Blood viscoelasticity measurement using steady and transient flow controls of blood in a microfluidic analogue of Wheastone-bridge channel

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Accurate measurement of blood viscoelasticity including viscosity and elasticity is essential in estimating blood flows in arteries, arterials, and capillaries and in investigating sub-lethal damage of RBCs. Furthermore, the blood viscoelasticity could be clinically used as key indices in monitoring patients with cardiovascular diseases. In this study, we propose a new method to simultaneously measure the viscosity and elasticity of blood by simply controlling the steady and transient blood flows in a microfluidic analogue of Wheastone-bridge channel, without fully integrated sensors and labelling operations. The microfluidic device is designed to have two inlets and outlets, two side channels, and one bridge channel connecting the two side channels. Blood and PBS solution are simultaneously delivered into the microfluidic device as test fluid and reference fluid, respectively. Using a fluidic-circuit model for the microfluidic device, the analytical formula is derived by applying the linear viscoelasticity model for rheological representation of blood. First, in the steady blood flow, the relationship between the viscosity of blood and that of PBS solution ($\mu_{\text{Blood}}/\mu_{\text{PBS}}$) is obtained by monitoring the reverse flows in the bridge channel at a specific flow-rate rate ($Q_{\text{PBS}}^{\text{SS}}/Q_{\text{Blood}}^{\text{L}}$). Next, in the transient blood flow, a sudden increase in the blood flow-rate induces the transient behaviors of the blood flow in the bridge channel. Here, the elasticity (or characteristic time) of blood can be quantitatively measured by analyzing the dynamic movement of blood in the bridge channel. The regression formula ($A_{\text{Blood}}(t) = A_{\alpha} + A_{\beta} \exp [-(t - t_0)/\lambda_{\text{Blood}}]$) is selected based on the pressure difference ($\Delta P = P_A - P_B$) at each junction (A, B) of both side channels. The characteristic time of blood (λ_{Blood}) is measured by analyzing the area (A_{Blood}) filled with blood in the bridge channel by selecting an appropriate detection window in the microscopic images captured by a high-speed camera (frame rate = 200 Hz, total measurement time = 7 s). The elasticity of blood (G_{Blood}) is identified using the relationship between the characteristic time and the viscosity of blood. For practical demonstrations, the proposed method is successfully applied to evaluate the variations in viscosity and elasticity of various blood samples: (a) various hematocrits from 20% to 50%, (b) thermal-induced treatment (50 °C for 30 min), (c) flow-induced shear stress (53 ± 0.5 mL/h for 120 min), and (d) normal rat versus spontaneously hypertensive rat. Based on these experimental demonstrations, the proposed method can be effectively used to monitor variations in viscosity and elasticity of bloods, even with the absence of fully integrated sensors, tedious labeling and calibrations. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4827355>]

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I. INTRODUCTION

Blood has two representative biophysical properties such as viscosity and elasticity. Among them, viscosity would play a significant role as a fluidic resistance in steady blood flows. It depends on several factors, such as deformability,^{1–5} aggregation,^{6,7} plasma viscosity,⁸ and haematocrit.⁹ Furthermore, the viscosity of blood is altered depending on the chemical and biological functions of red blood cells (RBCs).¹⁰ Therefore, viscosity has been clinically used to monitor patients with cardiovascular diseases (CVDs). On the other hand, the elasticity of blood, which is influenced by cytoskeleton and integral proteins and has been neglected in steady blood flows, has an influence on pulsatile blood flows in arteries and arterioles. In addition, the elasticity of RBC has been considered a dominant factor in the microcirculations in capillaries, where the diameter of the blood vessel is smaller than that of the RBC.

In general, normal RBCs have high flexibility, which allows these blood cells to pass through smaller capillaries and transport gases (O_2 , CO_2), nutrients, and wastes between blood and peripheral tissues. However, several CVDs, including diabetes and sickle cell anemia, exhibit impaired elasticity of RBCs, which hinders microcirculatory functions. For these reasons, the viscosity and elasticity of blood are essentially required to accurately estimate pulsatile blood flows in blood vessels, including arteries, arterioles, and capillaries. Exposure of RBC to high shear stresses due to large pressure difference developed in vascular systems can potentially damage the RBC membrane. Thus, the elasticity and viscosity of blood can be used to effectively evaluate the sub-lethal damage of RBCs. These physical properties of blood could be clinically considered as key indices in monitoring patients with CVDs. Compared with a bulky conventional viscometer,^{9,11} a microfluidic-based viscometer has distinctive advantages, such as small volume consumption, short measurement time, and promising feasibility of point-of-care-test (POCT). Recently, several researchers have measured viscosity or characteristic time of fluids using microfluidic devices. Fluid viscosity was identified using several methods, including micro-cantilever,¹² capacity sensor,¹³ capillary force,¹⁴ comparator,^{15,16} and laser-induced capillary wave.¹⁷ Most of these methods require fully integrated sensors or additional tedious calibration procedure using a standard reference. On the other hand, viscoelasticity measurement of fluids has been conducted using ferromagnetic micro-beads,¹⁸ optically-induced force,¹⁹ squeeze flows,^{20,21} flexible polymer-based deflection,^{22,23} micro-PIV,²⁴ and extensional flow.^{25,26} However, most of these previous studies focused on measuring the relaxation time rather than elasticity itself because these methods may not have the ability to concurrently measure fluid viscosity. In addition, these methods were only applied to identify relaxation time of fluids other than blood.

Simultaneous measurement of viscosity and elasticity is fundamentally required to investigate the biophysical properties of blood using a microfluidic platform. Sensorless and label-free detection are necessary to satisfy the promising potential of POCT. Recently, we have successfully demonstrated fluid viscosity measurement method by switching microfluidic flow in a microfluidic channel with label-free operation.²⁷ However, the previous method was only focused on measuring fluid viscosity.

Thus, in this study, we proposed a new measurement method, which can simultaneously measure viscosity and elasticity of blood by controlling the steady and transient blood flows in a microfluidic channel. The viscosity and elasticity of blood are sequentially identified with label-free and sensorless detection. First, the viscosity of blood is identified by monitoring the reversal of steady flows in the microfluidic channel. The elasticity of blood is then measured by analyzing the transient fluidic behaviors of blood in the microfluidic channel. Compared with the previous methods, the present method has the following three distinctive advantages. First, simultaneous identification of viscosity and elasticity of blood is made possible by the simple establishment of steady and transient blood flows. Second, the present method does not require fully integrated sensors, such as pressure sensors or flow meters. Third, fluorescent particle-based labeling in microfluidic channel is not required in this method.

To evaluate the performances of the proposed method, an analytical formula for the viscosity and elasticity of blood is derived by applying the linear viscoelasticity model for rheological

representation of blood, which behaves as a Newtonian fluid, especially above the shear rate of 1000. Based on the analytical formula, various representative parameters that influence the blood elasticity measurement are intensively studied to appropriately control transient flow of blood. In addition, elasticity effect owing to the microfluidic system is evaluated using pure liquid which does not include elasticity. Furthermore, using a diluted PEO solution as viscoelasticity fluid, variations in viscosity and elasticity are quantitatively evaluated with respect to four different concentrations.

