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Modeling and experimental evaluation of biomolecules' behaviors grafted on the blood vessels microchip under a pulsatile flow

Iqbal Kabir, Subin Mao, Sunney Que, Xuefeng Wang, and Long Que

***Abstract*— This paper reports on the numerical modeling and experimental evaluation of the behaviors of biomolecules immobilized on the surface of blood vessels on microchip under pulsatile flow conditions, which include velocity profiles of the fluids and shear stress profiles around the biomolecules. Using fluorescent *BSA, FITC conjugates* mimicking biomolecules, the behaviors of the biomolecules can be optically observed, facilitating the quantitative studies and analysis.**

I. INTRODUCTION

In our recent studies, we have developed microfluidic chips to study the behaviors of platelets by combining microfluidics and molecular tension sensors. Using these chips grafted with the integrin tension sensors (ITS) on the inner surfaces of microchannels, integrin $\alpha_{IIb}\beta_3$ force maps in platelets under relatively low flow status have been obtained [1]. In that research, the fluid flow always remained in the same direction. However, both the blood flow direction and the magnitude of the flow rate do not remain the same.

It is well known that the blood flow in animal or human body is highly pulsatile with zero or even reversing flow during systole within the coronary arteries [2]. As a result, the shear stress on the biomolecules such as platelets in blood vessel is also pulsatile and changes over time. Pulsatile blood flow, which is generated by the pulsatile heartbeat (1-2 Hz), plays a key role in the cardiovascular system [2-10].

Given the shear stress also depends on the locations, sizes and shapes of the blood vessels; in order to analyze the behaviors of molecules such as the platelets more accurately, it is important to study their spatiotemporal behaviors inside the blood vessel chip. This type of study can allow us to further understand cardiovascular diseases, hence mimicking hemodynamic conditions in pulsatile blood flow on chip is crucial. To this end, a system to achieve this goal has been developed.

II. MATERIALS AND METHODS

A. Materials

For chip fabrication: SU-8 photoresist was purchased from MicroChem, Inc. Deionized (DI) water was obtained from a DI water purification system (Millipore, France). Polydimethylsiloxane (PDMS) and curing agents (SYLGARD™ 184 Silicon Elastomer Kit, 2-part) were purchased from Dow Corning (Midland, MI, USA).

Chemicals for surface functionalization and shear stress assessment: 3-aminopropyltriethoxysilane (APTES), the linker glutaraldehyde, and acetic acid solution were purchased from Sigma-Aldrich (St. Louis, MO, USA). Albumin from Bovine Serum (BSA), FITC conjugate was obtained from ThermoFisher (Waltham, MA, USA). Polydimethylsiloxane (PDMS) and its curing agent were purchased from Dow Corning, Inc. (Midland, MI, USA)

Equipment for generating pulsatile flow: Four flow sensors, a microprocessor, four piezoelectric pumps, and all related accessories were purchased from Bartels Mikrotechnik GmbH (Dortmund, Germany).

B. Method

For numerical simulation and modeling, the finite element method (FEM) (COMSOL, Inc.) was used to calculate the profiles of the flow velocity and the shear stress on biomolecules in blood vessels microchannels under pulsatile flow conditions.

For experiments, biomolecules were firstly immobilized on the surface of the microfluidic channels inside the blood vessel chip through a surface functionalization process described in [11], allowing the biomolecules to be bonded to the surface through chemical bonds rather than physical adsorption. Then the chip was connected to a custom-designed pulsatile flow operational system as shown in **Figure 1** to assess the behaviors of biomolecules under pulsatile flowing conditions.

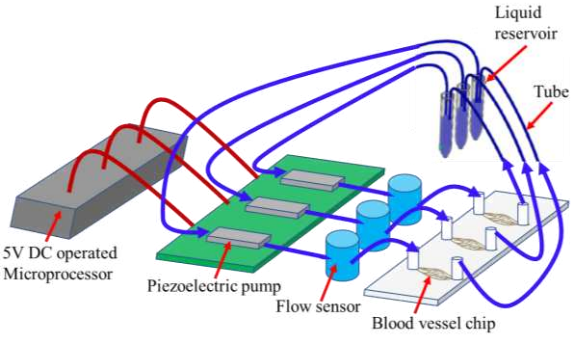

Fig. 1. Schematic illustration showing a system for generating pulsatile flow in the blood vessel chip (*not to scale*).

