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Microfluidic Biosensor for Monitoring Temporal Variations of Hemorheological and Hemodynamic Properties Using an Extracorporeal Rat Bypass Loop

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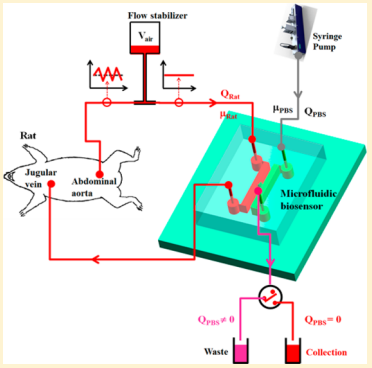
ABSTRACT: In this study, we propose a novel microfluidic biosensor for monitoring hemorheological and hemodynamic properties using an extracorporeal rat bypass loop. To monitor temporal variations of biophysical properties including viscosity, flow rate, and pressure of rat blood, a complex fluidic network is established by connecting the abdominal aorta and jugular vein to an extracorporeal bypass loop including a flow stabilizer and a microfluidic biosensor. Three biophysical properties are simultaneously measured through label-free operation and sensorless detection based on two sequential flow controls in the microfluidic channel. A discrete fluidic-circuit model is employed to derive analytical formulas for the complex fluidic network. First, to evaluate the measurement accuracy of the proposed method, a peristaltic pump is used as substitute for a rat. The flow rate and viscosity of 20% glycerin (test fluid) circulating within the fluidic network are measured, and then the results are compared with those obtained using conventional methods. The normal differences between two measurement methods are less than 4%. Then, the proposed method is used to monitor temporal variations in biophysical properties of blood circulating within the complex fluidic network under normal and continuous hemodilution conditions. Rats require at least 30 min to adapt to different fluidic environments. The flow rate, pressure, and hematocrit of rat blood tend to decrease gradually because of continuous hemodilution effect. Furthermore, the reduced flow rate increases blood viscosity under hemodilution condition. These experiments demonstrate that the proposed method can effectively monitor temporal variations of biophysical properties of rat blood under ex vivo conditions.



The biophysical properties of blood are distinctively influenced by the pathophysiological processes of circulatory vascular diseases or various clinical states.[1] Among them, blood viscosity is considered as a significant factor for monitoring variations in physiological and pathological conditions. In addition, blood viscosity is altered by several factors, such as hematocrit,[2] deformability,[3−6] aggregation,[7−9] and microvascular diameter.[10]

In vivo hemorheological studies in organs or tissues have been conducted by sensing pressure and flow rate in a perfusion circuit.[11] Blood viscosity has been measured by comparing the flow-rate ratio of blood to plasma in the same limb under the same arterial pressure.[11] In addition, to evaluate hemorheological alternations, a fluidic resistance of organs or tissues has been estimated by applying a linear relation between pressure and flow rate.[10,12]

Whittaker et al.[11] reported that blood viscosity obtained by an Oswald-viscometer (in vitro) is higher than blood viscosity estimated from data on pressure and flow rate from the hind limb of a dog (in vivo). The difference in blood viscosities, measured through in vivo and in vitro experiments, results from Fahraeus−Lindqvist effect depending on tube-diameter owing to the presence of a cell-free layer,[13−15] an axial migration of deformable red blood cells,[16,17] endothelial glycocalyx,[18] and vascular hindrance (or vasomotor control).[1,10,19] Since blood viscosity strongly depends on vessel diameters and tends to decrease at narrow conduits, the difference in the diameter of blood vessels and a conventional viscometer results in a difference in blood viscosity. The reason is that the diameter of blood vessels is significantly much smaller than that of the Oswald-viscometer. Therefore, a new measurement method which can identify blood viscosity under in vivo and in vitro environments is definitely required to resolve such difference in blood viscosity measurements. In other words, a new measurement technique which is fundamentally different from the conventional viscometer should be developed to measure blood viscosity with sufficient accuracy.

Recently, a microfluidic platform has been introduced for effectively manipulating small amounts of fluid in microfluidic channels for biomedical applications. Using such a microfluidic platform, various methods including a comparator with parallel flows,[20−24] fluidic Wheatstone-bridges with pressure sensors,[25]

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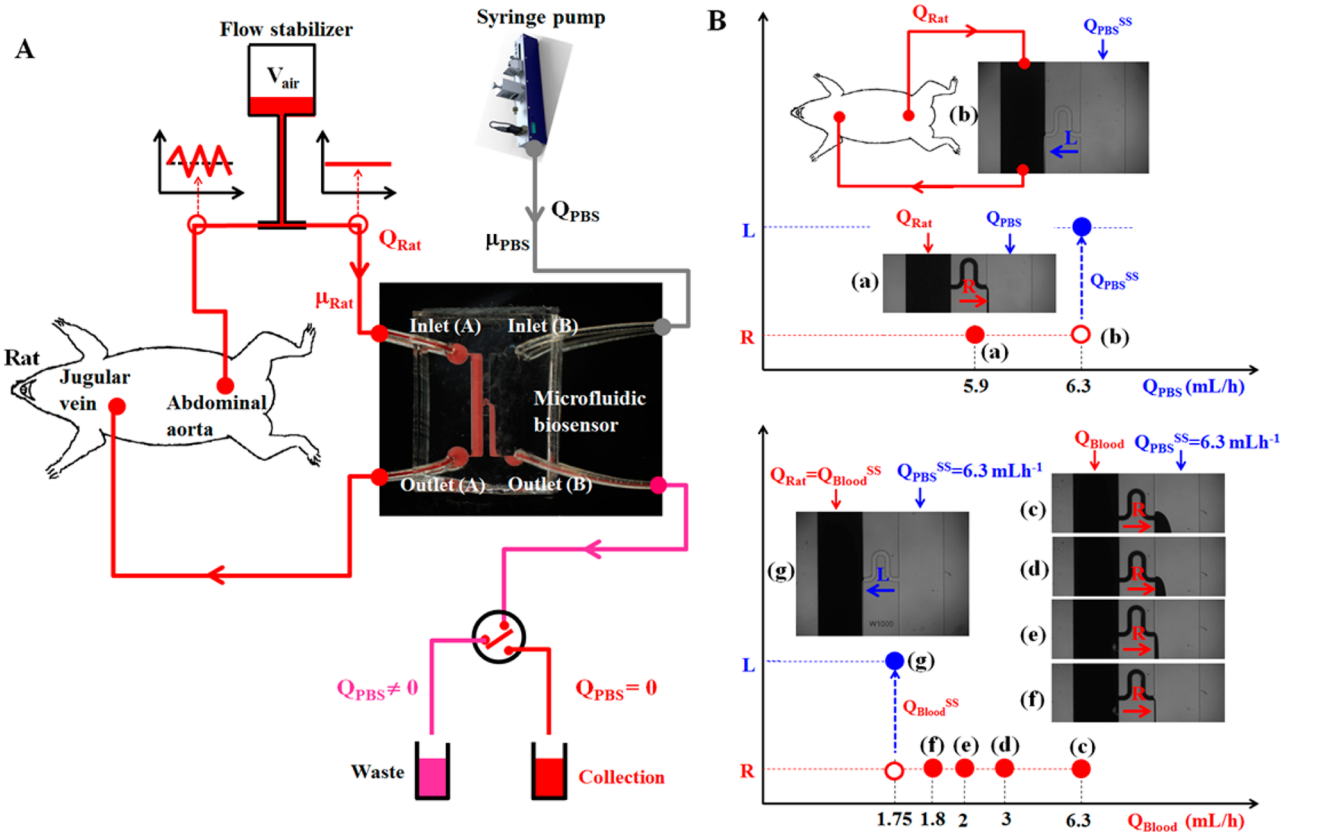