The proposed method is applied to demonstrate the usefulness in practical applications and evaluate the viscosity and elasticity of human blood with respect to (a) various hematocrits from 20% to 50%, (b) thermal-induced treatment (50 °C for 30 min), and (c) flow-induced shear stress (53 ± 0.5 mL/h for 120 min). The present method is also applied to compare the viscosity and elasticity of the blood samples, which are collected from a normal rat and a spontaneously hypertensive rat.

II. BLOOD VISCOELASTICITY MEASUREMENT BASED ON MICROFLUIDIC FLOW CONTROLS

A. The proposed method

In this study, simultaneous measurement method of the viscosity and elasticity of blood using steady and transient flow controls in a microfluidic analogue of Wheatstone-bridge channel is proposed without integrated sensors and additional labelling procedures. In other words, two sequential flow controls of blood are applied to identify the viscosity of blood under steady blood flows and elasticity (or characteristic time) of blood in transient blood flows. First, in steady blood flow, the viscosity of blood is identified by monitoring the reversal of steady flows at a specific flow-rate of each fluid in the bridge channel. Next, a sudden increase in the blood flow rate induces the transient behaviors of the blood flow in the bridge channel. Here, the elasticity (or characteristic time) of blood can be quantitatively measured by analyzing the dynamic movement of blood in the bridge channel, which allows the present method to sequentially measure the viscosity and elasticity of blood by simply controlling the flow rate of each fluid in steady and transient blood flows. As shown in Fig. 1A, a microfluidic analogue of Wheatstone-bridge channel, which is composed of two identical side channels and one bridge channel, is carefully designed and fabricated to demonstrate our proposed method. To deliver consistent flow rate of each fluid into the microfluidic channel, a flow stabilizer filled with 0.1 mL cavity volume is only installed between a syringe pump (neMESYS, Germany) for PBS solution and inlet (B) of the microfluidic device. However, to avoid time delay owing to the flow stabilizer under transient flow control of blood, blood flow is directly supplied into the inlet (A) of the microfluidic channel without the flow stabilizer. Blood (test fluid) and 1x phosphate buffered saline (PBS, reference fluid) are simultaneously supplied into the inlets of the microfluidic device. The viscosity of PBS solution is known in advance. The PBS solution does not possess elasticity.

Fig. 1B shows the schematics of the simultaneous measurement of viscosity and elasticity of blood using a microfluidic device. Given that blood viscosity (μ_{Blood}) is larger than that of the PBS solution (μ_{PBS}) at the same flow rate ($Q_{\text{PBS}}^L = Q_{\text{Blood}}^L$), the pressure (P_A) at the left junction (A) is greater than that (P_B) at the right junction ($P_A > P_B$). Thus, the blood unexceptionally tends to move toward the right direction in the bridge channel, as illustrated in Fig. 1B-(a). Next, when the flow rate of the PBS solution is increased to induce reversal of steady flow direction in the bridge channel (i.e., $Q_{\text{PBS}}^{\text{SS}} = \beta Q_{\text{Blood}}^L$), the PBS solution immediately moves from the right (R) direction to the left direction (L) in the bridge channel due to hydrodynamic force balance ($P_A \approx P_B$). Here, β indicates the viscosity of blood, which is to be measured in relation to that of the PBS solution (i.e., $\beta = \mu_{\text{Blood}}/\mu_{\text{PBS}} = Q_{\text{PBS}}^{\text{SS}}/Q_{\text{Blood}}^L$) [Fig. 1B-(b)]. Using a syringe pump, transient blood flows are induced in the left side channel, which is filled with blood, while the PBS solution is consistently supplied at the switching flow rate ($Q_{\text{PBS}}^{\text{SS}}$). When the flow rate of blood is abruptly increased from Q_{Blood}^L to $Q_{\text{Blood}}^{\text{TS}}$, blood as test fluid, immediately moves to the right direction (R) in the bridge channel, owing to pressure

FIG. 1. Simultaneous measurement of viscosity and elasticity of blood using flow controls in the microfluidic analogue of Wheatstone-bridge channel. (A) A schematic diagram of a microfluidic system composed of a microfluidic analogue of Wheatstone-bridge channel, tubes a flow stabilizer, and syringe pumps. The microfluidic device is composed of two inlets and two outlets, two side channels for delivering two fluids, and one bridge channel for detecting flow behaviors. (B) Schematics of sequential flow controls and a microfluidic device. (a) In a steady blood flow condition, when blood and PBS solution are simultaneously supplied into each inlet port [(A),(B)] at the same flow rate ($Q_{\text{Blood}}^L = Q_{\text{PBS}}^L$), blood moves toward the right direction (R) in the bridge channel owing to pressure difference ($P_A > P_B$) between the left and the right junctions, resulting from viscosity difference ($\mu_{\text{Blood}} > \mu_{\text{PBS}}$). (b) The PBS solution reversely flows toward the left direction (L) in the bridge channel at a specific flow-rate ratio ($Q_{\text{PBS}}^{SS}/Q_{\text{Blood}}^L$). The viscosity of blood in relation to that of the PBS solution can be measured by monitoring the reversal flow in the bridge channel at the specific flow-rate ratio. (c) When the flow rate of blood increases from Q_{Blood}^L to Q_{Blood}^{TS} , while that of the PBS solution is fixed at Q_{PBS}^{SS} , blood immediately moves to the right direction (R) in the bridge channel, and shows transient behaviors. The characteristic time can be characterized by analyzing the temporal variations of the area (A_{Blood}) filled with blood within specific detection window. (C) Microscopic images of blood flow in the bridge channel with respect to flow-rate ratio [(a) $Q_{\text{PBS}}/Q_{\text{Blood}}^L = 1$, (b) $Q_{\text{PBS}}/Q_{\text{Blood}}^L = 1.5$, (c) $Q_{\text{PBS}}/Q_{\text{Blood}}^L = 1.9$, (d) $Q_{\text{PBS}}/Q_{\text{Blood}}^L = 2.05$, and (e) $Q_{\text{PBS}}/Q_{\text{Blood}}^L = 2.1$] in steady blood flows. Blood reversely flows in the bridge channel from the right direction (R) to left direction (L), at a specific flow-rate ratio of $Q_{\text{PBS}}^{SS}/Q_{\text{Blood}}^L = 2.1$. When the flow rates of blood and PBS solution are set as $Q_{\text{Blood}}^{TS} = 1.5 \text{ mL/h}$ and $Q_{\text{PBS}}^{SS} = 2.1 \text{ mL/h}$, the area (A_{Blood}) occupied by blood within the detection window was quantitatively analyzed with respect to time [(f) $t = -0.5 \text{ s}$, (g) $t = 0 \text{ s}$, (h) $t = 0.3 \text{ s}$, (i) $t = 0.6 \text{ s}$, (j) $t = 0.9 \text{ s}$, and (k) $t = 1.2 \text{ s}$]. The blood flows in the bridge channel exhibit transient behaviors.

difference ($P_A > P_B$). The movement exhibits dynamically transient behaviors. In here, to quantify the transient fluidic motion without integrated sensors, the characteristic time (λ_{Blood}) is characterized by analyzing the temporal variation of the area (A_{Blood}) filled with blood within a specific detection window of the bridge channel [Fig. 1B-(c)]. Thereafter, the elasticity of blood can be evaluated through the relationship between the characteristic time and viscosity of blood sample.