III. CHIP FABRICATION

The chip was fabricated by soft lithography process. Briefly, an SU8 mold was fabricated using optical

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The authors are with the Department of Electrical and Computer Engineering and the Department of Physics, Iowa State University, Ames, IA 50011 USA, phone: 515- 294-6951; e-mail: lque@ iastate.edu. Sunney Que is with Ames High School and worked partly on this project through the NSF outreach program at ISU.

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lithography. Liquid PDMS was poured to the mold with curing agents, followed by 2 hr curing at a temperature of 70 °C. Then the PDMS layer peeled off the mold. Then the PDMS layer and a glass substrate were treated by oxygen plasma. Finally, the chip (**Figure 2**) was completed by bonding the glass substrate and PDMS layer.

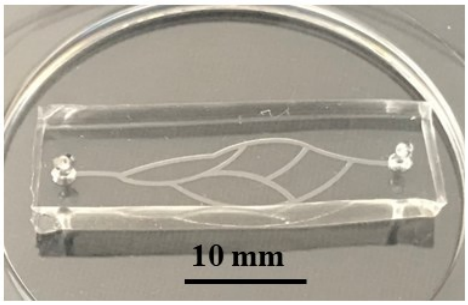

Fig. 2 Photo of a fabricated blood vessel chip.

IV. Experimental Setup and Procedure

A photo of the experimental setup is shown in **Figure 3**. It consists of commercially available piezoelectric pumps mounted on a printed circuit board, a microprocessor, blood vessel chips, and reservoirs for recirculating the fluids through the fluidic channels. A graphical user interface (GUI) on a computer is used to control the piezoelectric pumping system to operate the chip, thereby achieving the pulsatile flow in the chip. By this way, we can monitor the pulsatile flow effects on the biomolecules such as platelets. In following experiments, BSA, FTIC conjugate was used to mimic the biomolecules.

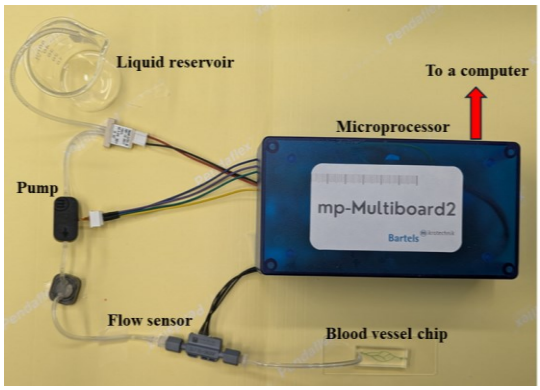

Fig. 3 Photo of the experimental setup: a microprocessor, which is connected to a computer with a GUI, is used to control the pulsatile flow in the blood vessel chip through a pump and flow sensor, which can adjust the flow rate and frequency.

V. Results and Discussions

A. Simulation results

Figure 4 shows a simplified blood vessel used for the simulation. It consists of *Inlet*, *Exit*, and *several microfluidic branches* between the Inlet and Exit. For the immobilized ***biomolecules*** in the microfluidic channels, it is assumed that their shape is an ellipse with a semi-major axis and a semi-minor axis of 30 µm and 15 µm, respectively.

A sketch showing the applied pulsatile flow on the chip at ***Inlet*** is given in **Figure 5a**. The representative shear stress profiles in the vessels at timepoint T1 and T2 are shown in **Figure 5b** and **Figure 5c,** respectively. At T1, the flow rate is in the increasing stage, while at T2, the flow rate is in the decreasing stage. Clearly, the profiles fluctuate at different time points, thereby verifying the temporal changes of the shear stress at different locations inside the microchannels of the chip.

