Elasto-inertial particle focusing under the viscoelastic flow of DNA solution in a square channel

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Particle focusing is an essential step in a wide range of applications such as cell counting and sorting. Recently, viscoelastic particle focusing, which exploits the spatially non-uniform viscoelastic properties of a polymer solution under Poiseuille flow, has attracted much attention because the particles are focused along the channel centerline without any external force. Lateral particle migration in polymer solutions in square channels has been studied due to its practical importance in lab-on-a-chip applications. However, there are still many questions about how the rheological properties of the medium alter the equilibrium particle positions and about the flow rate ranges for particle focusing. In this study, we investigated lateral particle migration in a viscoelastic flow of DNA solution in a square microchannel. The elastic property is relevant due to the long relaxation time of a DNA molecule, even when the DNA concentration is extremely low. Further, the shear viscosity of the solution is essentially constant irrespective of shear rate. Our current results demonstrate that the particles migrate toward the channel centerline and the four corners of a square channel in the dilute DNA solution when the inertia is negligible (elasticity-dominant flow). As the flow rate increases, the multiple equilibrium particle positions are reduced to a single file along the channel centerline, due to the elasto-inertial particle focusing mechanism. The current results support that elasto-inertial particle focusing mechanism is a universal phenomenon in a viscoelastic fluid with constant shear viscosity (Boger fluid). Also, the effective flow rate ranges for three-dimensional particle focusing in the DNA solution were significantly higher and wider than those for the previous synthetic polymer solution case, which facilitates high throughput analysis of particulate systems. In addition, we demonstrated that the DNA solution can be applied to focus a wide range of particle sizes in a single channel and also align red blood cells without any significant deformation. © 2016 AIP Publishing LLC.

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I. INTRODUCTION

Focusing particles within spatially narrow regions is a prerequisite to particle counting and separation in many applications.¹ Recently, microfluidic particle focusing has been extensively studied since it demands only a small sample volume, and it can be potentially integrated with other unit operations such as separation processes.^{2,3} Particle focusing can be categorized into active and passive methods.¹ Active methods require external force generators, and the device is usually composed of non-monolithic layers of different materials.¹ On the other hand, passive methods, not relying on any external forces such as electric force, utilize either the hydrodynamic interaction between the particles and an obstacle array⁴ or the lateral particle migration phenomena in nonlinear flows such as inertial and/or viscoelastic flow.⁵ The passive methods

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can be implemented with very simple channel structures compared with those required for active methods.

Viscoelastic particle focusing has attracted much attention as a promising passive method because this method can be applied to a wide range of materials including both micro- and submicron-sized particles, DNA molecules, and cells.^{6–13} The particle focusing by the viscoelasticity of suspending medium has been successfully utilized to separate particles by size or shape.^{8,14,15} It has also been demonstrated that the viscoelastic particle focusing can be utilized to measure cell deformability and damage, and the relaxation time of polymer solution can be determined by measuring the viscoelastic particle migration.^{16–18} Further, the flow rate for the particle focusing can be highly tunable, which potentially enables both phone-based applications (working at low flow rates due to the low frame rates of phone cameras) and high throughput analytical tools.¹¹ Viscoelastic particle focusing in polymer solutions arises from the imbalanced normal stress differences under pressure-driven channel flow.¹⁹

It has been observed that viscoelastic particle migration is significantly affected by the rheological properties of the suspending medium.²⁰ For instance, the particles migrate toward the channel centerline or mid-plane according to the channel geometry (tube or slit) when the shear viscosity of a medium is nearly constant.^{20,21} However, it was observed that some particles migrate toward the wall of a cylindrical tube when the viscosity of the medium is shear-thinning.²⁰ On the other hand, it is essential to understand the particle dynamics in a rectangular channel, which is common for most lab-on-a-chip applications. Yang *et al.* observed that particles migrate toward the channel centerline and the four corners of a square channel in viscoelastic fluids with constant shear viscosity when inertia is negligible and that the multiple equilibrium particle positions change to a single file along the channel centerline when the elastic and inertial forces are balanced (elasto-inertial particle focusing).²² Later, Del Giudice *et al.* reported that the particles migrate toward the channel centerline in a square channel when the suspending medium is polyvinylpyrrolidone (PVP) solution with constant shear viscosity and when inertia is negligible.²³ They investigated the particle dynamics in a square channel at generally lower flow rates than the previous work²² with a slight overlap at the highest flow rates tested. In fact, the multiplicity of the equilibrium positions in the PVP solution has been observed in square channels by other research groups.^{9,24} Therefore, there remains a question about whether the multiplicity of the equilibrium positions occurs for specific polymer solutions and flow rate ranges.