Figure 1. Schematic diagram of the proposed method using two sequential flow controls in a microfluidic channel for monitoring temporal variations of the biophysical properties of rat blood circulating within a complex fluidic network. (A) A complex fluidic network established by connecting the abdominal aorta and jugular vein to an extracorporeal rat bypass loop. The extracorporeal rat bypass loop consists of a flow stabilizer (air-cavity volume [V_{air}] = 2 mL) and a microfluidic biosensor. To measure the three biophysical properties (viscosity, flow rate, pressure), PBS solution is delivered as reference fluid at a specified flow rate (Q_{PBS}) and viscosity (μ_{PBS}) by a syringe pump. (B) Two sequential flow controls for measuring the biophysical properties of rat blood within a complex fluidic network. First, rat blood is delivered into inlet (A). PBS solution is simultaneously supplied into inlet (B) at various flow rates ([a] Q_{PBS} = 5.9 mL/h, [b] Q_{PBS} = 6.3 mL/h) by a syringe pump. The switching flow rate of the PBS solution, which induces reverse flow in the bridge channel, is 6.3 mL/h. Rat blood is collected from outlet (B) after the syringe pump for delivering PBS solution is stopped. Second, PBS solution is supplied into inlet (B) at a flow rate of Q_{PBS}^{SS} = 6.3 mL/h by a syringe pump. The collected rat blood is simultaneously delivered into inlet (A) at various flow rates ([c] Q_{Blood} = 6.3 mL/h, [d] Q_{Blood} = 3 mL/h, [e] Q_{Blood} = 2 mL/h, [f] Q_{Blood} = 1.8 mL/h, [g] Q_{Blood} = 1.75 mL/h) by a syringe pump. The switching flow rate of rat blood is 1.75 mL/h. The viscosity and flow rate of rat blood within a complex fluidic network are 3.6 cP and 1.75 mL/h, respectively.

microcantilever resonator,[26] capillary-driven flow,[27−29] and droplet-based movements[30] have been employed to measure the viscosity of pure liquids. Most of these methods might require additional procedures such as labeling operation, sequential image processing, and calibration. Additionally, the flow-compartment method[31] and flow-switching method[32] have been suggested to measure blood viscosity with respect to a reference fluid with known viscosity. However, these proposed methods have been demonstrated, only at a given flow rate of each fluid. Thus, previous methods have technical limits in measuring the viscosity of blood circulating within a complex fluidic network (that is, ex vivo condition), wherein the flow rate is not specified. As such, a new measurement method, which can measure blood viscosity at unspecified flow-rate conditions, is required to measure ex vivo hemorheological properties.

In this study, we propose a microfluidic biosensor for monitoring temporal variations of hemorheological and hemodynamic properties using an extracorporeal rat bypass loop. To monitor the temporal variations of the biophysical properties of blood under ex vivo conditions, a complex fluidic network is established by connecting the abdominal aorta and jugular vein to an extracorporeal rat bypass loop.[33] Based on two sequential flow controls in the microfluidic channel, three biophysical properties, namely, blood flow rate, blood viscosity, and pressure are simultaneously measured via label-free operation and sensorless detection. The proposed method has three distinctive advantages over conventional viscosity measurement methods. First, the proposed method can simultaneously measure three biophysical properties (viscosity, flow rate, and pressure) of blood circulating within a complex fluidic network (that is, ex vivo hemorheological and hemodynamic properties), wherein the blood flow rate is not specified. Second, blood viscosity values measured under ex vivo and in vitro conditions exhibits no difference. That is, all biophysical properties can be measured with the microfluidic biosensor under ex vivo or in vitro condition. Lastly, the proposed method does not require a flow-rate sensor, which usually causes serious contamination issues and requires tedious calibration procedures.

To demonstrate the feasibility and usefulness of the proposed method, a simple analytical study is conducted using a discrete circuit model for a complex fluidic network. The analytical formulas of viscosity, flow rate, and pressure are