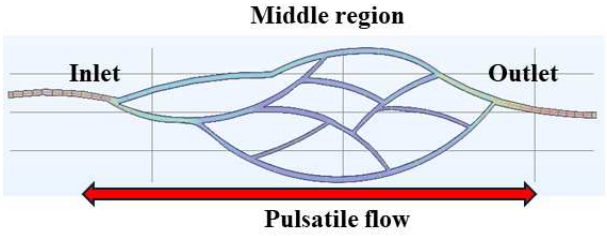

Fig. 4 The simulated blood vessel chip labeled with inlet, outlet, and middle regions

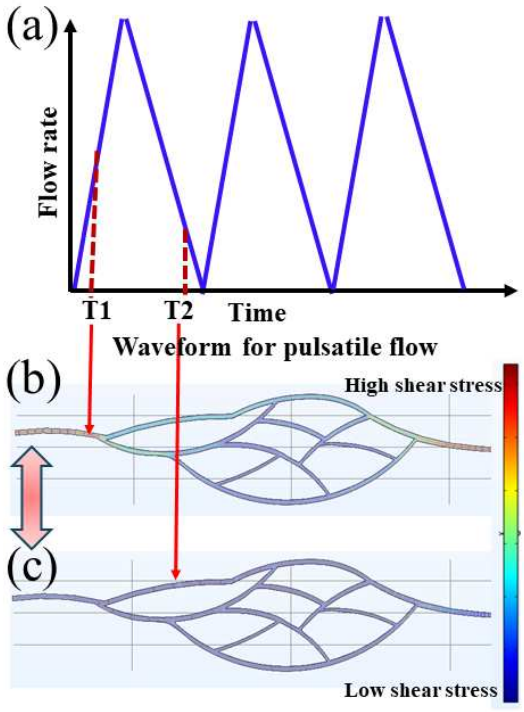

Fig. 5 (a) Sketch of the pulsatile flow rate and (b-c) representative shear stress profiles at different timepoints.

Shear stress profile for each pulsatile flow cycle: In each flowing cycle, the shear stress varies at different time points. **Figure 6** shows the calculated shear stress profile for three biomolecules at three different locations. In terms of the shear stress at different time points at the same location, its profile follows a similar pattern as the flow profile of the fluids at ***Inlet***. The magnitude of shear stress increases, reaches a peak, and then decreases. In terms of the shear

stress distribution at different locations, the shear stress is smaller in the middle region of the branches of the vessel, while the shear stress is larger close to the outlet, namely before and after flowing out of the branch regions for the blood vessel design in **Figure 4**.

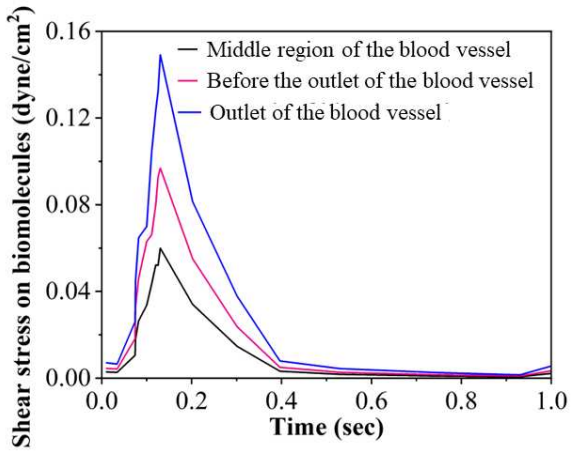

Fig. 6 Calculated shear stress profile on one biomolecule in one cycle of pulsatile flow at different locations of the blood vessel.

Peak shear stress at different locations under different flow rates: The calculations of the peak shear stress for the biomolecules at different locations in the blood vessel and under different flow rates have been carried out. Some representative simulation results of the blood vessel chip with immobilized biomolecules under a 1 Hz pulsatile flow are shown in **Figure 7**. For the same flow rate, the shear stress in the middle region is smaller than the shear stress in the regions close to the Exit (Figure 4). At the same location, the shear stress increases with the flow rates.