For the preliminary demonstration of the proposed method, blood sample (RBCs in PBS suspension, $H_{ct} = 40\%$) and PBS solution are simultaneously supplied into the two inlets of the microfluidic device. First, in steady blood flows, the flow rate of blood (Q_{Blood}^L) is fixed to 1 mL/h , which is carefully selected for Newtonian fluid.²⁷ The flow rate of PBS solution (Q_{PBS}) is then increased to monitor the occurrence of reverse flow in the bridge channel. Fig. 1C shows that the typical microscopic images of blood flows in the bridge channel were captured

with respect to the flow rates of blood [(a) $Q_{\text{PBS}}/Q_{\text{Blood}}^L = 1$, (b) $Q_{\text{PBS}}/Q_{\text{Blood}}^L = 1.5$, (c) $Q_{\text{PBS}}/Q_{\text{Blood}}^L = 1.9$, (d) $Q_{\text{PBS}}/Q_{\text{Blood}}^L = 2.05$, and (e) $Q_{\text{PBS}}^{SS}/Q_{\text{Blood}}^L = 2.1$]. This preliminary experimental result indicates that blood starts to reversely move in the bridge channel from right direction (R) to left direction (L) at a specific flow-rate ratio of $Q_{\text{PBS}}^{SS}/Q_{\text{Blood}}^L = 2.1$. Thus, the viscosity of blood in relation to that of the PBS solution is identified as 2.1 ($\mu_{\text{Blood}}/\mu_{\text{PBS}} = 2.1$). The evaluation of the transient behaviors of blood in transient blood flows shows that the flow rate of blood immediately changes from 1 mL/h ($Q_{\text{Blood}}^L = 1$ mL/h) to 1.5 mL/h ($Q_{\text{Blood}}^{TS} = 1.5$ mL/h), while the flow rate of the PBS solution (Q_{PBS}^{SS}) remains at 2.1 mL/h. Microscopic images of blood flows in the bridge channel are used to analyze the temporal variation of the area filled with blood (A_{Blood}) within the detection windows. Using digital image processing techniques, the area (A_{Blood}) filled with blood was quantitatively calculated according to time [(f) $t = -0.5$ s, (g) $t = 0$ s, (h) $t = 0.3$ s, (i) $t = 0.6$ s, (j) $t = 0.9$ s, and (k) $t = 1.2$ s]. The blood motion in the bridge channel exhibits a typical transient response. Therefore, the characteristic time of blood can be identified from the temporal variation of the area (A_{Blood}) filled with blood within the detection window.

B. Analytical regression formulae based on the linear viscoelasticity model

The elasticity of blood can only be identified under dynamic blood flow conditions. In other words, sudden changes in the flow rate of blood from Q_{Blood}^L to Q_{Blood}^{TS} should be applied to evaluate the elasticity of blood in left side channel of the microfluidic channel. At the blood flow rate of 1 mL/h ($Q_{\text{Blood}}^L = 1$ mL/h), the shear rate in the side channel filled with blood is estimated to be $\dot{\gamma} = 1.3 \times 10^4 \text{ s}^{-1}$. The blood then behaves as a Newtonian fluid in the microfluidic channel.²⁷ In this study, the linear viscoelasticity model with constant properties (viscosity, elasticity) can be appropriately applied to represent viscoelasticity of the blood, which consists of viscosity element and elasticity element in series. In other words, when measuring viscosity and elasticity of blood simultaneously, the transient flow conditions of blood should be carefully selected to satisfy that the blood viscosity remains constant irrespective of shear rate (or flow rate). The PBS solution, which does not possess elasticity, is only modeled as viscosity element. The flow rate of PBS solution consistently remained at a specific value (Q_{PBS}^{SS}). For convenience, the elasticity effect contributed by the components of microfluidic system including a flexible tube and polydimethylsiloxane (PDMS) microfluidic channel is neglected to simplify governing equation. The elasticity effect owing to the system is experimentally evaluated using glycerin solution which does not include elasticity effect. As shown in Fig. 2, the left lower side channel filled with blood is

FIG. 2. A lumped parameter model for rheological representation of blood based on linear viscoelasticity model under transient fluidic manipulations. Flow rate of blood is suddenly controlled from Q_{Blood}^L to Q_{Blood}^{TS} at transient fluidic manipulation ($t > t_0$) when PBS solution was consistently delivered at Q_{PBS}^{SS} . Left lower side channel filled with blood is modeled by elasticity element (G_{Blood}) and viscosity element (μ_{Blood}) connected in series. In addition, right lower side channel filled with PBS solution is modeled by single viscosity element (μ_{PBS}).

modeled by elasticity element ($\mathbf{G}_{\text{Blood}}$) and viscosity element (μ_{Blood}) connected in series.²⁸ Using the linear viscoelasticity model, a governing equation for the blood viscoelasticity in the left lower side channel is derived as

$$\dot{\tau}_w(t) + \frac{\tau_w(t)}{\lambda_{\text{Blood}}} = \mathbf{G}_{\text{Blood}} \dot{\gamma}_w(t), \quad (1)$$

where $\tau_w(t)$ and $\dot{\gamma}_w(t)$ denotes the shear stress and the shear rate that act on the channel wall. The characteristic time (λ_{Blood}) of blood is given as $\lambda_{\text{Blood}} = \mu_{\text{Blood}} / \mathbf{G}_{\text{Blood}}$. Using the definition of a hydraulic diameter (D) for the rectangular channel (width = w , depth = h),^{27,29} a shear rate formula in the right side of Eq. (2) is derived as,

$$\dot{\gamma}_w(t) = \frac{32Q_{\text{Blood}}(t)}{\pi D^3}. \quad (2)$$

To convert the shear stress (τ_w) into the pressure at a junction (P_A), the force balance equation is applied to the lower left side-channel filled with blood. Based on the force balancing equation, the relationship between the shear stress and the pressure is derived as,

$$\tau_w = \frac{D}{4L} P_A. \quad (3)$$

In Eq. (3), L represents the channel length of the lower left or right side channel. By substituting Eqs. (2) and (3) into Eq. (1), the pressure (P_A) at the left junction (A) can be derived as,

$$\dot{P}_A(t) + \frac{P_A(t)}{\lambda_{\text{Blood}}} = \frac{128 L \mathbf{G}_{\text{Blood}}}{\pi D^4} Q_{\text{Blood}}(t). \quad (4)$$

