Supplementary Materials

Article

Microfluidic Quantification of Blood Pressure and Compliance Properties Using Velocity Fields under Periodic on-off Blood Flows

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S1. Blood sample preparation

Ethics Committee of Chosun University Hospital (CHOSUN 2018-05-11) approved that all experimental procedures were appropriate and humane. Both fresh frozen plasma (FFP) and concentrated RBCs were bought from a blood bank (Gwangju, Korea). Because the concentrated RBCs were stored in citrate phosphate dextrose adenine (CPDA), washing procedures were required to separate CPDA from the RBCs. The RBCs (~7 mL) and 1× PBS (pH 7.4, Gibco, Life Technologies, New York, NY, USA) (~7 mL) were infused in a 15-mL tube. After fitting the tube into a centrifugal separator, it had operated at 4,000 rpm for 10 min. Pure RBCs (i.e., lower layer) were then collected after removing the PBS (i.e., upper layer). Because FFP had kept at -20 ºC, it thawed at the room temperature of 25 ºC. After filtering debris with a syringe filter (i.e., mesh size = 5 μm), autologous plasma was then collected in a 15-mL tube. The RBCs and plasma were stored at 4 ºC prior to the experiment.

Various blood samples were carefully prepared to varying degrees for pressure and equivalent compliance measurements. Apart from experiments investigating the contributions of hematocrit to blood pressure and compliance, hematocrit of blood sample set to Hct = 50%. First, to measure effect of the hematocrit on blood pressure and equivalent compliance, the hematocrit of blood sample set to Hct = 30%, 40%, and 50% by adding normal RBCs into diluent (1× PBS, plasma). Second, to stimulate RBCs aggregation of blood sample, three concentrations of dextran solution (i.e., C_{dex} = 5, 10, 15 mg/mL) were prepared by adding dextran powder (*Leuconostoc* spp., MW = 450–650 kDa, Sigma–Aldrich, USA) into 1× PBS and mixing them. Blood sample (Hct = 50%) was then prepared by inserting RBCs to individual dextran solution. Third, to vary the degree of RBC deformability, normal RBCs were chemically fixed using a glutaraldehyde (GA) solution. Six different concentrations of GA solutions (C_{GA} = 0, 2, 4, 6, 8, and 10 μL/mL) were prepared by adding them (Grade II, 25% in H_2O, Sigma-Aldrich, USA) into 1× PBS. Homogeneous fixed RBCs were gathered after exposing normal RBCs to individual GA solution sufficiently. The fixed blood sample (Hct = 50%) was prepared by inserting fixed RBCs into 1× PBS.

S2. Contribution of erythrocyte sedimentation rate (ESR) in driving syringe to pressure and equivalent compliance

While supplying blood samples with a syringe pump, continuous ESR occurred inside the driving syringe. For this reason, the hematocrit of the blood sample supplied into the microfluidic channel tended to change over time. Because the syringe pump was installed in a horizontal direction, it was expected that the ESR inside the driving syringe contributed to the decreasing hematocrit of blood samples flowing in microfluidic channels after a certain time. To evaluate the effect of the ESR in the driving syringe on blood pressure and equivalent compliance, a dextran solution was employed to enhance RBCs aggregation or ESR in driving syringe. The blood sample (Hct = 50%) was prepared by adding normal RBCs into a specific concentration of dextran solution (C_{dex} = 0, 5, 10, and 15 mg/mL).

As shown in **Figure S1(A-a)**, corresponding microscopic images for the blood sample with different concentrations of dextran solution were captured at specific time (t = 120, 1140 s). With an elapse of long time, RBCs flowing in the main channel decreased substantially at higher concentrations of dextran solution. Below C_{dex} = 5 mg/mL, the image intensity of blood flow did not show a significant difference. However, above C_{dex} = 10 mg/mL, the image intensity of blood flow tended to increase because the numbers of RBCs (i.e., hematocrit) decreased considerably. This result indicated that the ESR in a driving syringe caused to decrease the hematocrit of the blood sample flowing in the main channel. To evaluate the image intensity of blood flow, a specific ROI (150 × 250 pixels) was selected within the main channel. The averaged image intensity of blood flow ($\langle I_{mc} \rangle$) was obtained by conducting digital image processing for each microscopic image. **Figure S1(A-b)** showed temporal variations of $\langle I_{mc} \rangle$ with respect to C_{dex}. Blood sample with C_{dex} = 0 or 5 mg/mL showed similar trends when compared with a periodic flow-rate profile. After turning off the syringe pump (Q_0 = 0), $\langle I_{mc} \rangle$ tended to increase because of RBCs aggregation. After t = 60 s,

