Stretching DNA with electric fields beneath submicron interfacial constriction created by a closely fitting microdroplet in a microchannel

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In this article, we report an alternative strategy for stretching single DNA molecules with electric fields. The approach invokes a closely fitting microdroplet in a microchannel, creating a natural converging geometry for stretching DNA within a submicron interfacial constriction. We demonstrate that DNA molecules can undergo prestretching within the constriction, pseudotethering entropic trap, and rapid extension within the thin film underneath the droplet. An elastic dumbbell model is derived to account for the observed stretch behavior, with predictions in good agreement with the experimental results. © *2008 American Institute of Physics*. [DOI: 10.1063/1.2969047]

Stretching DNA is of long-standing interest in biotechnology and engineering science because it not only can assist in extracting genetic information[1] but also offers a microscopic view for understanding the rheological behavior of polymers under the influence of external force fields.[2] To achieve a significant extension of a DNA molecule from its natural coil state, it is necessary to apply a sufficiently large stretching force over it to overcome the elastic recoil of the chain. Along this line, successful stretching of DNA can be realized by many means. Aside from standard stretching techniques,[3–5] the recent advance in nano-/microtechnology further permits judicious control of conformation changes in DNA within nano- or submicron structures.[6] The up-to-date development for linearization of DNA in nanofluidic channels can be found in a recent review.[7] In this article, we present an alternative strategy of stretching single DNA molecules with electric fields. As is sketched in Fig. 1, our approach invokes the formation of a submicron confinement using a closely fitting oil slug (of 550 μm in length) in a Polydimethylsiloxane (PDMS) microchannel (of 120 μm in height and 200 μm in width), creating a natural converging geometry that enables us to stretch DNAs underneath the slug with an amplified electric field. The experimental setup and some other details can be seen in the supplemental information.[8]

Figure 2 displays the sequential images for the motion of *T*4 DNA molecules (165 kilo-base-pairs with contour length of 56.4 μm), due mostly to electrophoresis, beneath the front cap of the slug. As shown in these images, when a DNA arrives at the cap, it is temporally blocked by the constriction due to entropic trap but allowed to follow the cap and migrate laterally toward the wider side gap regions [Fig. 2(a)]. At the same time, the DNA is also gradually unraveled and extended by a local elongational field around the tip of the cap [Fig. 2(b)]. Upon overcoming the entropic barrier by unraveling itself, the DNA starts to escape the trap and translocates into the underneath film [Fig. 2(c)]. During this translocation process, the front part of such a nearly escaping DNA feels an intensified electric field within the film and is quickly ejected from the trap, while its tail still remains restrained and moves slowly within the constriction. As the trapping effect on the tail now renders pseudotethering and aids in the direct pulling of the front,[5] the combined effects thus lead to rapid unraveling and extension of a DNA molecule [Fig. 2(d)]. Figure 3 provides a closer look at the dynamics of single DNA molecules in this stretching process, clearly indicating substantial DNA stretch by electric fields. Such prestretching, pseudotethering, and stretching phenomena seem in part similar to those in Ref. 5, in which stretching DNA was realized in a converging channel having a gel matrix placed ahead of the channel. Yet, the difference here is that we do not invoke any gel-like medium to create these effects. Rather, they are realized using the unique confinement geometry created by a closely fitting slug.

Figure 4 shows the measured extensional fraction and prestretch ratio of DNAs as a function of the electric field E_f within the film. The total extension increases with the field until $E_f \sim 80$ V/cm, after which it falls slightly.[8] As for the prestretch ratio, it grows with field at a much smaller rate and quickly saturates at about 20% extension. This is expected because the prestretching is made by the local elongational field with a much weaker intensity than the field in the film. Nevertheless, the prestretching in effect lowers the free energy of a DNA, making it more extendible at the primary stretching stage when entering the film, which explains why the measured total extension continues to grow with field even though the prestretch length remains nearly unchanged. To quantify the measured results shown in Fig. 4, we employ an elastic dumbbell model to describe the dynamics of a

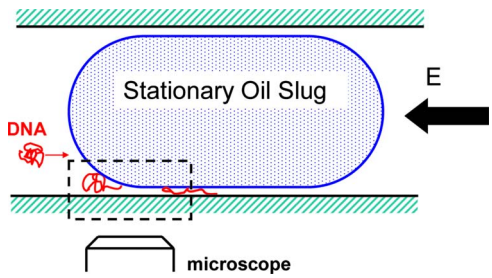

FIG. 1. (Color online) Stretching DNAs with an electric field within a submicron constriction (highlighted area) created by a closely fitting slug. The motion of DNAs is observed by a microscope positioned below the constriction.