Thereafter, the flow rate of blood is given, depending on steady flow-rate condition ($t = t_0$) and transient flow-rate condition ($t > t_0$):

$$Q_{\text{Blood}}(t) = \begin{cases} Q_{\text{Blood}}^L, & t = t_0 \\ Q_{\text{Blood}}^{\text{TS}}, & t > t_0 \end{cases}. \quad (5)$$

Solving the ordinary differential equation for the flow-rate conditions of blood using Eqs. (4) and (5), analytical formula of the pressure (P_A) with respect to time is expressed as follows:

$$P_A(t) = \begin{cases} \frac{128 \mu_{\text{Blood}} L}{\pi D^4} Q_{\text{Blood}}^L, & t = t_0 \\ \frac{128 \mu_{\text{Blood}} L}{\pi D^4} (Q_{\text{Blood}}^L - Q_{\text{Blood}}^{\text{TS}}) \exp\left(-\frac{t - t_0}{\lambda_{\text{Blood}}}\right) + \frac{128 \mu_{\text{Blood}} L}{\pi D^4} Q_{\text{Blood}}^{\text{TS}}, & t > t_0 \end{cases}. \quad (6)$$

On the other hand, the lower right side channel filled with PBS solution is modeled by single viscosity element (μ_{PBS}). And, the flow rate of the PBS solution (Q_{PBS}) is fixed at $Q_{\text{PBS}}^{\text{SS}}$. Thus, the pressure (P_B) at the right junction (B) is simply derived as,

$$P_B(t) = \frac{128 \mu_{\text{PBS}} L}{\pi D^4} Q_{\text{PBS}}^{\text{SS}}. \quad (7)$$

According to Eqs. (6) and (7), particularly for steady blood flows ($t = t_0$), the same pressure condition ($P_A \approx P_B$) at both junctions (A, B) satisfies the following relationship between viscosity and flow rate for each fluid,

$$\frac{\mu_{\text{Blood}}}{\mu_{\text{PBS}}} = \frac{Q_{\text{PBS}}^{\text{SS}}}{Q_{\text{Blood}}^L}. \quad (8)$$

According to Eq. (8), the viscosity of blood in relation to that of the PBS solution is related to a specific flow-rate ratio ($Q_{\text{PBS}}^{\text{SS}}/Q_{\text{Blood}}^{\text{L}}$), at which reverse flow occurs in the bridge channel. In other words, the viscosity of blood can be easily measured by monitoring the specific flow-rate ratio under the given viscosity of PBS solution as the reference fluid.

Under transient blood flows ($t > t_0$), the motion of blood in the bridge channel depends on the pressure difference ($\Delta P = P_A - P_B$) at the two junction points. In this study, a microfluidic device was designed to satisfy the condition that the fluidic resistance in the lower left side channel (R_S) is much greater than that of the bridge channel (R_B) (i.e., $R_S \gg R_B$). Considering the fluidic resistance relation between the side channel and the bridge channel, we assumed that the elasticity effect contributed by the bridge channel which is partially filled with blood is negligible under transient fluidic manipulations of blood. In other words, the blood sample would only participate as a label to characterize fluidic motions in the bridge channel. According to Eqs. (6)–(8), the pressure difference ($\Delta P = P_A - P_B$) at both junctions is expressed as,

$$\Delta P(t) = \frac{128\mu_{\text{Blood}}L}{\pi D^4} (Q_{\text{Blood}}^{\text{TS}} - Q_{\text{Blood}}^{\text{L}}) \left(1 - \exp\left(-\frac{t - t_0}{\lambda_{\text{Blood}}}\right) \right). \quad (9)$$

According to Eq. (9), the pressure difference depends on several factors, including flow rate difference ($Q_{\text{Blood}}^{\text{TS}} - Q_{\text{Blood}}^{\text{L}}$), blood viscosity (μ_{Blood}), and channel dimensions. However, the characteristic time (λ_{Blood}) is only coupled with the elapsed time ($t - t_0$). In addition, as illustrated in Fig. 1C, the simple mathematical formula for the pressure difference (ΔP) properly follows the transient response of the area (A_{Blood}) filled with blood, with respect to time. Thus, based on the mathematical formula for pressure difference [Eq. (9)], the following regression formula, which is composed of constant and exponential terms, is employed to mathematically express the area (A_{Blood}) filled with blood in the detection window:

$$A_{\text{Blood}}(t) = A_x + A_y \exp\left(-\frac{t - t_0}{\lambda_{\text{Blood}}}\right). \quad (10)$$

In the left side of Eq. (10), the area ($A_{\text{Blood}}(t)$) filled with blood can be quantitatively obtained by analysing the microscopic images, which were sequentially captured by the high-speed camera. A least squares method is then applied to determine three unknown parameters (A_x , A_y , and λ_{Blood}) in Eq. (10). The characteristic time (λ_{Blood}) can be calculated from the three parameters. At last, the elasticity of blood (G_{Blood}) is identified using the relaxation formula ($\lambda_{\text{Blood}} = \mu_{\text{Blood}}/G_{\text{Blood}}$) and the viscosity ratio [Eq. (8)], which was measured by monitoring the reversal of flow in the bridge channel at a specific flow rate ($Q_{\text{PBS}}^{\text{SS}}$).

C. Parameter studies for effective measurement of the characteristic time

When the flow rate of PBS solution is set at values of the switching-flow condition ($Q_{\text{PBS}}^{\text{SS}}$), the PBS solution moves to the left direction in the bridge channel, as shown in Fig. 3A-(a). According to Eq. (8), the viscosity of blood in relation to that of the PBS solution equals to the specific flow-rate ratio (i.e., $\mu_{\text{Blood}}/\mu_{\text{PBS}} \approx Q_{\text{PBS}}^{\text{SS}}/Q_{\text{Blood}}^{\text{L}}$). At a specific transient flow rate of blood ($Q_{\text{Blood}}^{\text{TS}}$), blood should unexceptionally move from the left direction (L) to the right direction (R) in the bridge channel. The switching-flow phenomena in the bridge channel were extensively studied to establish this transient flow confidently with varying representative parameters, including (a) the viscosity ratio of blood in relation to that of the PBS solution ($\mu_{\text{Blood}}/\mu_{\text{PBS}}$), (b) the channel-width ratio (W_B/W_S , W_B : channel width of the bridge channel, W_S : channel width of the side channel), (c) the fluidic-resistance ratio (R_B/R_S , R_B : fluidic resistance of the bridge channel, R_S : fluidic resistance of the lower side channel), and (d) the transient flow rate of blood ($Q_{\text{Blood}}^{\text{TS}}$).