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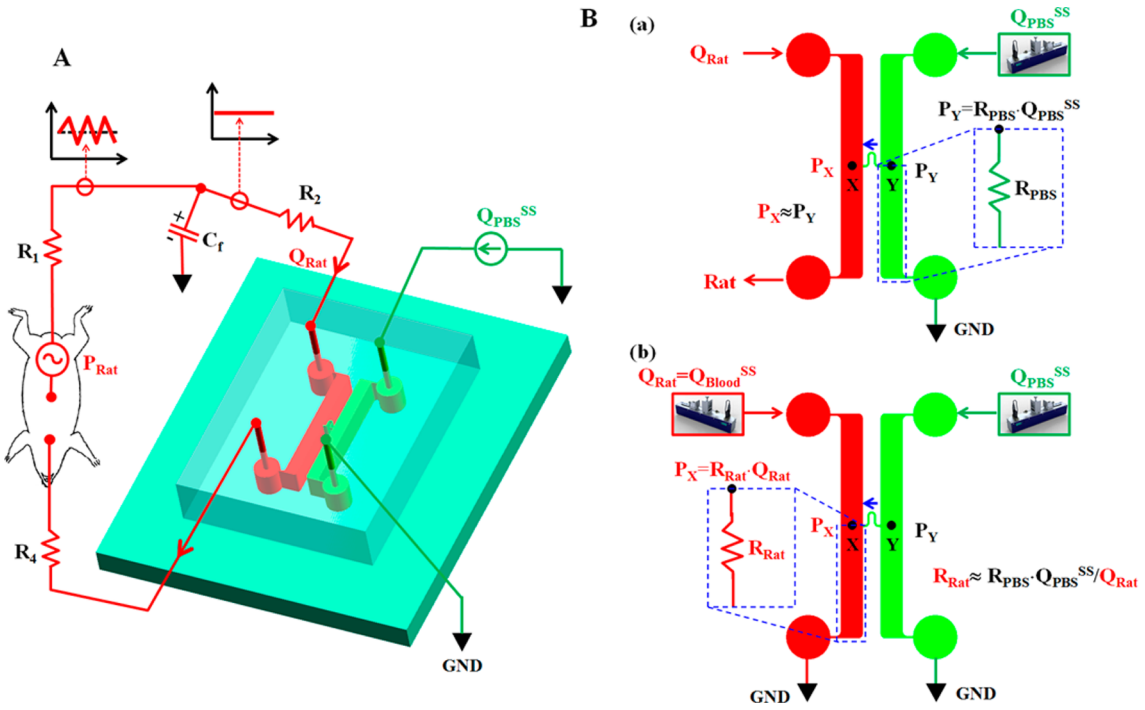

Figure 2. A discrete fluidic circuit model for a complex fluidic network comprising a rat, a flow stabilizer, and a microfluidic biosensor. (A) The fluidic circuit is composed of fluidic resistances (R_1, R_2, R_4, R_{Rat}, R_{PBS}), air compliance (C_f) resulting from the flow stabilizer, flow rates (Q_{Rat}, Q_{PBS}), and pressure at the abdominal aorta (P_{Rat}). (B) A mathematical model for balancing pressure (P_X, P_Y) at each junction (X, Y) in the two flow controls. (a) Balancing pressure P_Y at a switching flow rate of PBS solution (Q_{PBS}^{SS}) and (b) balancing pressure P_X at a switching flow rate of rat blood (Q_{Blood}^{SS}).

derived based on two sequential flow controls to induce the hydrodynamic balancing in the microfluidic channel. To evaluate the measurement accuracy of the proposed method, a peristaltic pump is used as substitute for a rat. Under various pumping speeds, flow rate and viscosity of 20% glycerin (test fluid) circulating within a complex fluidic network are measured using the proposed method. The measurement results are compared with the ones obtained through conventional methods such as application of a microbalance and a conventional viscometer. The proposed method is also employed to monitor temporal variations in the biophysical properties of blood circulating within the complex fluidic network using an extracorporeal rat bypass loop. Under continuous hemodilution condition by adding phosphate buffered saline (PBS) solution to the fluidic network, the proposed method is then applied to monitor temporal variations of biophysical properties including viscosity, flow rate, pressure, and hematocrit.

EXPERIMENTAL SECTION

Measurement of Biophysical Properties Using Two Sequential Flow Controls. A novel microfluidic biosensor is proposed to monitor hemorheological and hemodynamic properties using an extracorporeal rat bypass loop. Three biophysical properties, namely, flow rate, viscosity, and pressure are measured simultaneously based on two sequential flow controls in the microfluidic channel. To demonstrate the proposed method, a microfluidic Wheastone-bridge biosensor is designed with two inlets (A, B), two identical and parallel side channels (width = 1000 μm, length = 10 mm), one bridge channel (width = 100 μm) connected to the middle of the side channels, and two outlets (A, B). Channel depth is fixed at 50 μm. As illustrated in Figure 1A, a complex fluidic network for ex vivo experiments is established by connecting the abdominal aorta and jugular vein to an extracorporeal rat bypass loop, which includes a flow stabilizer with 2 mL air cavity[34] and a microfluidic biosensor. Rat blood (test fluid) is supplied consistently from the abdominal aorta to the microfluidic biosensor with the help of the flow stabilizer, which acts as a fluidic low-pass filter. However, the flow rate (Q_{Rat}) of rat blood is not specified because of the absence of a flow-rate sensor. To measure the viscosity (μ_{Rat}) and flow rate (Q_{Rat}) of rat blood, 1× PBS solution (reference fluid) is delivered at a specific flow rate (Q_{PBS}) by a syringe pump. The viscosity of PBS solution (μ_{PBS}) was measured in advance using a conventional viscometer. The left side channel and bridge channel are filled with rat blood under suitable flow-rate control of PBS solution. Thus, rat blood only returns from outlet (A) to the jugular vein. However, the right side channel is partially filled with rat blood and PBS solution, which is discarded from outlet (B) as a waste. Rat blood is collected only after the syringe pump delivering the PBS solution is stopped.

As shown in Figure 1B, two flow controls are applied to measure the three biophysical properties of blood circulating within a complex fluidic network. In the first step, rat blood is supplied consistently into the inlet (A) at an unspecified flow rate of Q_{Rat}. PBS solution is simultaneously delivered into the inlet (B) at a specific flow rate of Q_{PBS} by a syringe pump. As shown in Figure 1B-(a) and 1B-(b), when the flow rate of PBS solution is changed from (a) Q_{PBS} = 5.9 mL/h to (b) Q_{PBS} = 6.3 mL/h, both side channels immediately achieve a hydrodynamically balanced condition.[32] Reverse flow of rat blood occurs in the bridge channel at a switching flow rate of Q_{PBS}^{SS} = 6.3 mL/h. Equilibrium pressure at the left and right side junctions can be estimated by a linear relationship between pressure and flow

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rate. After the syringe pump supplying the PBS solution is stopped, pure rat blood is collected into a plastic bottle from the outlet (B). In the second step, the rat blood collected during the first step is supplied into the inlet (A) by a syringe pump. PBS solution is supplied simultaneously into inlet (B) at the same switching flow rate (Q_{PBS}^{SS}) as that in the first step. To induce the same flow-switching direction from right to left as the first step, the flow rate of rat blood (Q_{Blood}) is reduced from (c) Q_{Blood} = 6.3 mL/h to (g) Q_{Blood} = 1.75 mL/h, while change in flow direction of rat blood in the bridge channel is monitored. As shown in Figure 1B-(c)−1B-(g), reverse flow occurs in the bridge channel at a switching flow rate of Q_{Blood}^{SS} = 1.75 mL/h. The unknown flow rate of rat blood (Q_{Rat}) is evaluated as 1.75 mL/h ($Q_{Rat} = Q_{Blood}^{SS}$) from this experiment. Based on the hydrodynamic balancing, the viscosity formula of rat blood (μ_{RAT}) is derived as $\mu_{RAT} = \mu_{PBS} \times Q_{PBS}^{SS}/Q_{RAT}$. Thus, the viscosity of rat blood circulating within the complex fluidic network including the extracorporeal rat bypass loop at the blood flow rate of 1.75 mL/h is 3.6 cP.