In this study, we investigated elasto-inertial particle focusing under the viscoelastic flow of a double-stranded (ds) λ -DNA solution in a square microchannel. It was previously demonstrated that DNA can be used as an efficient elasticity enhancer.¹¹ The elastic property of the DNA solution is significant even when a very small amount of DNA is added to the medium.¹¹ The shear viscosity of the very dilute DNA solution does not significantly deviate from the viscosity of the medium, i.e., the shear viscosity is nearly constant irrespective of shear rate.¹¹ Therefore, the dilute DNA solution is ideally suited to investigate particle dynamics in a viscoelastic fluid with constant shear viscosity (Boger fluid²⁵).

Simultaneously, the dilute DNA solution has advantages over other known viscoelastic media such as PVP and poly (ethylene oxide) (PEO) solutions.¹¹ For instance, the particle focusing is achieved at high flow rates despite its low viscosity, i.e., particle focusing is less sensitive to inertial force when compared with synthetic polymer solutions.¹¹ The current work demonstrates that DNA can be utilized to focus particles of various sizes, which is promising for the analysis of particulate samples involving heterogeneous sizes. We also show that red blood cells (RBCs) can be efficiently aligned without significant cell deformation in the DNA solution, which could be beneficial in analyzing morphology changes in single red blood cells.

II. THEORETICAL BACKGROUND

The spatial segregation of particles in channel flow has been a longstanding issue because of its importance in both theory and practical flow systems such as blood flow.²⁶ It is predicted that no lateral particle migration occurs, due to time reversibility conditions, when the

suspending medium is Newtonian and inertia is negligible, i.e., the Reynolds number is much less than unity (Stokes flow).²⁷ The Reynolds number denotes the relative ratio of inertial to viscous forces. On the other hand, the particles laterally migrate if the medium is a non-Newtonian viscoelastic fluid such as a polymer solution.²⁸ Ho and Leal predicted that a particle laterally migrates in second-order fluid by an imbalanced first or second normal stress difference.¹⁹ Lateral particle migration in viscoelastic fluids has also been predicted with computational simulations based on nonlinear constitutive modeling such as Oldroyd-B and Giesekus models^{29–34} or molecular dynamics.³⁵

The equilibrium particle positions in a viscoelastic channel flow are determined by various factors such as the rheological properties of the suspending medium, and the competition between inertial and elastic forces if the Reynolds number is non-negligible.^{20,22,24,36} It was previously reported that some particles migrate toward the channel walls in shear-thinning fluids, as opposed to the single-line focusing along the channel centerline in viscoelastic fluids with constant shear viscosity.²⁰ Analysis of lateral particle migration in shear-thinning fluids is further complicated, since secondary flow can be generated in non-circular conduits.³² Therefore, the shear-thinning viscoelastic fluid has been considered to be not suitable for practical applications such as particle focusing.³⁷

In a viscoelastic fluid with constant shear viscosity, it has been shown that particles migrate toward low first normal stress difference regions which correspond to the channel centerline and the four corners in a square channel.²² Meanwhile, Del Giudice *et al.* reported that particles migrate solely toward the channel centerline in a square channel even when inertia is negligible and the shear viscosity is constant.²³ They suggested that the multiple equilibrium particle positions in the previous work²² might possibly originate from the difference in the hydrophilicity between the top and bottom layers of a microchannel. However, this hypothesis needs to be precluded since the channel materials in the previous work were all polydimethylsiloxane (PDMS).²² They also proposed that the shear-thinning behavior occurring at high shear rates, which is hard to measure with conventional rotational rheometers, can be a possible reason for the multiple equilibrium particle positions.²³ Therefore, it is necessary to investigate whether the shear-thinning in viscosity is a valid premise for the quintuple equilibrium particle positions.