After measuring blood viscosity by manipulating the switching flow rate of PBS solution (i.e., $\mu_{\text{Blood}}/\mu_{\text{PBS}} \approx Q_{\text{PBS}}^{\text{SS}}/Q_{\text{Blood}}^{\text{L}}$), the instant of reversal in flow direction [i.e., left direction (L) to right direction (R)] was monitored by varying steady switching flow-rate of blood

FIG. 3. (A) Switching flow-rate of blood in steady blood flows ($Q_{\text{Blood}}^{\text{SW}}$) to induce reverse flows from the left direction (L) to the right direction (R) in the bridge channel with respect to various specific flow-rate ratios ($Q_{\text{PBS}}^{\text{SS}}/Q_{\text{Blood}}^{\text{L}}$). The switching flow-rate of the blood ($Q_{\text{Blood}}^{\text{SW}}$) is independent of the specific flow-rate ratio ($Q_{\text{PBS}}^{\text{SS}}/Q_{\text{Blood}}^{\text{L}}$) ($R^2 \approx 0.05$), which should be greater than 1.3 mL/h to obviously induce reversal flow in the bridge channel. (B) Variations in the switching flow-rate of blood in steady flows ($Q_{\text{Blood}}^{\text{SS}}$) with varying fluidic resistance ratios ($R_{\text{B}}/R_{\text{S}}$) of the bridge channel (R_{B}) to the side channel (R_{S}). (C) Variations of the switching flow rate of blood in steady flow ($Q_{\text{Blood}}^{\text{SS}}$) for various width ratios ($W_{\text{B}}/W_{\text{S}}$) of the bridge channel (W_{B}) to the side channel (W_{S}). (D) Variations of characteristic time which depends on (a) width ratio ($W_{\text{dw}}/W_{\text{B}}$) of the detection window (W_{dw}) to the bridge channel (W_{B}) and (b) transient flow rate of blood ($Q_{\text{Blood}}^{\text{TS}}$).

($Q_{\text{Blood}}^{\text{SS}}$). Under various blood viscosity ratios, the steady switching flow-rate of blood ($Q_{\text{Blood}}^{\text{SS}}$) was measured with varying viscosity ratio or specific flow-rate ratio (i.e., $\beta \approx Q_{\text{PBS}}^{\text{SS}}/Q_{\text{Blood}}^{\text{L}}$) as shown in Fig. 3A-(b). The switching flow-rate of blood is independent of the switching flow-rate ratio (or viscosity ratio of blood). In addition, the steady switching flow-rate should be maintained above 1.3 for a successful reversal of blood flow in the bridge channel. This result implies that the viscosity of blood in relation to that of the PBS solution ($\mu_{\text{Blood}}/\mu_{\text{PBS}}$) does not have any noticeable influence on the switching flow-rate of blood. Instead, the hysteresis phenomena (i.e., $Q_{\text{Blood}}^{\text{SS}} \neq Q_{\text{Blood}}^{\text{L}}$) of blood flow in the bridge channel would be related to other factors rather than viscosity of blood in relation to that of the PBS solution. Thus, in this study, we selected two representative factors, namely, ratio of fluidic resistance ($R_{\text{B}}/R_{\text{S}}$) and ratio of channel width ($W_{\text{B}}/W_{\text{S}}$), which are probably expected to have an influence on the hysteresis behaviors.

In this study, microfluidic devices were specifically designed to satisfy various fluidic resistance ratios that range from 0.1 to 35 ($R_{\text{B}}/R_{\text{S}} = 0.1\text{--}35$). At an extreme case, the switching flow-rate ratio of the PBS solution to the blood was fixed at 4.25 ($Q_{\text{PBS}}^{\text{SS}}/Q_{\text{Blood}}^{\text{L}} = 4.25$). Fig. 3B shows that the steady switching flow-rate of blood ($Q_{\text{Blood}}^{\text{SS}}$) is dependent on the ratio of fluidic resistance ($R_{\text{B}}/R_{\text{S}}$). These experiment results show that the steady switching flow-rate of blood ($Q_{\text{Blood}}^{\text{SS}}$) is strongly related to the ratio of fluidic resistance ($R_{\text{B}}/R_{\text{S}}$). In other words, the steady switching flow-rate of blood tends to increase at lower fluidic resistance ratios but remains constant above a fluidic resistance ratio of 5.

Microfluidic devices with various channel width ratios that range from 0.03 to 1 ($W_{\text{B}}/W_{\text{S}} = 0.03\text{--}1$) were carefully designed and fabricated to maintain the fluidic-resistance ratio at 0.1 ($R_{\text{B}}/R_{\text{S}} = 0.1$) and evaluate the effect of the ratio of channel width ($W_{\text{B}}/W_{\text{S}}$) between

the bridge (W_B) and side channels (W_S) on the switching flow-rate of blood. Fig. 3C shows that the steady switching-flow rate of blood ($Q_{\text{Blood}}^{\text{SS}}$) tends to decrease gradually at lower channel-width ratio. The same channel-width ratio (i.e., $W_S \approx W_B$) induces a maximum switching flow-rate. These experimental results show that for various fluidic-resistance ratios and channel-width ratios, the steady switching flow-rate of blood ($Q_{\text{Blood}}^{\text{SS}}$) is strongly related to these two parameters compared with the ratio of blood viscosity. If the microfluidic channel was designed to possess minimal hysteresis behavior, blood would show reverse flows even at small fluidic fluctuations that result from a syringe pump. In this case, evaluating the blood motion in the bridge channel is difficult. Therefore, the microfluidic device with a little hysteresis behaviors is required to definitely monitor the blood motion in the bridge channel at a specific transient flow condition. After taking into account all these aspects, a microfluidic device for the performance demonstration of the proposed method was appropriately designed to have the bridge channel (width = 243 μm and length = 1800 μm) and both side channels (width = 50 μm and length = 1600 μm). The channel depth was fixed at 50 μm .

In this study, an area (A_{Blood}) filled with blood within the detection window, which acts as a virtual sensor in the bridge channel, was used to analyze the temporal variations of blood motions in the bridge channel. Using a commercial software (Matlab, Mathworks, USA), a gray image was converted into a binary image by setting an optimum threshold value based on the Otsu's method. The blood area (A_{Blood}) was quantitatively evaluated by selecting an appropriate size of detection window for each image and counting the area occupied by blood sample as shown in Fig. 1C. The size of detection window (W_{dw}) should be appropriately selected to effectively measure the characteristic time of blood in transient blood flow. Extensive studies determine the flow rate of blood that induces transient blood flows ($Q_{\text{Blood}}^{\text{TS}}$). For this study, blood (RBCs in PBS suspension, $H_{\text{ct}} = 40\%$) was tested. First, in a steady blood flow, the reverse flow of blood in the bridge channel occurs at a switching flow-rate ratio of 2.1 ± 0.05 ($Q_{\text{PBS}}^{\text{SS}}/Q_{\text{Blood}}^{\text{L}} = 2.1 \pm 0.05$) as shown in Fig. 1C. The viscosity of the PBS solution was measured as 1.0 ± 0.01 mPa·s by using a conventional viscometer (DV-II, Brookfield, USA) at a consistent room temperature of 25 °C. The conventional viscometer consumes 16 mL volumes for each viscosity measurement. Using Eq. (8), the viscosity of blood is identified as 2.1 ± 0.05 mPa·s. The flow rate of blood ($Q_{\text{Blood}}^{\text{TS}}$) was immediately set to 1.5 mL/h to induce transient blood flow, while the flow rate of PBS solution remained at 2.1 mL/h. Owing to a pressure difference ($\Delta P = P_A - P_B$) at the two junctions [Eq. (9)], blood moves to the right direction (R) in the bridge channel. A high-speed camera (PCO, Germany) was employed to capture microscopic images of blood motions in the bridge channel. For all experiments, the frame rate and total number of images were fixed at 200 Hz and 1424, respectively. Given that the suitable size of the detection window has an influence on relaxation identification, the characteristic time was evaluated with varying width ratios (W_{dw}/W_B) of the detection window (W_{dw}) to the bridge channel (W_B), as shown in Fig. 3D-(a). The width ratios were selected from 0.125 to 0.875. The length ratio (L_{dw}/L_B) of the detection window (L_{dw}) to the bridge channel (L_B) was carefully determined as 0.7 to avoid singular flow patterns in the region near both the junctions. Consequently, the characteristic time (λ_{Blood}) is consistently identified as 0.37 ± 0.01 s, particularly when the width ratio is longer than 0.5. Thus, in this study, the width ratio (W_{dw}/W_B) and the length ratio (L_{dw}/L_B) of the detection window were determined at 0.5 and 0.7, respectively.