Analytical Study Using a Discrete Fluidic-Circuit Model. As shown in Figure 2A, the flow direction of rat blood is changed from right to left in the bridge channel, under a hydrodynamically balanced condition ($P_X \approx P_Y$). A simple fluidic circuit model for the present fluidic network consisting of a rat, a flow stabilizer, and the microfluidic biosensor is established using fluidic resistances (R_1, R_2, R_4, R_{Rat}, R_{PBS}), air compliance (C_f) resulting from the flow stabilizer, flow rates (Q_{Rat}, Q_{PBS}^{SS}), and pressure at the abdominal aorta (P_{Rat}). Unstable flow fluctuations in the abdominal aorta are stabilized by a flow stabilizer. Thus, the flow rate of rat blood is supplied consistently into the inlet (A). However, the flow rate of rat blood (Q_{Rat}) is not specified. PBS solution is delivered simultaneously into the inlet (B), at a switching flow rate of Q_{PBS}^{SS}. Through the simple fluidic circuit model for the complex fluidic network as shown in Figure 2A, a governing equation describing the relationship between pressure at the abdominal aorta (P_{Rat}) and balancing pressure at the left junction (P_X) is derived as

$$\alpha \frac{dP_X(t)}{dt} + \beta P_X(t) = P_{Rat}(t) \tag{1}$$

In eq 1, the coefficients α and β are given as

$$\alpha = R_1 C_f \left(2 + \frac{R_2}{R_{Rat}} - \frac{2}{1 + \frac{R_{Rat}}{R_4}} \right) \tag{2}$$

$$\beta = 2 + \frac{R_1}{R_{Rat}} + \frac{R_2}{R_{Rat}} - \frac{2 + \frac{R_1}{R_2}}{1 + \frac{R_{Rat}}{R_4}} \tag{3}$$

In eq 3, the pressure at the abdominal aorta (P_{Rat}) which consists of the mean pressure (P_0) and oscillating pressure (P_n) with a fundamental period (T) is described as

$$P_{Rat}(t) = P_0 + \sum_{n=1}^{m} P_n \sin\left(\frac{2\pi n t}{T}\right) \tag{4}$$

By substituting eq 4 into eq 1, the following equation can be derived as

$$\tau_f \frac{dP_X(t)}{dt} + P_X(t) = \frac{P_0}{\beta} + \sum_{n=1}^{m} \frac{P_n}{\beta} \sin\left(\frac{2\pi n t}{T}\right) \tag{5}$$

In eq 5, τ_f denotes a time constant which is expressed as $\tau_f = \alpha/\beta$. If the time constant is sufficiently larger than the period (T), then the dynamic term in eq 5 is negligible. Thus, eq 5 is simplified as

$$P_X(t) = \frac{P_0}{\beta} \tag{6}$$

Therefore, the mean pressure at the abdominal aorta is proportional to the balancing pressure at the left junction (P_X). With the flow stabilizer, the balancing pressure measured at the left junction (P_X) can be used to monitor temporal variations of the mean pressure of the abdominal aorta.

As illustrated in Figure 2B-(a), the pressure at the left junction (P_X) can be approximately estimated by the pressure at the right junction (P_Y) under a hydrodynamically balanced condition. On the basis of the Poiseuille flow formula (that is, pressure drop is product of fluidic resistance and flow rate), the analytical formula for the balancing pressure at the left junction is approximately estimated as $P_X \approx P_Y = R_{PBS} \cdot Q_{PBS}^{SS}$. For a rectangular channel (width = w, depth = h), the balancing pressure at the left junction (P_X) is analytically derived as

$$P_X = \frac{12\mu_{PBS} Q_{PBS}^{SS} L_{eq}}{wh^3} \left[1 - \frac{192}{\pi^5} \frac{h}{w} \sum_{n=1,3,5,\ldots}^{\infty} \frac{1}{n^5} \tanh\left(\frac{n\pi w}{2h}\right) \right]^{-1} \tag{7}$$

In eq 7, L_{eq} denotes the equivalent length of the side channel. As shown in eq 7, the balancing pressure at the left junction (P_X) can be used to monitor the temporal variations of the mean pressure at the abdominal aorta, only by measuring the switching flow rate of PBS solution (Q_{PBS}^{SS}).

Then, the other hydrodynamic condition is induced by controlling the flow rate of rat blood, as shown in Figure 2B-(b). PBS solution is supplied into inlet (B) at the switching flow rate of Q_{PBS}^{SS} obtained during the first step. Rat blood collected during the first step is simultaneously delivered by a syringe pump. The flow rate of rat blood is set to Q_{Blood}^{SS}, which induces reverse flow of rat blood occurs in the bridge channel under hydrodynamically balanced condition. Therefore, the flow rate of rat blood (Q_{Rat}) circulating within the fluidic network with the extracorporeal rat bypass loop is equal to Q_{Blood}^{SS} ($Q_{Rat} = Q_{Blood}^{SS}$). In addition, under a hydrodynamically balanced condition ($P_X \approx P_Y$), the same pressure at each junction satisfies the following relationship:

$$R_{Rat} \cdot Q_{Blood}^{SS} = R_{PBS} \cdot Q_{PBS}^{SS} \tag{8}$$

Because fluidic resistance is only proportional to fluid viscosity for each side channel with identical physical dimensions, an analytical formula for rat blood (μ_{Rat}) is simply derived as

$$\mu_{Rat} = \mu_{PBS} \frac{Q_{PBS}^{SS}}{Q_{Blood}^{SS}} \tag{9}$$

Therefore, the viscosity of rat blood is identified by measuring the switching flow rates of PBS solution and rat blood (Q_{PBS}^{SS}, Q_{Blood}^{SS}), wherein reverse flows occur in the bridge channel under hydrodynamically balanced condition. On the other hand, based on the definition of a hydraulic diameter (D) for a rectangular channel, the shear rate of blood flow is approximately estimated as

