) flow field can be generated by the electrokinetics-induced flows in a microscale cross-channel.

Figure 3 compares the electric lines of the electric field (thin solid lines) and the streamlines of the pressure-driven flow (dashed lines) with streamlines of the pure elongational flow (thick solid lines) for the first quarter of the intersection area in the cross-channels. Since no electric source is inside the microchannel, the Laplace equation is satisfied for the electric potential ϕ and the electric-field vector $\mathbf{E} = -\nabla\phi$ can then be calculated. The streamlines of the pressure-driven flow are calculated using the steady state Stokes equation (Re = 0). Because of the existence of sharp channel corners, both the electric lines and the streamlines of the pressure-driven flow are different from

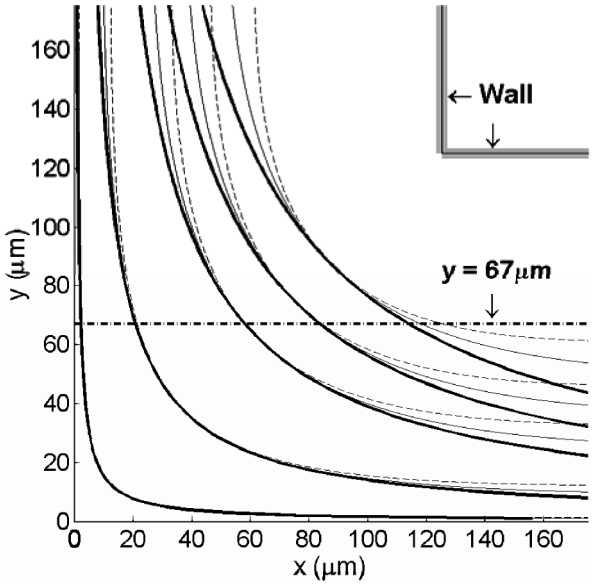


FIG. 3. Comparison of the electric lines, the streamlines of pressure-driven flow, and the streamlines of pure elongational flow.

the streamlines of the pure elongational flow near the channel wall. However, we can still see that the electric lines are closer to the streamlines of the pure elongational flow than the streamlines generated by the hydrodynamic pressure. At $y = 67$ μm, the electric lines agree closely with the streamlines of the pure elongational flow up to around $x = 90$ μm, which supports our results in Fig. 2.

We have used the Brownian dynamics method with the wormlike chain model [14–16] to simulate the coil-stretch transition of DNA molecules. The governing equation for the movement of the ith bead is given as follows [16]:

$$\zeta \dot{\mathbf{r}}_i = \zeta \mathbf{v}_i + \mathbf{F}_i^{\text{Brownian}} + (\mathbf{F}_i^S - \mathbf{F}_{i-1}^S) + q\mathbf{E}_i, \qquad (3)$$

where $\mathbf{r}_i$ is the ith bead's coordinates, $\dot{\mathbf{r}}_i$ is its derivate with respect to time, ζ is the drag coefficient, $\mathbf{v}_i$ is the flow velocity generated by electro-osmosis, $\mathbf{F}_i^{\text{Brownian}}$ is the Brownian force, $\mathbf{F}_i^S - \mathbf{F}_{i-1}^S$ is the total spring force for the ith bead, and $q\mathbf{E}_i$ is the electric force. Following the examples of [14,15], we used a 20-bead chain (20 beads and 19 springs) with persistence length (half of one Kuhn step length) of 0.066 μm to simulate the movement of λ-DNA.

Because the counterions move in a direction opposite to the DNA molecules and screen the hydrodynamic interactions over a distance larger than the Debye length (only several nanometers) [17], we can neglect the hydrodynamic interactions in Eq. (3). The flow cell is fabricated using PMMA, which has a surface with a very low charge density in the buffer solution. Thus the electro-osmotic effect is much weaker than the electrophoretic effect. Since the flow generated by electro-osmosis is similar to the electric field [18,19], we can neglect the electro-osmosis in Eq. (3) by simply combining this effect into the "effective" charge density q of the DNA molecule. In the simulation, $\dot{\mathbf{r}}_C \approx q\mathbf{E}_C/\zeta$, where $\dot{\mathbf{r}}_C$ is the velocity for the center of mass of DNA and $\mathbf{E}_C$ is the electric-field at $\mathbf{r}_C$ (the location for the center of mass of DNA). By fitting the center of mass velocity of DNA with the electric field, we found $q/\zeta = -11\,600$ $\mu\text{m}^2/(\text{V s})$, the value used in the Brownian dynamics simulation.