The characteristic time was measured with varying transient flow-rate of blood from 1.3 mL/h to 2.0 mL/h to evaluate the relationship between characteristic time (λ_{Blood}) and transient flow-rate of blood ($Q_{\text{Blood}}^{\text{TS}}$), as shown in Fig. 3D-(b). The relaxation is not significantly changed when the transient flow rate of blood is above 1.7 mL/h. This observation implies that the minimum characteristic time is identified at 0.25 ± 0.03 s. As the transient flow rate of blood is increased from 1.3 mL/h to 1.7 mL/h, the characteristic time tends to decrease from 0.46 ± 0.06 s to 0.25 ± 0.03 s. In other words, higher transient flow rates increase the pressure difference (ΔP) at both junctions [Eq. (9)], which results in reducing the elapsed time to fill the detection window. Based on these results, the transient flow rate of blood ($Q_{\text{Blood}}^{\text{TS}}$) was fixed at 1.5 mL/h to induce reliable transient flow of blood throughout all experiments.

D. Evaluation of elasticity effect on a microfluidic system

To carefully investigate elasticity effect contributed by a microfluidic system, four different concentrations of glycerin solution (5%, 10%, 20%, and 40%), which behave as Newtonian fluid and does not include elasticity, were prepared. First, viscosity measurement was conducted using the proposed method and the conventional viscometer. As shown in Fig. 4A, the viscosity values identified by the proposed method show sufficient accuracy within 7% normalized difference (ND) between the proposed method and the conventional method. Next, after completing viscosity identification of glycerin solutions, flow rate of the glycerin solution is immediately changed to induce transient fluidic behaviors. Characteristic time (λ) for each glycerin solution is estimated using the proposed method. Here, the elasticity measured by the proposed method is only contributed by the components of the microfluidic system. Among components of the microfluidic system, flexible tube (ID = 250 μm , OD = 760 μm , and L = 400 mm) and the PDMS microfluidic channel would have a strong influence on a characteristic time. In addition, the glycerin solution as test fluid does not include elasticity effect. In other words, the elasticity contributed by the microfluidic system is estimated as $G_{\text{sys}} = \mu_{\text{glycerin}}/\lambda - G_{\text{glycerin}} \approx \mu_{\text{glycerin}}/\lambda$, by

FIG. 4. (A) Quantitative comparison of viscosity values obtained by the proposed method and the conventional method using four concentrations of glycerin (5%, 10%, 20%, and 40%). (B) Quantitative evaluation of elasticity effect contributed by the microfluidic system composed of flexible microfluidic channel and connecting tubes. (C) Variation of relaxation time measured by a conventional rotational rheometer using PEO (0.25%) and glycerine (10%) solutions with respect to shear rate. (D) Variations of relaxation time estimated at a shear rate of $1.3 \times 10^4 \text{ s}^{-1}$. (E) Variations of viscosity for diluted PEO solutions (0.06%, 0.13%, 0.19%, and 0.25%) with respect to shear rate. (F) Variations of elasticity measured by the proposed method and the conventional method with respect to various concentrations of PEO solution. The inset shows that the elasticity measured by the proposed method (Pm) is comparable to the result obtained by the conventional method (Cm).

selecting pure liquid which does not include with elasticity. According to experimental results as shown in Fig. 4B, the characteristic time (λ) tends to increase depending on viscosity of glycerin as expected. However, elasticity contributed only by the microfluidic system is consistently remained at 0.73 ± 0.04 mPa without respect to viscosity values. Taking into account the fact that the consistent elasticity is only contributed by the microfluidic system, the minimum elasticity value by the proposed method is about 0.73 ± 0.04 mPa, which is considered as a threshold value and should be compensated in the measurement of elasticity of each fluid.

On the other hand, to quantitatively evaluate the elasticity contributed by the proposed method, we measured elasticity of glycerin (10%) solution using a conventional rotational rheometer (HAAKE MARS III, Thermo Fisher Scientific, Germany). For convenience, frequency was fixed to 0.5 Hz during viscoelasticity measurement. Due to operation limitations, the maximum shear rate of the conventional rheometer was fixed at 1020 s^{-1} . As shown in Fig. 4C, relaxation time tends to increase linearly with respect to shear rate. According to linear regression analysis, the relaxation time is linearly proportional to shear rate ($R^2 \approx 0.9682$). At the same shear rate condition of $1.3 \times 10^4 \text{ s}^{-1}$ in the microfluidic channel, the characteristic time of test fluid solution is roughly estimated as 927.25 ± 44.9 ms by applying the regression formula as shown in Fig. 4D. Thus, the effect contributed by the conventional rotational rheometer is estimated to be 1.5 ± 0.07 mPa. Comparing the values of elasticity contributed by the proposed method and the conventional rotational rheometer, we found that the elasticity effect contributed by the present microfluidic system is comparable to the result obtained by the conventional rotational viscometer, even under high relaxation time.