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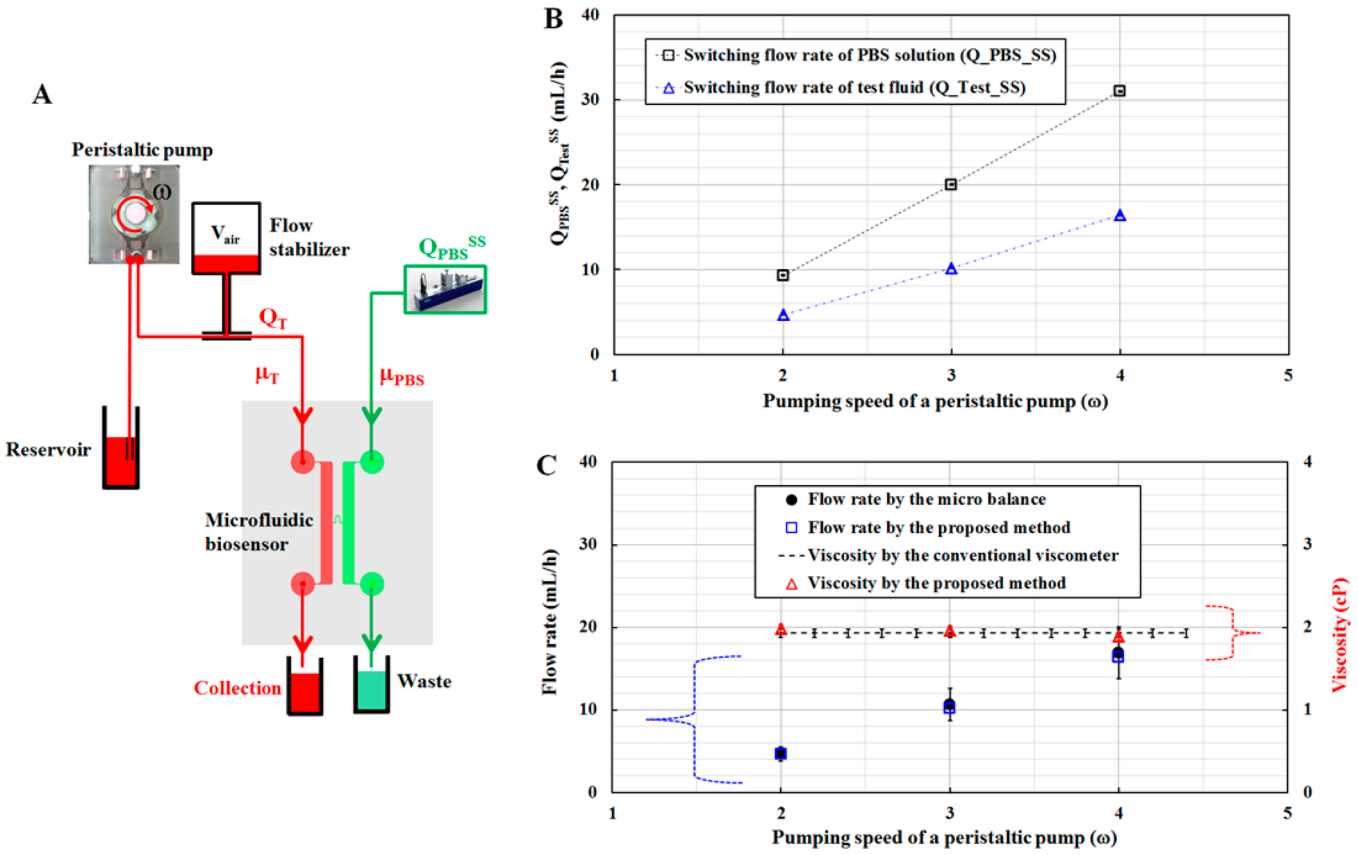

Figure 3. (A) A schematic diagram of the experimental setup used to evaluate performance of the proposed method with a 20% glycerin as test fluid circulating within a complex fluidic network. The fluidic network consists of a reservoir, a peristaltic pump, a flow stabilizer, and the microfluidic biosensor. PBS solution as reference fluid is supplied into inlet (B) by a syringe pump. (B) Variations of switching flow rates of PBS solution (Q_{PBS}^{SS}) and the test fluid (Q_{Test}^{SS}) with respect to various pumping speeds of a peristaltic pump (ω). (C) Comparison of the viscosity (μ_T) and flow rate (Q_T) of the test fluid measured by the proposed method and the conventional method, with respect to various pumping speeds of the peristaltic pump (ω).

$$\dot{\gamma} = \frac{32Q_{Rat}}{\pi D^3} \tag{10}$$

Fabrication of the Microfluidic Biosensor and the Experimental Setup. A rectangular master replica molder (depth = 50 μm) is fabricated using conventional micro-electromechanical system (MEMS) technologies, including photolithography and deep reactive-ion etching. Polydimethylsiloxane (PDMS, Sylgard 184, Dow Corning, U.S.A.) is poured into the master molder. After curing at 70 °C for 1 h, the PDMS block is peeled off the master molder. After treating the PDMS block and a glass substrate with oxygen plasma (CUTE, Femto Science, Korea), the microfluidic biosensor is prepared by bonding the PDMS block and the glass substrate. The microfluidic biosensor is mounted on an optical microscope (Nikon, Tokyo, Japan) equipped with a charge-coupled device (CCD) camera (PCO, Germany). Rat blood is circulated under ex vivo condition within a complex fluidic network, including an extracorporeal bypass loop consisting of a flow stabilizer and the microfluidic biosensor. PBS solution (1×, pH 7.4, Bio Solution, Korea), which is used as the reference fluid to prevent rupture of the red blood cell (RBC) membrane because of different osmotic pressure, is supplied simultaneously into the inlet (B) using a syringe pump (neMESYS, Centoni Gmbh, Germany) to detect a hydrodynamically balanced condition at a switching flow rate of Q_{PBS}^{SS}. Next, rat blood and PBS solution are supplied into the microfluidic biosensor by the syringe pump under in vitro condition to measure the flow rate and viscosity of rat blood. The switching flow rates of the reference fluid and rat blood are measured by continuously monitoring switching flow direction in the bridge channel based on two sequential flow controls proposed in this study. All experiments are conducted at consistent room temperature (25 °C). The viscosity of PBS solution was measured in advance using a commercial viscometer (DV-II, Brookfield, U.S.A.) is 1.00 ± 0.05 cP.