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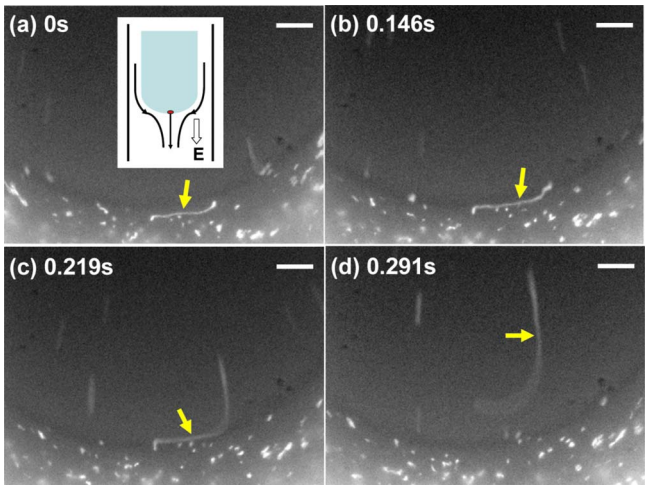

FIG. 2. (Color online) Sequential images for the DNA motion beneath the front cap of the slug. As illustrated in the inset in (a), these images are taken from the bottom view of the highlighted area in Fig. 1. The electric field is E_f=81.8 V/cm in the film. Scale bar is 10 μm.

DNA chain as this model is simple and capable of capturing the essential physics of the dynamics.[9] In this model, a DNA molecule is modeled as two beads connected by a linear Hookean chain of spring constant k. Let x_1 and x_2 be the respective positions of the front and rear beads of the chain in a nonuniform electric field E. With the aid of a simple force balance between chain tension, electric, and frictional forces for each bead, the chain dynamics can be simplified to the motion of the two beads, which is governed by the following coupled set of equations:[9]

$$dx_1/dt = -(x_1 - x_2)/\tau + \mu E(x_1) \quad \text{and}$$

$$dx_2/dt = (x_1 - x_2)/\tau + \mu E(x_2), \tag{1}$$

where t is time and $\tau=\zeta/k$ is the chain relaxation time of ~0.6 s for $T4$ DNA with electrophoretic mobility μ and friction coefficient ζ of the chain.[10] As the stretching process involves two stages: the pseudotethering beneath the cap and the primary pulling action at the entrance to the film, we use Eq. (1) to determine the extension (x_1-x_2) of a DNA for each stage.

During the prestretching stage, a DNA is trapped within the constriction and extended by the elongational field $E=G\,z$ around the tip, where z is the arc distance measured from the tip, as defined in the inset in Fig. 4. Here G measures the strength of the field gradient and can be estimated as E_f/a with $a\sim 60$ μm being the radius of the cap. Replacing x by z in Eq. (1) and letting $Z\equiv z_1-z_2$, we solve for Z with the combined equation of Eq. (1) and find the prestretch length $\ell\equiv Z$ $(t=\tau_{\text{trap}})$ during the trapping $t\leq\tau_{\text{trap}}$:

$$\ell = Z_0 \exp[(\mathrm{De}-2)\tau_{\text{trap}}/\tau], \tag{2}$$

where $\mathrm{De}=\mu G\tau$ is the Deborah number and Z_0 is the initial length of the chain (of ~10 μm estimated from the images) prior to the trapping. Here the trapping time $\tau_{\text{trap}} \approx 0.023 \exp(117.1/E_f)$ (with E_f in V/cm) is obtained from the best fitted Arrhenius equation by plotting the measured τ_{trap} against $1/E_f$. The measured mobility μ is 2.6×10^{-4} cm^2/s V. In addition, the distance that the DNA travels laterally during the trapping can also be found by solving

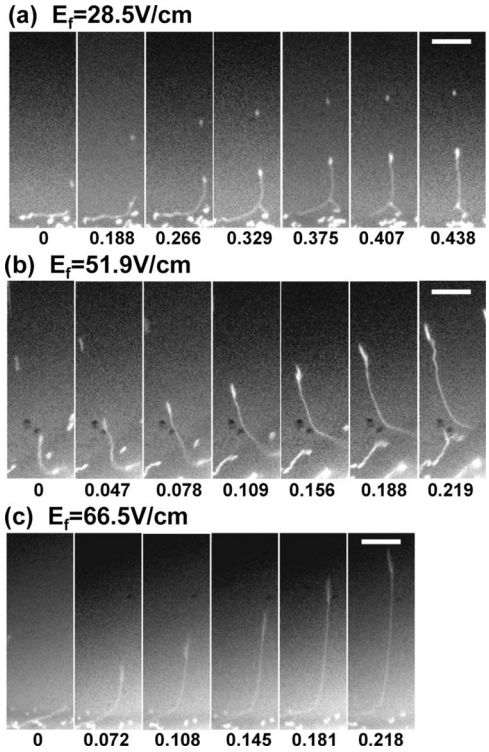

FIG. 3. Snapshots for stretching DNA with different electric fields. The number below each panel is the time frame (in seconds). Scale bar is 10 μm.

the front end position z_1 and evaluating it at $t=\tau_{\text{trap}}$, yielding $\ell_{\text{trap}}=[Z_0\exp(\mathrm{De}\ \tau_{\text{trap}}/\tau)+\ell]/2$.