E. Viscoelasticity measurement of diluted PEO solution

Lastly, the proposed method is applied to measure viscosity and elasticity of diluted PEO (Polyethylene Oxide, MW = 2M, Sigma-Aldrich, USA) solution, which behaves as a non-Newtonian fluid and includes elasticity. Four concentrations of the PEO solution [(a) $C_{\text{PEO}} = 0.06\%$, (b) $C_{\text{PEO}} = 0.13\%$, (c) $C_{\text{PEO}} = 0.19\%$, and (d) $C_{\text{PEO}} = 0.25\%$], which were diluted using DI-water, were prepared. To validate the performance of the proposed method, the values of elasticity of four different concentrations of PEO solution were measured using the proposed method and the conventional rotational rheometer. As shown in Fig. 4C, the relaxation time of each PEO solution was measured using the conventional method at shear rates ranging from 100 to 1020 s^{-1} . A linear regression formula for each fluid was obtained through performing regression analysis. As shown in Fig. 4D, the relaxation time for each PEO solution was roughly estimated at a shear rate of $1.3 \times 10^4 \text{ s}^{-1}$, which corresponds to the wall shear rate in the microfluidic system. As a result, the relaxation time of PEO solutions tends to decrease with increasing the concentration of PEO solution. Based on the relaxation time and viscosity measured by the conventional method, the elasticity of each PEO solution is estimated using the formula of relaxation time (i.e., $G_{\text{PEO}} = \mu/\lambda - G_{\text{sys}}$). Here, the viscosity of each PEO solution (μ) obtained by using the proposed method is used, because the conventional method cannot measure viscosity of each fluid, especially at a high shear rate of $1.3 \times 10^4 \text{ s}^{-1}$. Second, the elasticity of each PEO solution was measured using the proposed method. To clearly visualize interface between two fluids in side channel, the glycerin (5%) was used as reference fluid, instead of the PBS solution. First, viscosity of each PEO solution was measured using the proposed method, under various shear rates. As shown in Fig. 4E, the PEO solution behaves as a non-Newtonian fluid, where viscosity tends to decrease at high shear rates gradually. In addition, the viscosity tends to increase depending on the concentration of the PEO solution, and remains constant above 10000 of shear rate ($\dot{\gamma} > 10000 \text{ s}^{-1}$). Especially at the shear rate of $1.3 \times 10^4 \text{ s}^{-1}$, the proposed method can be applied to measure elasticity of the PEO solution. Fig. 4F shows the variations of elasticity measured by the proposed method and the conventional method with varying the concentrations of PEO solution. The elasticity tends to increase gradually with increasing concentration of PEO solution. For four concentrations, the PEO solution has distinctively different elasticity ($P < 0.05$). The present data are comparable with the result in a previous study.³⁰ The values of elasticity measured by the proposed method are

roughly comparable to those obtained by the conventional method. The inset shows that the both methods are strongly correlated with each other ($R^2=0.9827$). The proposed method (Pm) seems to underestimate the elasticity of PEO solution about 10%, compared to the conventional method (Cm). These results support that the present method proposed in this study is capable of measuring elasticity of viscoelastic fluids with reasonable accuracy.

III. RESULTS AND DISCUSSION

A. Blood viscosity and elasticity versus hematocrits (H_{ct})

The blood samples tested in this study were provided by a blood bank (Daegu-Kyeongbuk Blood Bank, Korea) other than rat blood. We prepared blood samples with different hematocrits that range from 20% to 50% to evaluate variations in viscosity and elasticity of blood with respect to hematocrits.³¹ The hematocrit of each blood sample was properly adjusted by adding RBCs into the PBS solution. The PBS solution (1x, pH 7.4, Bio Solution, Korea), which behaves as Newtonian fluid and does not have elasticity effect, was used as the reference fluid. Fig. 5A shows the variations of viscosity, elasticity, and characteristic time of blood with respect to the hematocrit that ranges from 20% to 50%. The viscosity of blood for each hematocrit is found to be (a) 1.35 ± 0.03 mPa·s for $H_{ct}=20\%$, (b) 1.75 ± 0.03 mPa·s for $H_{ct}=30\%$, (c) 2.1 ± 0.05 mPa·s for $H_{ct}=40\%$, and (d) 2.50 ± 0.05 mPa·s for $H_{ct}=50\%$. The viscosity of blood gradually increases with the increase of hematocrit. This increasing tendency of blood viscosity is also shown in the previous study.²⁸ In addition, the characteristic time gradually decreased with respect to hematocrit. The blood elasticity (G_{Blood}) was identified using the relaxation formula ($\lambda_{Blood} = \mu_{Blood}/G_{Blood}$). The corresponding elasticity of blood for each hematocrit was found to be (a) 2.92 ± 0.26 mPa for $H_{ct}=20\%$, (b) 4.01 ± 0.21 mPa for $H_{ct}=30\%$, (c) 4.99 ± 0.25 mPa for $H_{ct}=40\%$, and (d) 6.64 ± 0.68 mPa for $H_{ct}=50\%$. When blood moves from a sufficiently large channel to a narrow channel (diameter < 300 μ m), the hematocrit of blood tends to decrease abruptly, depending on the channel size due to the existence

FIG. 5. (A) Variations of viscosity and elasticity of bloods with various hematocrits of human blood (RBCs in PBS suspension) ranging from 20% to 50%. (B) Variations in viscosity and elasticity of human blood (RBCs in PBS suspension, $H_{ct}=40\%$) exposed to the high temperature of 50 °C for 30 min. (C) Variations in viscosity, elasticity, and hematocrit of human blood (RBCs in PBS suspension, $H_{ct}=40\%$) under extremely high flow rate of $Q=53 \pm 0.5$ mL/h for 120 min. (D) Comparison of (a) plasma viscosity (μ_{plasma}) of blood drawn from a normal rat and spontaneously hypertensive rate, (b) the variations in viscosity and elasticity of blood (RBCs in PBS suspension, $H_{ct}=25\%$) extracted from both rats.

of cell-free layer.³² For this reason, the present result obtained in the narrow microfluidic channel (i.e., equivalent diameter = $50\text{ }\mu\text{m}$) would be quantitatively different from the result measured by the conventional rheometer. However, the present result is comparable with the previous result, in the point of dependency on hematocrit.²⁸ In other words, viscosity and elasticity of blood have higher values at higher hematocrits, even in narrow microfluidic channels ($P < 0.05$). In conclusion, this proposed method is capable of simultaneously measuring viscosity and elasticity of various blood samples, depending on varying hematocrits that range from 20% to 50%.

B. Human blood with thermal-induced treatment

The proposed method was applied to evaluate the sub-lethal damage of RBC under thermal-induced treatment. First, 5 mL of human blood (RBCs in PBS suspension, $H_{ct} = 40\%$) was transferred into a plastic bottle. The plastic bottle was immersed in a controllable water bath. The bottle was then heat-treated at the temperature of $50\text{ }^{\circ}\text{C}$ for 30 min. After the heat treatment, the plastic bottle was cooled to room temperature of $25\text{ }^{\circ}\text{C}$. Thereafter, the viscosity and characteristic time of the heat-treated blood were measured using the proposed method. As shown in Fig. 5B, experimental results were represented with respect to the exposure time from 0 min to 30 min at the temperature of $50\text{ }^{\circ}\text{C}$. The viscosity of blood slightly increased from $2.1 \pm 0.05\text{ mPa}\cdot\text{s}$ at $t = 0\text{ min}$ to $2.25 \pm 0.05\text{ mPa}\cdot\text{s}$ at $t = 30\text{ min}$. By considering the fact that blood viscosity is consistently maintained for 30 min, the RBC membrane still keeps mechanical function up to 30 min ($P > 0.05$). On the other hand, the elasticity of blood decreases gradually for the initial 20 min, and thereafter shows a rapid decrease at 30 min ($P < 0.05$). This result indicates that the elasticity of blood is largely influenced by the thermal-induced treatment. In other words, the heat treatment of blood may induce sub-lethal damage to RBCs with rapid decrease in elasticity.