Rat Preparation Procedure. According to POSTECH Ethics Committee, all experiments are performed while ensuring that the procedures are appropriate and humane. Two male Sprague−Dawley rat (12−13 weeks old, body weight of 389.6 ± 7.8 g) are assigned for normal condition and hemodilution condition. Each rat is anesthetized using an intramuscular-injected ketamine (100 mg/kg) and xylazine (10 mg/kg). The rat is laid in a supine position on a plastic pad after anesthesia injection. In all experiments, heparin (1000 IU/mL/kg) is injected into the tail vein of the rat to prevent blood clotting in the vascular conduits. After injecting heparin, the rat is placed on the plastic pad for 5 min. The extracorporeal bypass loop is established by connecting the flow stabilizer to the microfluidic biosensor using a polyethylene tube (ID = 0.58 mm). The bypass loop is filled with heparin (10 IU/mL). The abdominal aorta is cannulated with one end of the extracorporeal bypass loop using a 20G catheter. The other end of loop is connected to the right jugular vein. As such, rat

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blood is supplied into the extracorporeal bypass loop, and returned to jugular vein of the rat. The rat is sacrificed under an anesthetic state at the end of the experiment.

RESULTS AND DISCUSSION

Performance of the Proposed Method. To evaluate the performance of the proposed method, a peristaltic pump (MP-1000, Tokyo Rikakikai Co., Japan) is used as a substitute for the rat. Thus, the fluidic network includes a reservoir, a peristaltic pump, a flow stabilizer, and the microfluidic biosensor, as shown in Figure 3A. Glycerin, as the test fluid, is diluted to 20% (vol/vol) with deionized water (DI-water). The test fluid in the reservoir is supplied into the microfluidic biosensor by the peristaltic pump. The flow stabilizer has a role in removing flow fluctuations caused by the peristaltic pump. The viscosity (μ_T) and flow rate (Q_T) of the test fluid are not specified. The switching flow rate of PBS solution (Q^{SS}_{PBS}) is measured by monitoring the reverse flow of the test fluid in the bridge channel based on the two flow controls. The switching flow rate of the test fluid (Q^{SS}_{Test}) collected from outlet (A) is measured by controlling the flow rate of the test fluid, which induces reverse flow in the bridge channel. PBS solution is supplied into inlet (B) at the switching flow rate of Q^{SS}_{PBS}. Experimental measurements are repeated three times (n = 3). The unknown flow rate of the test fluid in the proposed method is identified as $Q_T = Q^{SS}_{Test}$. From eq 9, the viscosity of the test fluid (μ_T) is measured as $\mu_T \approx \mu_{PBS} \cdot Q^{SS}_{PBS}/Q^{SS}_{Test}$.

As shown in Figure 3B, the switching flow rates of the test fluid (Q^{SS}_{Test}) and PBS solution (Q^{SS}_{PBS}) are measured with respect to various pumping speeds of the peristaltic pump, which ranges from ω = 2 to ω = 4. As a result, the flow rate of the test fluid ($Q_T = Q^{SS}_{Test}$) and the switching flow rate of PBS solution (Q^{SS}_{PBS}) are linearly proportional to the pumping speed. To evaluate the accuracy of the flow rate obtained by the proposed method, the flow rates of the test fluid are measured by using a microbalance (AP250D, Ohaus, U.S.A.) at the same pumping speeds. As shown in Figure 3C, the flow rates obtained by the proposed method are in good agreement with those measured by the microbalance. The normal difference between the both methods is less than 4% with respect to pumping speed. This result indicates that the proposed method can measure the flow rate of the test fluid circulating within a complex fluidic network with high accuracy.

In addition, using the viscosity formula (eq 9), the viscosity of the test fluid is measured with respect to the pumping speed as shown in Figure 3C and found to be consistent at 1.94 ± 0.05 cP. This result supports the hypothesis that Glycerin behaves as a Newtonian fluid, which is expected. To compare with the viscosity obtained by the proposed method, the viscosity of the test fluid is measured with a conventional viscometer. The viscosity of the test fluid is found to be 1.93 ± 0.05 cP using the conventional viscometer. This comparative study indicates that the proposed method can measure the viscosity of a test fluid circulating within a complex fluidic network with sufficient accuracy.

In summary, the proposed method can measure the viscosity and flow rate of a test fluid with sufficient accuracy and consistency, as shown by the experimental demonstrations.

Biophysical Properties under Normal Condition. To allow rat blood to circulate within a complex fluidic network, the abdominal aorta and jugular vein are connected to an extracorporeal bypass loop, which includes a flow stabilizer and the microfluidic biosensor as shown in Figure 1A. To monitor the temporal variations of the biophysical properties of rat blood circulating within the complex fluidic network, the switching flow rate of each fluid, which induces reverse flows in the bridge channel, is measured at intervals of 10 min for 130 min. As shown in Figure 4A, after 30 min, the switching flow rate of PBS solution (Q^{SS}_{PBS}) and the balancing pressure (P_X) at the left junction (X) remain consistent at 9.23 ± 0.32 mL/h and 11.92 ± 0.29 kPa, respectively. Therefore, a normal rat requires at least 30 min to adapt to the different fluidic environments established in the extracorporeal rat bypass loop.