Overall, the shear stress on the immobilized biomolecules clearly varies both spatially and temporally

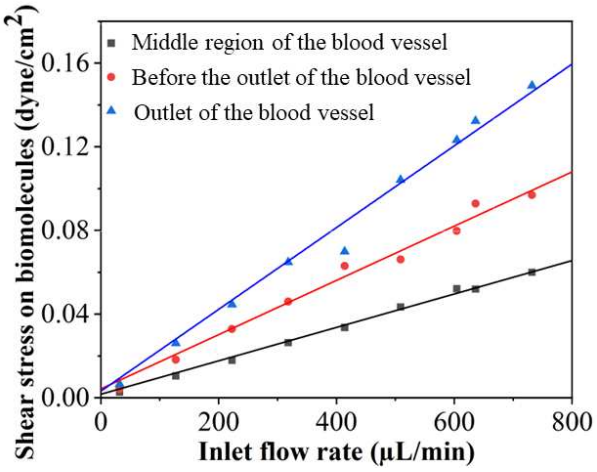

Fig. 7. Calculated shear stress (peak magnitude) on one biomolecule at three different locations of the blood vessel with different flow rates.

B. Experimental results

Using the fabricated blood vessel chip, the inner surfaces of the microfluidic channels were first coated with biomolecules (BSA, FITC conjugates). Representative optical and fluorescence images of branches of a blood vessel chip are captured as shown in **Figure 8a-b**, verifying that the biomolecules are immobilized inside the microchannels.

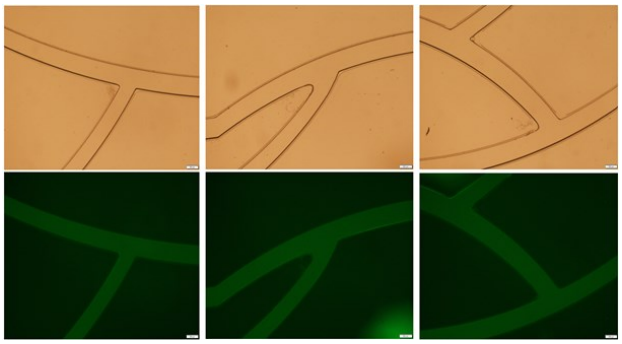

Fig. 8. Top three and bottom three are the optical and fluorescence images of branches of a blood vessel chip, respectively before a pulsatile flow is applied.

Then, a pulsatile flow was applied on the chip for the experiments. A measured flow profile (1 Hz) of deionized (DI) water with a maximum flow rate of 100 µL/min to the chip is shown in **Figure 9a**. Different pulsatile flow (i.e., different rates and frequencies) can be readily tuned by a flowing control system in our lab.

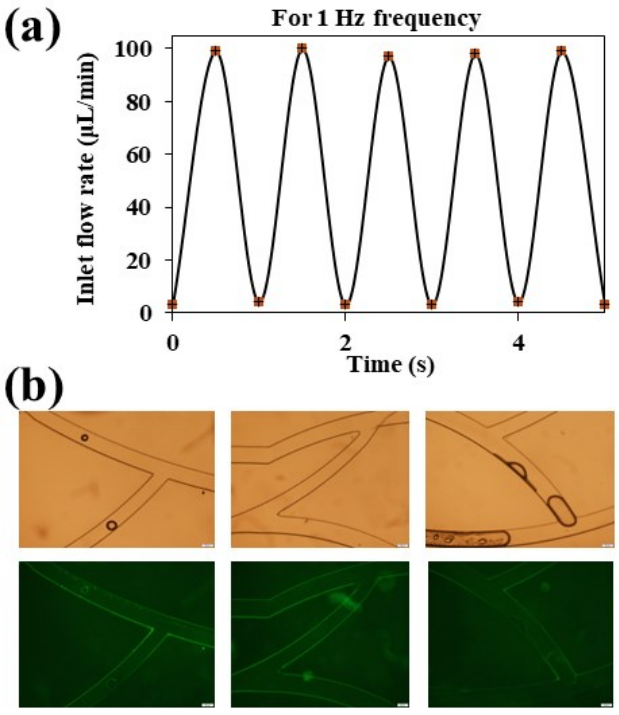

Fig 9 (a) One measured representative pulsatile flow profile; (b) top three and bottom three are the optical and fluorescence images of blood vessel respectively after a pulsatile flow.