It has been found that both the initial conformation and the residence time of DNA molecules inside the pressure-

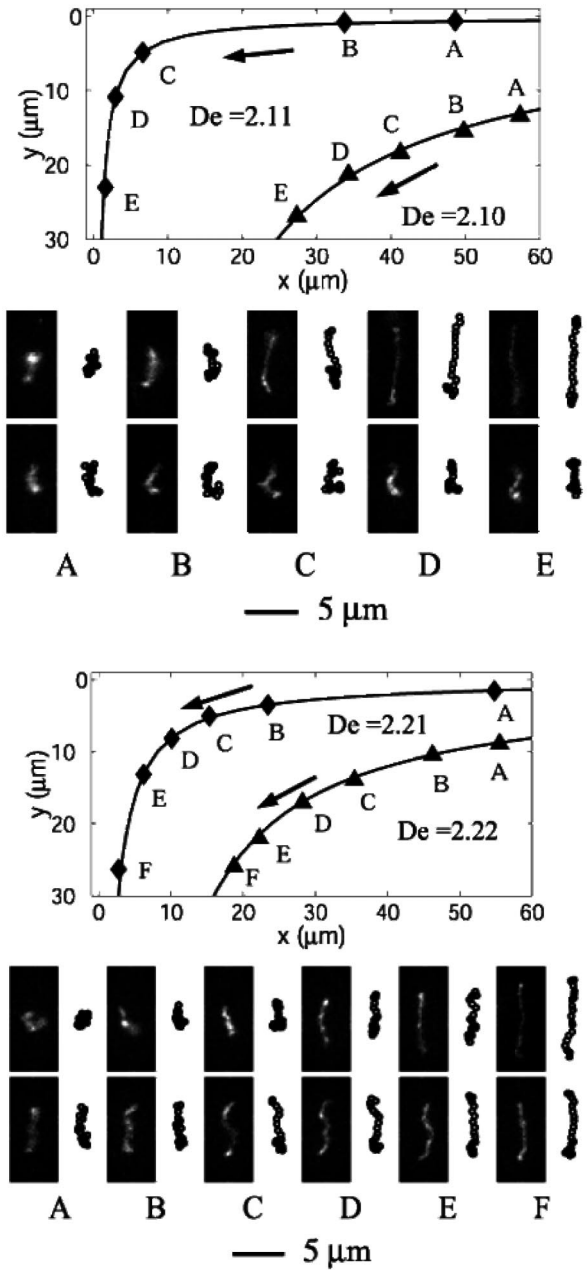


FIG. 4. Experiment and simulation of the movements of two DNAs in the intersection region (a) with similar initial conformation but different residence time and (b) with different initial conformation and residence time.

driven elongational flow field play important roles in DNA stretching [3]. This is also true in the electrokinetics-induced stagnation flow. In our DNA stretching experiments, the ''electrokinetics-induced'' Deborah number (De) [20] was calculated to be $2.1 \sim 2.2$, which is larger than the critical value of 0.5 for molecule stretching. With a similar initial conformation (a semi-dumbbell shape in this case), a DNA molecule with a longer residence time can be stretched more than the one with a shorter residence time, as shown in Fig. 4(a). On the other hand, two DNA molecules with different initial conformations (a curled and a partially stretched shape in this case) may end with a similar extent of stretching as shown in Fig. 4(b), even though the residence times are substantially different. Our coarse-grain molecular simulation by using corresponding initial DNA shapes agrees qualitatively with the experimental observations.

In conclusion, we have demonstrated that an elongational flow field can be generated by using electrokinetic forces in a microscale cross-channel. The velocity distribution of DNA molecules shows that the flow field is fairly homogeneous and two-dimensional (planar). The initial conformation and the residence time of the DNA molecules inside the electrokinetics-induced flow field play important roles in the extent of DNA stretching. The same observation has been reported by others using hydrodynamic force [14,21,22]. The use of coarse-grain molecular modeling is able to qualitatively simulate the conformational changes of DNA molecules observed in the experiments.

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