it remained constant over time. However, after turning on the syringe pump (Q_0 = 1.5 mL/h), <I_{mc}> tended to decrease because of RBC disaggregation. After an elapse of a certain time, it remained constant over time. The blood sample with C_{dex} = 10 or 15 mg/mL contributed to substantially increasing <I_{mc}> when compared with the blood sample with C_{dex} = 0 or 5 mg/mL. With respect to the blood sample with C_{dex} = 10 mg/mL, <I_{mc}> increased and fluctuated significantly after t = 720 s. The blood sample with C_{dex} = 15 mg/mL contributed to fluctuating <I_{mc}> significantly after t = 240 s. Higher concentrations of dextran solution (i.e., C_{dex} >= 10 mg/mL) contributed to inducing unstable behavior of <I_{mc}>. The instability of <I_{mc}> initialized within a short time at a higher concentration of dextran solution. From the results, higher concentrations of dextran solution caused enhanced ESR in the driving syringe. The hematocrit of the blood sample flowing in the main channel tended to decrease over time. <I_{mc}> was employed effectively to monitor variations of RBCs flowing in the main channel.

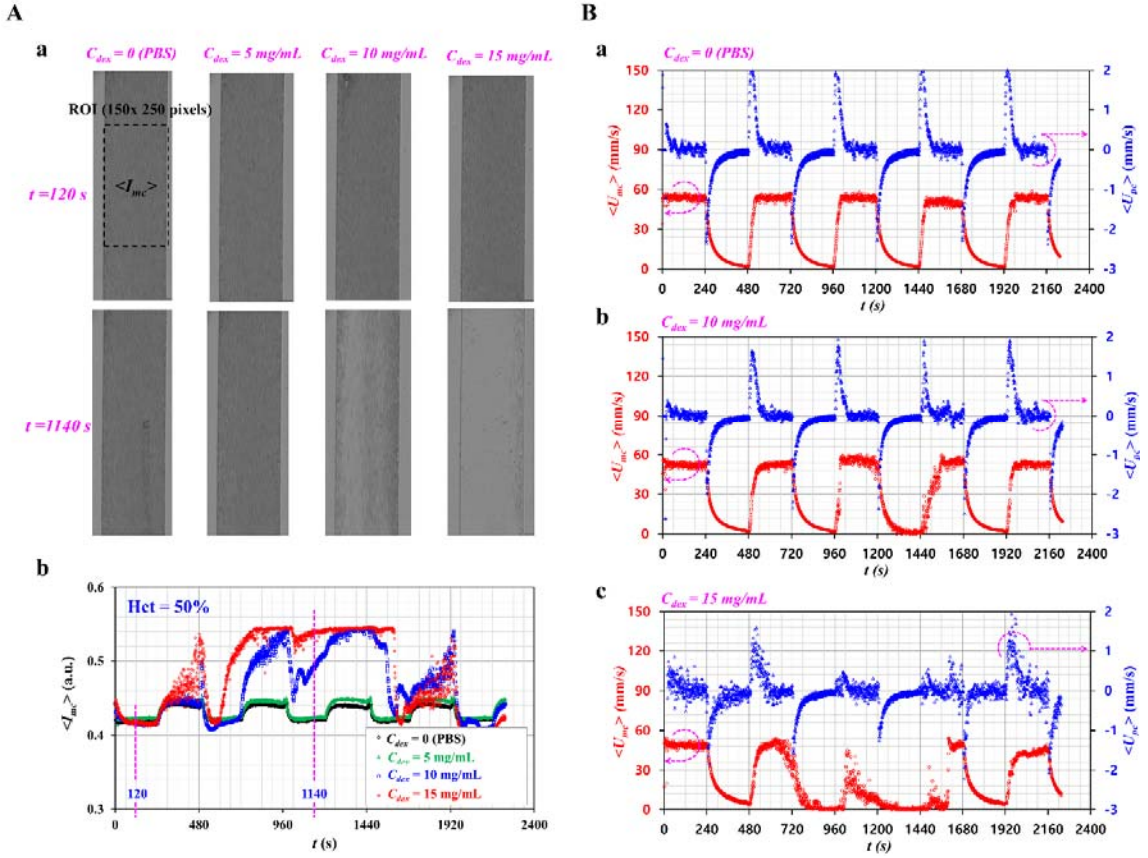

Figure S1. Contribution of dextran solution to averaged image intensities (<I_{mc}>) and averaged velocities (<U_{mc}>, <U_{pc}>). Here, the blood sample (Hct = 50%) was prepared by adding normal RBCs into a specific concentrations of dextran solution (C_{dex} = 0, 5, 10, and 15 mg/mL). **(A)** Variations of image intensity with respect to C_{dex}. **(a)** Microscopic image captured for the blood sample having a specific concentration of dextran solution at specific times (t) (t = 120, and 1140 s). **(b)** Temporal variations of <I_{mc}> with respect to C_{dex}. **(B)** Variations of averaged velocities (<U_{mc}>, <U_{pc}>) with respect to concentrations of dextran solution (C_{dex}): **(a)** C_{dex} =0 (1× PBS), **(b)** C_{dex} =10 mg/mL, and **(c)** C_{dex} = 15 mg/mL.