As for the pulling stage $(t>\tau_{\text{trap}})$, the bead positions in Eq. (1) are now redefined as y with respect to the place $(y=0)$ where the DNA starts to translocate into the film (see the inset in Fig. 4). As the front end (at $y=y_1>0$) of the DNA is pulled by the nearly uniform field E_f in the film while the rear (at $y=y_2<0$) advances with the velocity $\mu G z_2$ due to the elongational field at distance $z_2=\ell_{\text{trap}}+y_2$ away from the tip, the bead equations now read

$$dy_1/dt = -Y/\tau + \mu E, \quad dy_2/dt = Y/\tau + \mu G z_2, \tag{3}$$

where $Y\equiv y_1-y_2$ measures the total extension starting from the prestretch length $Y(t=\tau_{\text{trap}})=\ell$. Since we expect that most

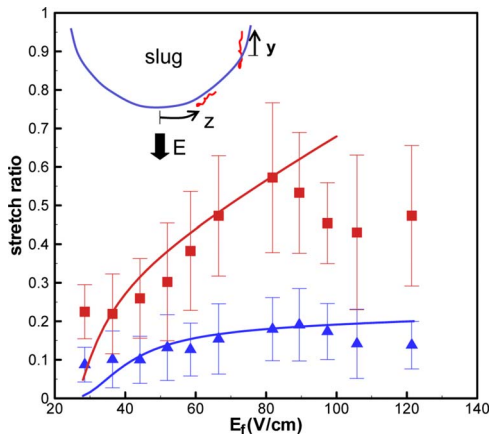

FIG. 4. (Color online) Plot of measured total extensional fractions (squares) and prestretch ratios (triangles) against the film electric field. Solid curves are the results predicted by our dumbbell model. The inset shows the coordinates defined in the model.

of the contributions to Y come from y_1, we solve Y by approximating $z_2 \approx \ell_{\text{trap}}$ in Eq. (3):

$$Y = \ell \exp(-2t_1/\tau) + Y_\infty[1 - \exp(-2t_1/\tau)], \quad (4)$$

where $t_1 = t - \tau_{\text{trap}}$ and $Y_\infty = \mu(E_f - G\ell_{\text{trap}})\tau/2$ is the maximum extension due to the velocity mismatch between the two ends of the chain. As indicated by Eq. (4), Y consists of two contributions: prestretching along the cap (the ℓ term) and primary stretching in the film (the Y_∞ term). Now, as the chain will be kept extending until its tail disengages the trap, the extent of the total stretch is determined at $t_1 = t_{\text{escape}}$, the moment when the rear end of the chain exits the constriction, i.e., $y_2(t_1 = t_{\text{escape}}) = 0$. Substituting Eq. (4) into Eq. (3), we solve y_2 as

$$y_2 = 0.5(\ell - Y_\infty)[1 - \exp(-2t_1/\tau)] + \mu E_{\text{av}} t_1 - \ell. \quad (5)$$

Here $E_{\text{av}} = (G\ell_{\text{trap}} + E_f)/2$ is the average of the elongational field and the film field and taken to be the effective field across the constriction. The escape time t_{escape} then can be determined by setting $y_2 = 0$ at $t_1 = t_{\text{escape}}$ in Eq. (5). For a significant stretch observed in the experiment, t_{escape} cannot be too short compared to the chain relaxation time τ so that the DNA has time to be extended by the electric field. To first approximation by neglecting the exponential term in Eq. (5), we find $t_{\text{escape}} \approx (Y_\infty + \ell)/(2\mu E_{\text{av}})$, which is simply the average of $Y_\infty/\mu E_{\text{av}}$, the time required to pull the chain to the maximum extension, and $\ell/\mu E_{\text{av}}$, the time for a prestretched DNA to cross the constriction without changing its length. The total extension is therefore evaluated by $L \equiv Y$ $(t_1 = t_{\text{escape}})$ according to Eq. (4). Plotting both L and ℓ against E_f, we find that the theoretical curves indeed capture the respective trends of the data, except for the overestimate of the total extension at high fields, which can be explained as follows. At high fields, a fast moving DNA could have a shorter escape time than expected and hence have less time to be extended by electric fields. Besides, the observed chain might have been detached from the trap and undergoing a rapid recoil with $Y \propto \exp(-2t_1/\tau)$.

In conclusion, we demonstrate stretching DNAs with electric fields within a submicron confinement created by a closely fitting slug in a regular 100 μm sized microchannel. This approach has potentials in manipulation of confined DNAs at microscales without needing fabrication of sophisticated channel geometries or fine structures. We identify that the stretching in essence combines three distinct processes: prestretching within the constriction, pseudotethering by entropic trap, and rapid chain extension by an intensified electric field within the film. The measured stretch ratios are in good agreement with those predicted by our simple dumbbell model.

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