Representative optical and fluorescence images are shown in **Figure 9b** after performing pulsatile flow for several minutes. Clearly some biomolecules were flushed out of the branches, while some remained attached to the surface of the microchannels.

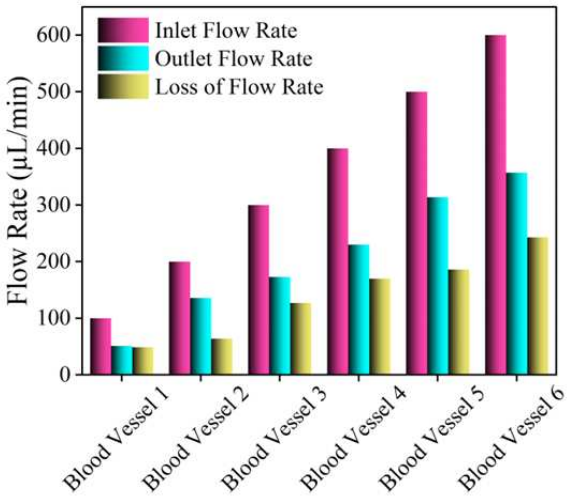

Fig 10 Measured flow rates at inlet and outlet for several blood vessel chips of different dimensions.

The measured flow rates at the inlet and the outlet of blood vessel (Figure 4) are shown in **Figure 10**. As shown, the flow rate at inlet is always larger than that at outlet for each blood vessel of different dimensions, resulting in an obvious loss of the flow from the inlet to the outlet.

Based on the fluorescence images of the blood vessel, the measured intensity of the fluorescence images of the branches of chips is shown in **Figure 11** using ImageJ [12]. As shown, the fluorescence intensity is dependent on the flow rates (i.e., the flow rates at inlet). The fluorescence intensity decreases with the increased flow rates, indicating the increased shear stress applied on the biomolecules, as a result, more biomolecules have been washed away from the vessel and then out of the chip.

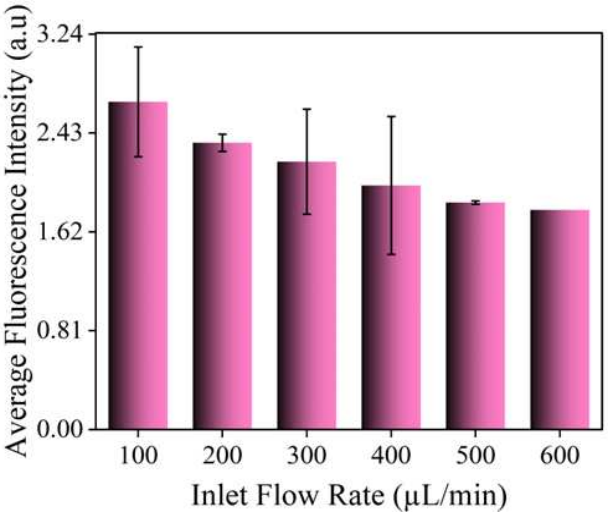

Fig. 11. Measured fluorescence intensities with different flow rates at Inlet of the chip.

VI. Conclusion

A pulsatile microfluidic system has been developed to introduce pulsatile flow in a blood vessel chip. In this effort, the behaviors of the biomolecules immobilized on the surface of the microchannels of the chip have been simulated using a finite-element method and experimentally studied, mainly focusing on the flow frequency of 1Hz. It has been found that the velocity and shear stress profiles in the blood vessel is dependent on the locations of the biomolecules inside the blood vessel.

The next step of this research includes systematic evaluation of the behaviors of biomolecules when fluids flow from wide microchannels to narrow microchannels, and from narrow microchannels to wide microchannels, to mimic the *stenosis* in blood vessels [13-14].

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