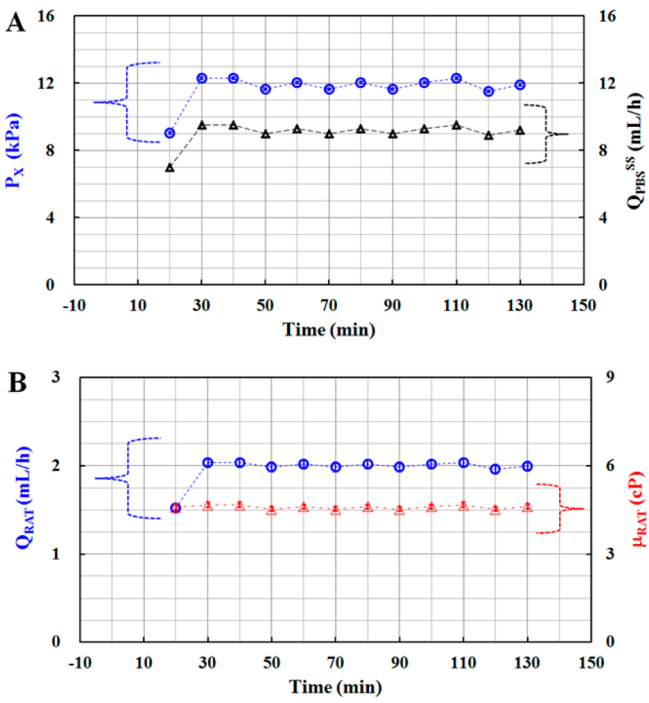

Figure 4. (A) Temporal variations of switching flow rate of PBS solution (Q^{SS}_{PBS}) and balancing pressure (P_X) of rat blood circulating within a complex fluidic network under normal condition. After 30 min, Q^{SS}_{PBS} and P_X maintain consistent at 9.23 ± 0.32 mL/h and 11.92 ± 0.29 kPa, respectively. (B) Temporal variations of the flow rate (Q_{Rat}) and viscosity (μ_{Rat}) of rat blood measured using the proposed method under normal conditions. After 30 min, Q_{Rat} and μ_{Rat} remain consistent at 2.01 ± 0.02 mL/h and 4.6 ± 0.06 cP, respectively.

After monitoring the switching flow rate of PBS solution for 130 min, we collect pure rat blood from outlet (B) after stopping the syringe pump delivering PBS solution. The switching flow rate of the rat blood ($Q^{SS}_{Blood} = Q_{Rat}$) is measured using the collected blood by varying the switching flow-rate of PBS solution. As shown in Figure 4B, after 30 min, the flow rate (Q_{Rat}) and viscosity (μ_{Rat}) of the rat blood remain consistent at 2.01 ± 0.02 mL/h and 4.6 ± 0.06 cP, respectively. The hematocrit of the rat blood is measured at 39.8 ± 0.6% using a centrifugal-hemocytometer.

As biophysical properties of the rat model tested under the normal condition, the blood pressure and hematocrit are comparable to the mean arterial pressure and hematocrit which were reported in previous studies.[7,13] In addition, the rat model has been kept sufficiently consistent for 130 min as shown in Figure 4.

These results show that the proposed method can monitor the temporal variations of biophysical properties effectively

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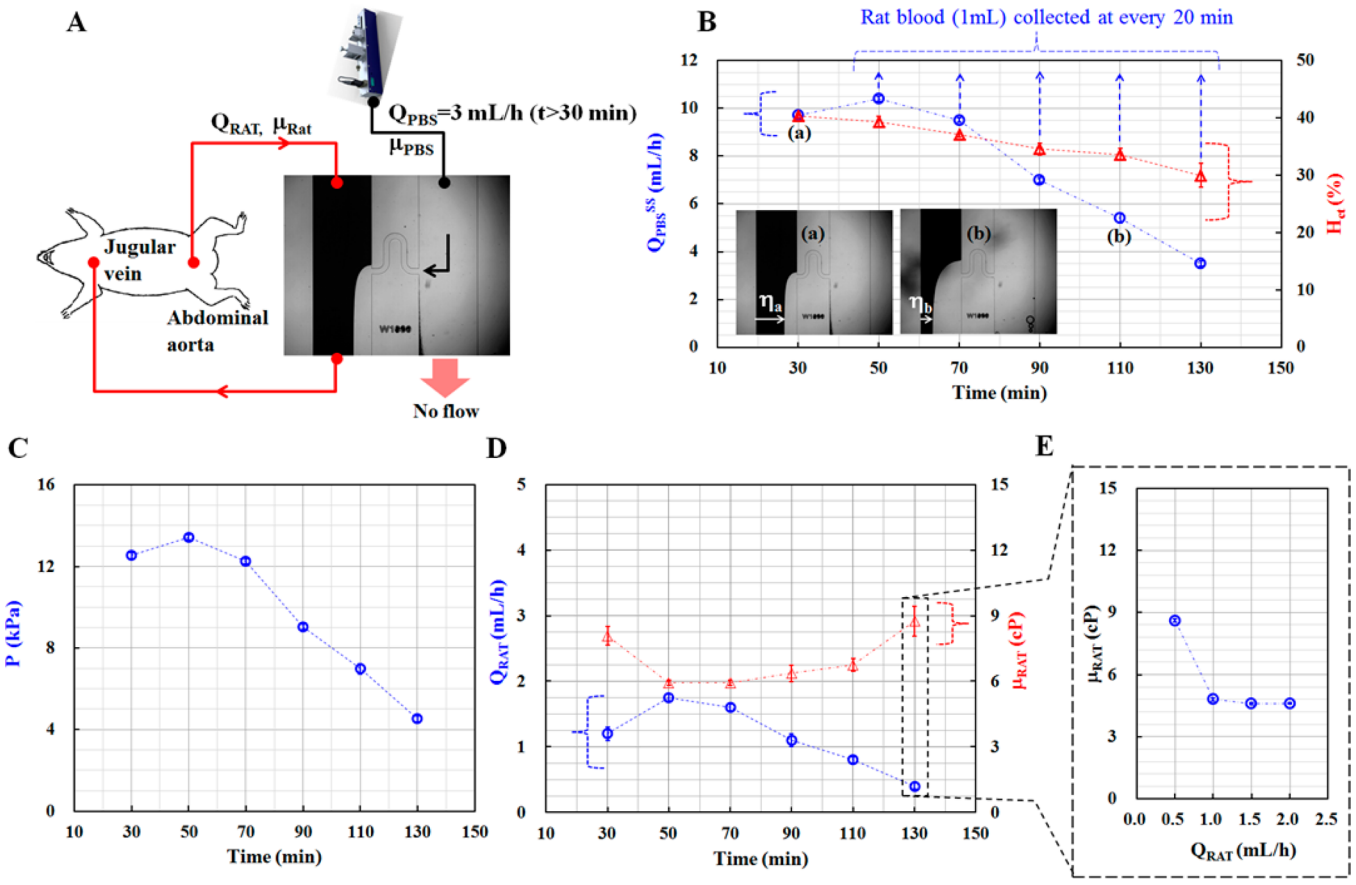