In this work, we investigated lateral particle migration in a square channel where the suspending media are λ -DNA solutions. It was demonstrated that the elasticity is relevant, even when a very tiny amount of λ -DNA (concentration (c) = 5 ppm (0.0005 (w/v)%); overlapping concentration (c^*) of λ -DNA $\approx$ 84 ppm) is added to a solvent.¹¹ The shear viscosity of the very dilute DNA solution is almost identical to the solvent viscosity, and consequently, the shear viscosity of the DNA solution is essentially constant irrespective of shear rate.¹¹ Therefore, we can consider the dilute DNA solution as a viscoelastic fluid with constant shear viscosity. On the other hand, the quintuple positions (the channel centerline and the four corners) are reduced to a single-line alignment along the channel centerline when the elastic and inertial forces are synergistically balanced, termed “elasto-inertial particle focusing.”²² Here, we briefly introduce its principles (the reader can refer to the details presented in our previous work²²).

The viscoelastic flow of a polymer solution can be characterized with the three dimensionless parameters: Weissenberg (Wi), Reynolds (Re), and elasticity (El) numbers.³⁸ The Weissenberg number denotes the relative ratio of elastic to viscous properties.³⁹ These dimensionless parameters are defined in a square channel by $Wi = \frac{2\lambda Q}{h^3}$ and $Re = \frac{\rho Q}{\mu h}$, respectively. λ denotes the relaxation time of a polymer solution, Q is flow rate, h is channel height, and ρ and μ correspond to fluid density and viscosity, respectively.³⁸ The elasticity number is defined by $El \equiv \frac{Wi}{Re}$, which denotes the relative importance of elastic to inertial forces.³⁸ The lateral particle migration velocity (V), which originates from the elastic force, can be semi-empirically predicted with¹¹

$$\frac{V}{\langle U \rangle} \sim \frac{c}{c^*} Wi \left(\frac{a}{h} \right)^2 \hat{\nabla} \dot{\gamma}^2, \quad (1)$$

where $\langle U \rangle$ is the average streamwise velocity, a is the particle radius, and $\hat{\gamma}$ is the non-dimensionalized strain rate. The length scale and velocity are non-dimensionalized with half the channel height ($h/2$) and $\langle U \rangle$, respectively. Equation (1) can be derived by balancing the elastic force ($F_{elastic}$) with the drag force ($F_{drag} = 6\pi\mu aV$) exerted on a sphere.^{21,40} The elastic force ($F_{elastic}$) is semi-empirically represented by $F_{elastic} \sim a^3 \nabla N_1$,^{21,40} and the viscoelastic fluid with constant shear viscosity can be modeled with the Oldroyd-B model.³⁹ When the elasticity is dominant over the inertial force, Eq. (1) predicts that a particle migrates toward low shear rate regions, which correspond to the channel centerline and the four corners in a square channel. When the inertial force ($F_{inertial}$) is relevant, it is possible that the inertial lift force pushes particles away from the corners while not significantly affecting the particles along the channel centerline.²² Consequently, for a specific range of flow rates, the particles are focused along the channel centerline, termed elasto-inertial particle focusing.²² The principle of elasto-inertial particle focusing is schematically presented in Fig. 1(a).

III. EXPERIMENTAL

A. Microchannel geometry and fabrication

The schematic diagram for the channel is presented in Fig. 1(a). The cross-sectional shape is a square with width $\times$ height = $50\ \mu\text{m} \times 50\ \mu\text{m}$, and the channel length is 5 cm.²² The four-walled PDMS microchannel, composed of a PDMS replica from a SU-8 pattern and a flat slab of PDMS, was fabricated following the conventional soft lithography technique.⁴¹ The prepolymer base and curing agent of PDMS (10:1) (Sylgard 184, Dow) were thoroughly mixed and then degassed in a vacuum chamber (≈ 40 min). The mixture was poured onto the silicon master mold (Microfit, Korea) including the microchannel geometry embedded with a SU-8 photoresist (Microchem), and onto a bare Si wafer to fabricate a PDMS slab. Both the PDMS channel and slab were cured at 150°C for 20 min and then peeled off the silicon wafers. Holes for both inlet and outlet were made with a punch (World Precision Instruments, 0.75 mm diameter). The surfaces of both the PDMS channel and slab were exposed to air plasma (PDC-32G, Harrick) at 250 mTorr for 2 min and then immediately contacted together. The four-walled PDMS microchannel was baked at 180°C for 2 h to enhance the bonding strength. The syringe and the microchannel were connected through a Tygon tube.