As shown in **Figure S1B**, temporal variations of averaged velocities (<U_{mc}>, <U_{pc}>) were obtained with respect to C_{dex} (**[a]** C_{dex} = 0 (1× PBS), **[b]** C_{dex} = 10 mg/mL, and **[c]** C_{dex} = 15 mg/mL). Below C_{dex} = 10 mg/mL, both velocities showed periodic variations over time consistently. However, blood samples with C_{dex} = 15 mg/mL contributed to fluctuating <U_{mc}> and <U_{pc}> after t = 600 s. As shown in **Figure S1(A-a)**, the

hematocrit of the blood sample flowing in the main channel decreased significantly at t = 1,140 s. Because there were no sufficient RBCs for fluid tracers in the main channel, velocity fields of blood flow did not provide consistent results over time. Although blood flow rate was precisely controlled with a syringe pump, $\langle U_{mc} \rangle$ did not give consistently periodic patterns over time. Additionally, because the pressure channel was not filled with sufficient RBCs, $\langle U_{pc} \rangle$ did not provide periodic patterns consistently. From the results, higher concentrations of dextran solution (i.e., C_{dex} = 15 mg/mL) caused unstable variations of $\langle U_{mc} \rangle$ and $\langle U_{pc} \rangle$ because the channels were not filled with sufficient RBCs as a fluid tracer. As shown in **Figure S1B**, based on temporal variations of $\langle U_{mc} \rangle$ and $\langle U_{pc} \rangle$, evaluations of pressure (P_x) and equivalent compliance (C_{eq}) were obtained with respect to C_{dex}.

Figure S2(A-a) showed variations of β with respect to C_{dex}. β tended to decrease with respect to C_{dex}. Because the dextran solution contributed to increasing fluidic resistance (or blood viscosity), it hindered blood flows in the pressure channel.