Figure 5. (A) Schematic diagram of the experimental setup used for measuring temporal variations of biophysical properties such as viscosity, flow rate, and balancing pressure of rat blood circulating within a complex fluidic network under continuous hemodilution condition. After closing outlet (B), PBS solution is supplied into inlet (B) at a flow rate of 3 mL/h after 30 min to induce continuous hemodilution within the complex fluidic network. (B) Temporal variations of the switching flow rate of PBS solution (Q_{PBS}^{SS}) and the hematocrit (H_{ct}) of rat blood. Insets a and b show microscopic images captured at t = 30 min (before hemodilution) and t = 110 min (during hemodilution). Rat blood is collected from outlet (B) at intervals of 20 min after the syringe pump delivering PBS solution is stopped. (C) Temporal variations of balancing pressure (P_X). (D) Temporal variations of the flow rate (Q_{Rat}) and the viscosity (μ_{Rat}) of rat blood. (E) Variations of the viscosity of rat blood collected at t = 130 min with respect to flow rates.

including viscosity, flow rate, and pressure of blood circulating under ex vivo condition.

Hemodynamic and Hemorheological Properties under Hemodilution Condition. To monitor hemorheological and hemodynamic properties including viscosity, flow rate, and pressure of blood under continuous hemodilution condition,[35] a complex fluidic network is established by connecting the abdominal aorta and jugular vein to an extracorporeal rat bypass loop as shown in Figure 5A. After 30 min, based on the two-step flow controls proposed in this study, the switching flow-rate of PBS solution (Q_{PBS}^{SS}) is measured by monitoring the reverse flow in the bridge channel. Rat blood (1 mL) is collected from outlet (B) after the syringe pump delivering PBS solution is stopped. After closing outlet (B), PBS solution is supplied into inlet (B) of the microfluidic biosensor at a flow rate of 3 mL/h. This procedure is repeated at intervals of 20 min for 130 min. During this procedure, rat blood is diluted continuously. Since the supplied volume of PBS solution for 20 min (Q_{PBS} = 1 mL) is equal to the removed rat blood (Q_{Blood} = 1 mL), the overall volume of rat blood is maintained constantly under continuous hemodilution condition.

As shown in Figure 5B, the switching flow rate of PBS solution (Q_{PBS}^{SS}) and hematocrit (H_{ct}) are measured for 130 min. Insets a and b show typical microscopic images captured at t = 30 min (before hemodilution) and t = 110 min (during hemodilution). As expected, the channel width (η_a, η_b) filled with rat blood in the left side channel tends to decrease ($\eta_a > \eta_b$) because of the hemodilution effect. In addition, the hematocrit of each rat blood sample is measured using a centrifugal-based hemocytometer. As shown in Figure 5B, hematocrit tends to decrease over time. This experiment demonstrates that hemodilution decreases hematocrit in rat blood circulating within a complex fluidic network.

On the basis of the switching flow rate of PBS solution (Q_{PBS}^{SS}) measured at intervals of 20 min for 130 min, the balancing pressure at the left junction (P_X) is estimated using the eq 7. As represented in Figure 5C, balancing pressure is decreased gradually over time, as expected. Therefore, blood pressure in the abdominal aorta tends to decrease because of continuous hemodilution effect.

In addition, the switching flow rate of rat blood (Q_{Blood}^{SS}) is measured by monitoring the reverse flow in the bridge channel according to proposed method to measure the viscosity of six rat blood samples collected for 130 min. As shown in Figure 5D, the flow rate (Q_{Rat}) and viscosity (μ_{Rat}) of rat blood are measured at intervals of 20 min for 130 min. After 50 min, the flow rate of rat blood tends to decrease gradually. However, the

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viscosity of rat blood increases. Variations in the viscosity of rat blood with respect to flow rate (Q_{Rat}) collected at t = 130 min are shown in Figure 5E. This result indicates that rat blood tested in this study behaves as a non-Newtonian fluid. Considering the fact that hemodilution decreases flow rate of rat blood, the reduced flow rate resulting from this process increases the viscosity of the rat blood.

These experimental demonstrations confirm that the proposed method with two sequential flow controls in a microfluidic channel can effectively monitor the temporal variations of hemorheological and hemodynamic properties including viscosity, flow rate, and pressure of rat blood circulating within a complex fluidic network.

CONCLUSIONS

In this study, we proposed a novel microfluidic-based biosensor for monitoring temporal variations of the biophysical properties of rat blood circulating within a complex fluidic network, which was established by connecting the abdominal aorta and jugular vein to an extracorporeal rat bypass loop. The extracorporeal rat bypass loop is composed of a flow stabilizer and a microfluidic biosensor. Three biophysical properties, namely, flow rate, viscosity, and pressure were simultaneously measured by label-free and sensorless detection based on two sequential flow controls in the microfluidic biosensor. The analytical formulas for viscosity, flow rate, and balancing pressure in the bridge channel were derived using a discrete circuit model for a complex fluidic network. First, a peristaltic pump was used as a substitute for a rat to evaluate the measurement accuracy of the proposed method. The flow rate and viscosity of 20% glycerin (test fluid) circulating within a complex fluidic network were measured at various pumping speeds. For the comparison, the flow rate and viscosity of the test fluid were also measured using a microbalance and a conventional viscometer, respectively. The normal differences between the two measurement methods are less than 4%. Then, the proposed method was employed to monitor temporal variations of the biophysical properties of rat blood circulating within a complex fluidic network. This result indicated that a normal rat requires at least 30 min for acclimating to different fluidic environments established in the extracorporeal rat bypass loop. The flow rate and viscosity of rat blood remained consistent at 2.01 ± 0.02 mL/h and 4.6 ± 0.06 cP, respectively. Lastly, under continuous hemodilution condition, the proposed method was used to measure temporal variations of the biophysical properties of blood circulating within the complex fluidic network by supplying PBS solution into the network at a flow rate of 3 mL/h. The result showed that the flow rate, pressure, and hematocrit of rat blood tended to decrease gradually because of continuous hemodilution effect. In addition, the reduced flow rate increased the viscosity of rat blood.

From these experimental demonstrations, the proposed method was found to be capable of effectively monitoring the biophysical properties (viscosity, flow rate, pressure) of rat blood circulating within a complex fluidic network (ex vivo condition), without fully integrated sensors, labeling operations, and tedious calibration procedures. In the future, the proposed method will be used to evaluate real-time monitoring of the biophysical properties of animal models with various cardiovascular diseases.

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Notes

The authors declare no competing financial interest.

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