FIG. 1. (a) Schematic illustration for particle focusing under the viscoelastic flow of DNA solution in a square channel. The randomly distributed particles at the inlet are gradually focused along the channel centerline as the particles move downstream. The elastic force ($F_{elastic}$) drives particles toward the channel centerline and the four corners, whereas the inertial force ($F_{inertial}$) pushes the particles off the walls. The particles form a single file along the channel centerline when the elastic and inertial forces are synergistically balanced (elasto-inertial particle focusing).²² (b) Particle focusing in a 5 ppm λ -DNA solution in a 13.8 wt.% sucrose $1\times$ TE buffer solution at various flow rates (particle diameter = $10\ \mu\text{m}$). The images were captured 4 cm downstream from the inlet, and the 1000 images were stacked with the “min intensity” option in ImageJ software.

B. Materials and image acquisition

In this work, a Newtonian medium was prepared by dissolving 13.8 wt. % sucrose solution (Sigma-Aldrich) in $1\times$ Tris-EDTA (TE) buffer solution (10 mM Tris-HCl and 1 mM EDTA, Sigma-Aldrich). The sucrose was added to match the medium's density with that of the polystyrene (PS) beads (1.05 g/cm^3) to preclude particle sedimentation. For the particle migration experiments, the λ -DNA solutions with different concentrations (2.5, 5, 10, and 50 ppm) were used as viscoelastic media and were prepared by dissolving λ -DNA (New England Biolabs) in the Newtonian medium. Additionally, a 5 ppm λ -DNA solution in a phosphate-buffered saline solution without sucrose ($1\times$ PBS, Sigma-Aldrich) was prepared for the focusing experiment with the fresh rat RBCs (purchased from Orient Bio, Seongnam City, Korea), and the whole rat blood was provided as stored in K_2EDTA -treated tubes (BD Vacutainer[®]). The viscosities of all the solutions were measured at 20°C with a rotational rheometer (AR-G2, TA Instruments) equipped with a cone and plate geometry (diameter = 60 mm; angle = 1°). The PS beads with three different diameters (6, 10, 15 μm ; Polysciences) were used for the particle migration experiments. The particle concentration for each size was 0.1 vol. % in this work if there is no special note. A tiny amount of surfactant (0.01 vol. %, TWEEN 20; Sigma-Aldrich) was added to minimize particle-particle adhesion for the particle migration experiments. The particles in the microchannel were observed by two optical microscopes (BX-60, Olympus; IX-71, Olympus). The images were acquired with a high speed camera (MC2, Photron) for the PS bead migration experiments (15–60 frames per second (fps)) or a high-resolution CCD (charge-coupled device, DMK-23U445, ImagingSource) for the RBC experiments (30 fps). The flow rates were controlled with a syringe pump (11-plus, Harvard Apparatus). All the images were processed with ImageJ software (NIH), and the PS bead location was determined following the previous work.²² The RBC location was determined by manually clicking the center of each cell with ImageJ Software. The contrast of the snapshots in this work was uniformly enhanced.

IV. RESULTS AND DISCUSSION

In this research, the particle-suspended viscoelastic fluid was pumped through the straight square microchannel which is presented in Fig. 1(a). In the current experiments, the suspended particles were PS beads with a 10 μm diameter. The particle distribution was observed 1, 2, 3, and 4 cm downstream from the inlet under optical microscopes. The particles were randomly distributed at the inlet. As the particles move downstream from the inlet, the particles are gradually focused along the channel centerline, when the elastic and inertial forces are synergistically balanced²² (see Figs. 1(a) and 1(b); also refer to Section II). This elasto-inertial particle focusing was originally observed in a PEO aqueous solution in the same square microchannel as the current work.²² The three-dimensional particle focusing in the PEO solution was observed at a small range of flow rates, all near $200\text{ }\mu\text{l/h}$.²² In this study, we investigated elasto-inertial particle focusing in DNA solutions. The three-dimensional particle focusing in a cylindrical tube flow of DNA solution has been extensively investigated,¹¹ but there has not been a systematic study on particle focusing in a square channel.