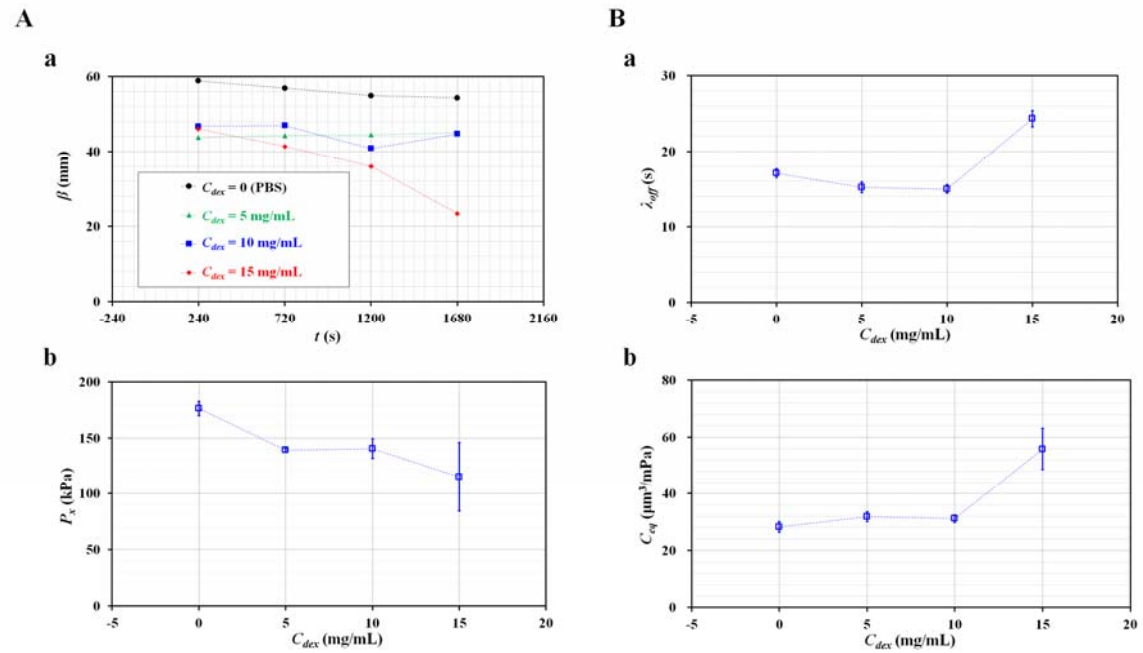

Figure S2. Quantitative evaluations of pressure (P_x) and equivalent compliance (C_{eq}) for the blood sample (Hct = 50%) comprising different concentrations of dextran solution (C_{dex} = 0, 5, 10, and 15 mg/mL). **(A)** Variations of P_x with respect to C_{dex}. **(a)** Temporal variations of β with respect to C_{dex}. **(b)** Variations of P_x with respect to C_{dex}. **(B)** Variations of λ_{off} and C_{eq} with respect to C_{dex}. **(a)** Variations of λ_{off} with respect to C_{dex}. **(b)** Variations of C_{eq} with respect to C_{dex}.

As discussed in **Figure S1B**, $\langle U_{pc} \rangle$ for blood samples with C_{dex} = 15 mg/mL was obtained inaccurately over time. Thus, β tended to decrease substantially over time. Using Equation (1) and **Figure 2D**, blood pressure (P_x) was obtained as P_x =3.1312·β. Four βs obtained for blood sample with each dextran solution were averaged to estimate P_x. As shown in **Figure S2(A-b)**, variations of P_x were obtained as mean ± standard deviation with respect to C_{dex}. P_x tended to decrease between C_{dex} = 0 and C_{dex} = 5 mg/mL. Above C_{dex} = 5 mg/mL, P_x remained constant, except for C_{dex} = 15 mg/mL. The blood sample with C_{dex} = 15 mg/mL showed a large fluctuation of P_x when compared with the blood sample having C_{dex} = 0, 5, 10 mg/mL. As shown in **Figure S1B**, using a periodic on–off behavior of $\langle U_{mc} \rangle$, variations of λ_{off} were obtained with respect to C_{dex}. As shown in **Figure 6(B-a)**, λ_{off} remained constant below C_{dex} = 10 mg/mL. With respect to the blood sample with C_{dex} = 15 mg/mL, λ_{off} increased distinctively. Using Equation (6), variations of C_{eq} were obtained

with respect to C_{dex}. As shown in **Figure S2(B-b)**, variations of C_{eq} were very similar to those of λ_{off}. Apart from C_{dex} = 15 mg/mL, equivalent compliance remained constant with respect to C_{dex}.

From these results, blood pressure (P_x) did not provide consistent variations with respect to C_{dex}. Additionally, equivalent compliance (C_{eq}) gave constant values with respect to C_{dex}. However, as shown in **Figure S1(A-b)**, the image intensity ($\langle I_{mc} \rangle$) showed significant difference with respect to C_{dex}. For this reasons, to effectively quantify RBCs aggregation or ESR, the previous method (i.e., image intensity-based quantification) might be considered effective when compared with the present method (i.e., blood pressure or equivalent compliance).