C. Human blood under high shear stress

For the third application, the proposed method was applied to investigate human blood, whose RBC membrane was damaged by mechanically-induced shear stress. 5 mL of human blood (RBCs in PBS suspension, $H_{ct} = 40\%$) was transferred into a plastic bottle. A fluidic circuit was constructed by connecting a silicon tube (ID = 1.05 mm, OD = 1.55 mm, and length = 1000 mm) between the plastic bottle and a peristaltic pump (MP-1000, Eyela, Japan), as shown in Fig. 5C.

The pump was operated at an extremely high flow-rate to induce mechanical damage on RBC membrane. The pump was operated for 120 min and the flow rate was measured at $53 \pm 0.5\text{ mL/h}$ by using an electronic balance (AP250D, Ohaus, USA). We hypothesized that RBC membrane would be damaged with membrane rupture under this experimental conditions. The variations in viscosity and elasticity of blood were monitored with respect to operation time in order to verify this hypothesis. The hematocrit variation was also measured to indirectly investigate the results identified by the proposed method. For this, we can see that viscosity, elasticity, and hematocrit of blood can be used to evaluate the damage of RBC membrane with respect to pump operation time. Fig. 5C represents the variations in viscosity, elasticity, and hematocrit of human blood, which was exposed to a mechanical shear stress for 120 min. The viscosity and elasticity of blood are consistently maintained for 120 min ($P > 0.05$). To indirectly compare with these results, variations in hematocrit were also monitored as a function of time. The hematocrit does not change significantly during pump operation for 120 min ($P > 0.05$). These results suggest that the damage of RBC membrane does not occur, contrary to our beginning hypothesis. Furthermore, the hematocrit of blood exhibits a similar trend to the viscosity and elasticity of blood identified by the proposed method. Therefore, we conclude that the proposed method is sufficient to investigate the damage of RBC membrane owing to unfavorable circumstances.

D. Comparison of normal rat versus hypertensive rat

For the last application, the proposed method was applied for whole blood sample extracted from a spontaneously hypertensive rat and a normal rat to measure the biophysical properties,

such as plasma viscosity, viscosity, and elasticity of RBCs. 5 mL of whole blood was drawn from the posterior vena cava.³³ After the plasma and RBCs were separated using a centrifuge, the hematocrit of blood was carefully adjusted to 25% by adding RBCs into PBS suspension. To separate the effects of plasma and RBCs, which have dominant influences on the viscosity and elasticity of blood, plasma viscosity was firstly measured to assess the rheological difference in plasma. Then, the rheological features of RBCs were investigated by measuring the viscosity and elasticity of the blood, which was prepared by adding the RBCs in PBS suspension. At first, the plasma viscosities of both rats were measured using the present method, as shown in Fig. 5D-(a). The plasma viscosity for the normal rat is $\mu_{\text{Plasma}} = 1.34 \pm 0.03$ mPa·s, while that of the hypertensive rat is 3.88 ± 0.05 mPa·s. This result indicates that the plasma viscosity of the hypertensive rat is much larger than that of the normal rat, as expected. The viscosity and elasticity of the blood for each rat were measured using RBCs in PBS suspension to investigate the changes in rheological properties of RBCs for each rat model, as shown in Fig. 5D-(b). The viscosity of blood extracted from the normal rat is measured to be 1.45 ± 0.03 mPa·s, while that collected from the hypertensive rat is identified to be 1.6 ± 0.05 mPa·s. In addition, the elasticity of blood extracted from the normal rat is 4.79 ± 0.88 mPa. But elasticity of blood collected from the hypertensive rat is 6.89 ± 1.25 mPa. These results show that the spontaneously hypertensive rat has higher values of viscosity and elasticity, compared with the normal rat ($P < 0.05$). Furthermore, this result well matched with previous result.^{34–36}

From these experimental demonstrations, we can draw a conclusion that the new method proposed in this study is capable of effectively evaluating physical properties of blood, including viscosity and elasticity, with reasonable accuracy.

IV. CONCLUSION

In this study, the viscosity and elasticity of blood were measured simultaneously using flow controls in a microfluidic device, without sensors and labeling. In steady blood flows, the viscosity of blood in relation to that of the PBS solution ($\mu_{\text{Blood}}/\mu_{\text{PBS}}$) was measured by monitoring the reverse flow in the bridge channel at a specific flow-rate rate ($Q_{\text{PBS}}^{\text{SS}}/Q_{\text{Blood}}^{\text{L}}$). In transient blood flows, the characteristic time of blood (λ_{Blood}) was measured by analyzing the area (A_{Blood}) filled with blood within the detection window, which acts as a virtual sensor in the bridge channel. The elasticity of blood (G_{Blood}) was then identified by applying the relationship between the characteristic time and the viscosity of test blood sample. Based on the linear viscoelasticity model that represents the viscoelastic behaviours of blood in a microfluidic channel, a viscosity formula of blood was analytically derived in the steady blood flow. Then, a regression formula was appropriately derived based on the pressure difference ($\Delta P = P_A - P_B$) at both junctions (A, B) in transient blood flows to estimate the characteristic time of blood. Among several parameters, which have an influence on the transient blood flows, the channel-width ratio and the fluidic-resistance ratio are strongly related to the switching flow-rate of blood in the bridge channel, rather than blood-viscosity ratio. The transient flow-rate of blood was determined at 1.5 mL/h ($Q_{\text{Blood}}^{\text{TS}} = 1.5$ mL/h) to establish the transient flow confidently. The area (A_{Blood}) filled with blood within the bridge channel was analyzed by applying the detection window (width ratio=0.5 and length ratio=0.7) to the microscopic images. Elasticity contributed by the microfluidic system, which is composed of flexible tube and microfluidic device, was consistently measured at 0.73 ± 0.04 mPa, which is considered as a threshold value for this proposed method. Furthermore, using diluted PEO solutions as viscoelasticity fluid, the proposed method was demonstrated to accurately measure viscosity and elasticity simultaneously. As a result, the proposed method underestimates the elasticity of PEO solution about 10%, compared to the conventional rotational viscometer. These results support that the proposed method is capable of measuring elasticity of viscoelastic fluids with reasonable accuracy. For practical demonstrations, the present method was used to evaluate changes in viscosity and elasticity of blood samples: (a) different hematocrits (20% to 50%), (b) thermal-induced treatment (50 °C for 30 min), and (c) flow-induced shear stress (53 ± 0.5 mL/h for 120 min). In addition, the biophysical properties of blood drawn from a normal rat and a

spontaneously hypertensive rat were also measured for comparison. From these experimental demonstrations, we can draw a conclusion that the proposed method is capable of effectively evaluating the biophysical properties of bloods, including viscosity and elasticity, without fully integrated sensors, and tedious labeling. In a near future, the proposed method will be applied to investigate the hemorheological properties of patients with CVDs.

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