The velocity profile in the square channel is not axisymmetric, which is quite different from the cylindrical tube case.²² The shear rate in a square has minimum values (=zero) at the channel centerline and at the four corners. Consequently, the elastic force, which is generated in a polymer solution, drives particles toward these low first normal stress areas ($N_1 \sim \dot{\gamma}^2$ when the viscoelastic flow is modeled with the Oldroyd-B model).²² When the elastic force is dominant (flow rate $\leq 200\text{ }\mu\text{l/h}$ ($Re = 0.71$; $Wi = 99.4$)), the particles migrate toward the channel centerline and four corners as shown in Fig. 1(b). The medium was a 5 ppm λ -DNA solution in a TE buffer solution with 13.8 wt. % sucrose. The relaxation time of the dilute DNA solution was estimated to be 0.11 s following the procedure in Kang *et al.*¹¹ The zero-shear viscosity is 1.65 cP and the power-law index is 0.99 when the shear viscosity is fitted with the Carreau model,³⁹ and this DNA solution has nearly constant viscosity (we note that the Reynolds and Weissenberg numbers were calculated based on the zero-shear viscosity). Therefore, our current

FIG. 2. Viscosities of the λ -DNA solutions at different concentrations. Medium was a $1\times$ TE buffer solution with 13.8 wt. % sucrose. The viscosity was measured at 20°C with a rotational rheometer (cone and plate geometry; diameter = 60 mm; angle = 1° ; AR G2, TA Instruments). Dotted lines denote the curves fitted with the Carreau model.

results clearly demonstrate that the quintuple equilibrium particle positions of the channel centerline and four corners (when elasticity is dominant at low flow rates) do not result from the shear-thinning viscosity but from the elastic forces in the viscoelastic fluid. On the other hand, Del Giudice *et al.* reported that the particles migrate toward the channel centerline when the suspending medium is a viscoelastic fluid with constant shear viscosity.²³ They fabricated the microchannel with poly (methyl methacrylate), utilizing micromilling. We surmise that the possibly uneven surface from the micromilling process may invoke additional inward particle migration at the corners, similar to the hoop-stress assisted focusing mechanism⁴²—a hypothesis which calls for further studies.

As the flow rate increases, the inertial wall lift force becomes relevant (flow rate $\geq 360\ \mu\text{L/h}$ ($Re = 1.28$; $Wi = 179$)). The particles moving along the corners are pushed off the walls by the

FIG. 3. Particle focusing by DNA concentration in a square channel: (a) 2.5 ppm, (b) 5 ppm, (c) 10 ppm, and (d) 50 ppm. The particle size was $10\ \mu\text{m}$ (diameter), and the particle distribution was observed 4 cm downstream from the inlet.

inertial lift force, while the particle equilibrium position along the channel centerline is not affected by the inertial force.²² The equilibrium particle position along the channel centerline is less affected by the inertial force since the magnitude of the inertial force depends upon the lateral location, and the wall lift force is significantly greater than the inertial force around the channel centerline.²² Consequently, the particles form a single file along the channel centerline as shown in Fig. 1(b). The inertial force eventually overwhelms the elastic force when the flow rate is further increased, and the particle focusing along the channel centerline significantly deteriorates, as shown in Fig. 1(b) (flow rate $\approx 3000 \mu\text{L/h}$ ($Re = 10.6$; $Wi = 1491$)). The particle dynamics observed in the DNA solution are qualitatively similar to those in the synthetic polymer solution,²² but the flow rate ranges of the elasto-inertial particle focusing were significantly higher and wider than those in the synthetic polymer solution.²² As previously discussed,¹¹ DNA has much longer relaxation time because of its structural rigidity, which is the origin for notably higher elastic property of DNA solution, as compared to the synthetic polymer. Therefore, the elastic force in the DNA solution is dominant over the inertial force up to higher flow rates than the synthetic polymer solution case.²² The elasticity number (El ; ratio of elastic to inertial forces) of the DNA solution is ≈ 140 , which is significantly higher as compared to the synthetic polymer solution case (≈ 22) in the previous work.²² Consequently, the particle focusing in the DNA solution is not deteriorated up to higher flow rate than in the synthetic polymer solution. Our results support that elasto-inertial particle focusing is not limited to a specific polymer solution but is a general phenomenon in dilute polymer solutions with constant shear viscosity.

Next, we investigated the effects of DNA concentration on particle focusing. As shown in Fig. 2, the viscosity becomes more shear-thinning with increasing concentration. The power-law indices fitted with the Carreau model are 0.99, 0.99, 0.97, 0.94, and 0.77 for the 2.5, 5, 10, 25, and 50 ppm solutions, respectively. As shown in Fig. 3, we observed that the distribution of

FIG. 4. Elasto-inertial particle focusing for $6 \mu\text{m}$ and $15 \mu\text{m}$ beads: (a) $6 \mu\text{m}$ and (b) $15 \mu\text{m}$. The observation was 4 cm downstream from the inlet. The particle distributions presented in the right columns of (a) and (b) were obtained using the “standard deviation” option of ImageJ software. The dotted yellow lines denote the channel boundaries, and the channel width is $50 \mu\text{m}$.

10 μm PS beads in the low concentration DNA solutions ($c \leq 10$ ppm; almost constant shear viscosity cases) is different than that from the high concentration case ($c = 50$ ppm; notable shear-thinning). It was observed that there are three different particle focusing modes according to the flow rates in the low concentration DNA solutions ($c \leq 10$ ppm), which is qualitatively the same as the synthetic polymer solution case:²² quintuple equilibrium positions along the channel centerline and in the four corners at low flow rates (phase I), single file along the channel centerline (elasto-inertial particle focusing mode, phase II), and deterioration of particle focusing at high flow rates (phase III). For the low concentration DNA solutions ($c \leq 10$ ppm), the elasto-inertial focusing (phase II) occurs at the lowest flow rate when $c = 2.5$ ppm. However, the tight focusing at the high flow rates (>1500 $\mu\text{l/h}$) was observed when $c = 5$ ppm (cf. PDFs at the flow rate = 1600 $\mu\text{l/h}$ in Fig. 3), which is beneficial for high throughput applications such as particle counting. Thus, we consider $c = 5$ ppm as an optimal concentration for particle focusing applications. On the other hand, it was observed that the particles were rather tightly focused along the channel centerline (with a very small portion of particles at the corners) even at a low flow rate (40 $\mu\text{l/h}$) when $c = 50$ ppm, but particle focusing along the channel centerline was significantly deteriorated when the flow rate was increased to 100 $\mu\text{l/h}$. Previous study found that when the suspending medium was shear-thinning, the particles were tightly focused along the channel centerline at low flow rates and particle focusing deteriorated with increasing flow rate.²⁴ Consequently, the particle dynamics at the concentration of 50 ppm was consistent with the previous observations in the shear-thinning fluid.

We also investigated particle focusing for other particle sizes (6 μm and 15 μm diameter ($\frac{2a}{h} = 0.12$ and 0.30, respectively)) when the DNA concentration is 5 ppm. As shown in Fig. 4, three different particle focusing modes, depending on flow rate, were observed, similar to the 10 μm particle case ($\frac{2a}{h} = 0.2$). Therefore, we can conclude that the three modes of particle focusing (phases I-III) are relevant for these particle sizes (6–15 μm) ($0.12 \leq \frac{2a}{h} \leq 0.30$). The starting flow rate for phase II is similar for all the particle sizes (>200 $\mu\text{l/h}$) and phase III occurs at

FIG. 5. Probability distribution functions at the different longitudinal locations from an inlet for different particle sizes; (a) 6 μm , (b) 10 μm , and (c) 15 μm . The comparative graph (1 cm downstream from the inlet) is also presented as (d). The flow rate was 640 $\mu\text{l/h}$. Suspending medium was a 5 ppm λ -DNA solution in a 13.8 wt. % sucrose $1 \times$ TE buffer solution.

relatively lower flow rates ($>640 \mu\text{L/h}$) in the $6 \mu\text{m}$ particle case (also see supplementary Fig. S1).⁴³ On the other hand, as shown in Figs. 5(a), 5(b), and 5(c), particles are gradually focused along the channel centerline as the particles move downstream from the inlet. The lateral migration speed is strongly dependent upon particle size^{21,40} ($|V| \propto (\frac{a}{h})^2$, also refer to Eq. (1)). Consequently, tighter particle focusing is observed for the larger particle size 1 cm downstream from the inlet, as shown in Fig. 5(d). The particle size-dependent migration speed can be utilized for size-based separation, and the current DNA-based approach will contribute to the enhancement of the separation throughput as compared to the synthetic polymer solution-based approach.¹⁴

Finally, we present some potential applications of the current work. As shown in Fig. 6(a), particles with different sizes ($6, 10$, and $15 \mu\text{m}$) are all aligned along the channel centerline in the range $360 \mu\text{L/h} \leq \text{flow rate} \leq 1300 \mu\text{L/h}$, which is achieved by the elasto-inertial focusing mechanism. The particles with three different sizes form an almost single line along the channel centerline at the flow rate $= 640 \mu\text{L/h}$. One drawback to the previous particle focusing methods is the limitation on applicable particle sizes.⁴⁴ The current work demonstrates that the DNA-based particle focusing method can enhance the range of particle sizes which can be focused along the channel centerline at the same flow rate. Therefore, it is expected that the current method can be applied to heterogeneous biological samples such as whole blood. We also applied the DNA-based approach to the focusing of fresh rat RBCs. The suspending medium was a $5 \text{ ppm } \lambda\text{-DNA}$ solution in a PBS buffered solution. The RBCs were aligned along the channel centerline at the flow rate of $20 \mu\text{L/h}$ as shown in Fig. 6(b) (Multimedia view). The

FIG. 6. (a) Stacked images according to flow rates (left column) and representative snap shot images (right column) for the heterogeneous particulate mixture of $6, 10$, and $15 \mu\text{m}$ diameter PS beads. The suspending medium was a $5 \text{ ppm } \lambda\text{-DNA}$ solution in a $13.8 \text{ wt. \%}$ sucrose $1 \times \text{TE}$ buffer solution. The particle concentration for each size was $0.033 \text{ vol. \%}$. (b) Focusing of fresh rat red blood cells at the flow rate of $20 \mu\text{L/h}$ (cell concentration $\approx 0.1 \text{ vol. \%}$): snap shot image (top left; Multimedia view), stacked image (bottom left), and probability distribution function (PDF; right). The suspending medium for the cell experiment was a $5 \text{ ppm } \lambda\text{-DNA}$ solution in a PBS buffered solution. All the images were acquired 4 cm downstream from the inlet. The dotted yellow lines denote the channel boundaries. The stacked images of (a) and (b) were obtained using the “standard deviation” option of ImageJ software. (Multimedia view) [URL: <http://dx.doi.org/10.1063/1.4944628.1>]

fresh RBCs are highly deformable and the deformability-induced lift force is relevant.^{10,45} Therefore, the RBCs are focused along the channel centerline at the low flow rate by the synergistic combination of elastic and deformability-induced wall lift forces.¹⁰ In fact, the focusing of RBCs was previously demonstrated in 6.8 wt. % PVP solution ($\mu = 90$ cP).¹⁰ However, the shear viscosity of the suspending medium in the current work is nearly identical to water viscosity and the focused cells experienced significantly less shear stress than in the PVP solution case (shear stress $\approx \mu \dot{\gamma}$). Consequently, the cell deformation can be significantly reduced in the DNA solution as compared with the synthetic polymer solution case.¹⁰ Therefore, the morphology of the focused RBC along the channel centerline as shown in Fig. 6(b) is almost the same as that of stationary cells. Therefore, we expect that RBC focusing, without significant deformation, in the DNA solution can be utilized to accurately analyze the morphology changes of disease-infected RBCs, such as malaria-infected RBCs.

V. CONCLUSIONS

We demonstrated that dilute DNA solutions are ideally suited to investigate particle dynamics in a viscoelastic fluid with constant shear viscosity. The viscosity of the dilute DNA solution is dominated by its medium, and is nearly identical to that of its medium, though the elastic property is very relevant, originating from the unique micromechanical properties of ds DNA molecules.^{46,47} It was shown that the particles migrate toward quintuple equilibrium positions along the channel centerline and the four corner under the elasticity-dominant condition when the suspending medium is very dilute. The quintuple equilibrium particle positions were observed for a wide range of particle sizes (6–15 μm). As the flow rate increases, the particles are aligned along the channel centerline by the synergistic combination of elastic and inertial forces. The particle dynamics observed in the current study are qualitatively the same as those using synthetic polymer solutions. Therefore, this study supports the hypothesis that elasto-inertial particle focusing is a universal phenomenon in dilute polymer solutions.

It was also demonstrated that DNA-based focusing can be applied to focus a wide range of particle sizes, which may contribute to the development of a novel image cytometer, applicable to the heterogeneous particulate systems that are relevant in most biological samples. It was also shown that the current method can be applied to focus RBCs without significant deformation, which may contribute to morphology-based analysis for disease-infected cells.

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