Using Microfluidics to study the Vascular System in a Freshman class

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Abstract
Vascular blood flow provides a unique opportunity to introduce microfluidics in biomedical engineering courses, allowing both experimentation and numerical modeling. Microfluidic-based flow networks can be used to illustrate fluid flow in the body and directly connect to normal and disease-state physiological function.

In an introduction to biomedical engineering class that combines labs with lectures, students visualize flow in a model vascular system using a poly(dimethyl)siloxane (PDMS) microfluidic device consisting of a network of various sized channels. The objective of this experiment is to characterize a simple model of the microvascular system using a PDMS microdevice. This devise is made using photolithography and simple microfabrication techniques. Students measure flow velocities of ten micron beads within the model network before and after blocking one of the channels and track the redistribution of flow when a channel is occluded. Using a MATLAB® simulation, students then calculate the resistance of the overall network as well as pressure and velocity in each channel before and after the blockage. These are then compared to experimentally measured velocities and calculated network resistance. This exercise allows students to visualize and model what happens to micro-vessels when blocked and how the surrounding vessels compensate and redirect flow. The use of a PDMS chip allows for reproducible and easily visualized results without the need for an animal model. Students learn microfluidics, image acquisition and analysis, microfabrication, and MATLAB® simulations as well as gain an appreciation of the fluid dynamics of microvascular blood flow in health and disease.

Introduction

Microfluidic devices have been applied to many areas of cutting-edge research, they allow for high throughput while using minimal supplies, more control over the environment, potential for automation, and ability to test different parameters easily [1]. Students typically learn fluid dynamics through lectures based in theory and mathematical equations. Providing students with hands-on models of fluid mechanics using microfluidic devices can provide students with tools to conceptualize the material and become aware of the interdisciplinary area of microfluidics that encompasses engineering, biology, and chemistry [2]. Exposing students to this growing field helps prepare the next generation of scientists and engineers to solve challenges in healthcare, biology, and the environment through microfluidics.

Development of microfluidic systems is particularly useful for studying vascular cell behavior as it can create more accurate *in vitro* environments [3]. Developing biologically accurate models that can simulate the effects of diseases is important for example, in atherosclerosis, fat build-up can lead to hardened arteries which is associated with increase in peripheral vascular resistance and decreased blood velocity as arteries narrow [4]. Microfluidic devices can study effects of shear stress and resulting elongation of endothelial cells [5]. A single chip can test multiple shearing regimes.

Using microfluidics in a teaching environment specifically doing hands on microfluidics experiments combined with modeling has a number of advantages. It allows students to contextualize research level techniques in an approachable learning environment and provide students with useful experimental and computational skills.

For this experiment, a microfluidic vascular model was designed to model the vascular system representing vessels of different sizes branching into parallel segments in the body. The model was evaluated experimentally with beads flowing in the channels and theoretically with MATLAB® to analyze flow velocities within the network. This model is as an example of how fluid resistance and flow can be characterized analogously to an electrical circuit of resistors.

The intended learning outcomes of this lab include students should be able to - understand the pressure - volumetric flow relationship; calculate resistance in a small flow network; apply a MATLAB® model to a simple network; use ImageJ - an image analysis tool to calculate velocities; conceptualize blood flow in the vascular system; practice teamwork and time management skills; and develop technical report writing skills.

Materials and Methods

The experiment reported was performed by students in a 1st year undergraduate engineering course entitled Introduction to Biomedical Engineering. This course is comprised of four modules covering key topics in Biomedical Engineering. Each module integrates lectures with a laboratory. The microfluidic lab described is in the Biofluids module. A class typically of 45 students is divided into groups of 3 to 4 students, and the lab is taught in three sections with 4 groups in each section. Prior to the lab, lectures on fluid flow in pipes, vascular flow and occlusion, and microfabrication were delivered to provide context. The lab was carried out during a 3-hour class period and the students were given three weeks to do the analysis and write a group report. A detailed lab procedure was provided to the students beforehand. The experiment proceeded as follows:

Each group was provided with a polydimethylsiloxane (PDMS) microfluidic device that had channels making up a simple microvasculature network. Figure 1 shows a schematic and image of the microfluidic device. The microfluidic device was made by the instructional staff using the process of soft lithography. PDMS (Sylgard 184 kit, DowCorning) was poured over a SU-8 mold containing the 4 replicates of the network pattern, the PDMS was cured at 60^{o}C for 24 hours, and the 4 devices cut and removed from the mold and sealed to a glass slide using a plasma cleaner (Harrick Plasma). Students measured flow velocities of beads representing cells within the PDMS channels of the model network before and after a channel blockage and tracked the redistribution of bead flow when a channel was occluded. Following the experimental portion of the lab, students theoretically calculated the volumetric flow in each channel before and after blockage using MATLAB®. The calculated velocities were compared with measured velocities.

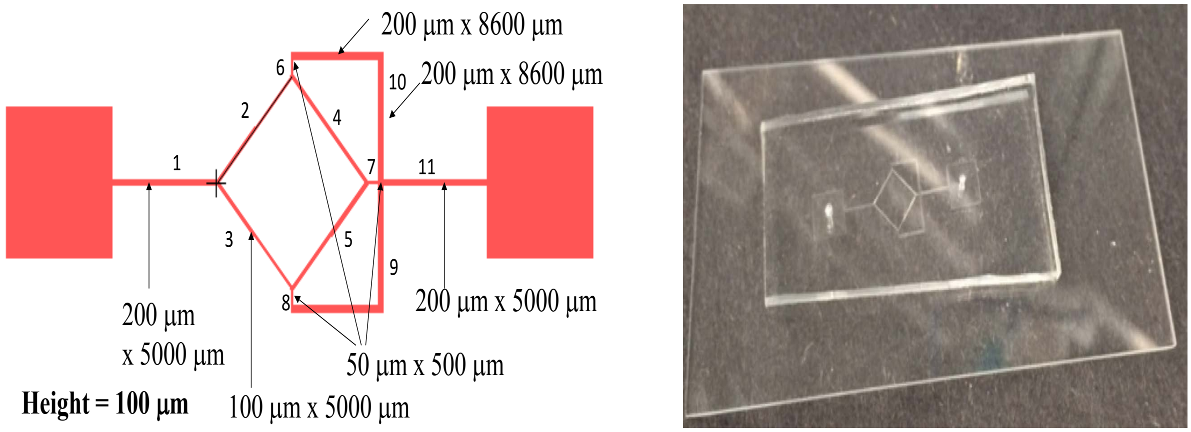

Figure 1: Schematic and Image of the Microfluidic device

In the first part of the experiment, students prepared a 5ml bead solution of 10 μm polystyrene beads [2010A, Duke Scientific] in a buffer solution (87.25% water, 12.5% glycerol, 0.25% sodium dodecyl sulfate - SDS) to mimic blood cells. A 3ml syringe with a 23G flat needle (Becton Dickson) was filled with the bead solution and placed in a syringe pump. The microfluidic device was connected to the syringe with tubing (0.02in ID, 0.02in wall) and placed on the stage of a stereo microscope (Nikon SMZ1000) with a 20X objective and a CCD camera (Sony DXC-390). The bead solution was pumped through the microfluidic device using a syringe pump (PHD2000, Harvard Apparatus) at a flowrate of 2 μl/min. Three second videos were captured of each channel at a frame rate of 30 frames per sec. using the software Streampix (NorPix, Inc.).

In the second part of the experiment, the microfluidic device was blocked at Channel 3 and the process repeated. The channel was blocked by punching a hole in the channel with an 23G needle, removing the plug, and injecting a few drops of silicone sealant (SS-67A, Silicone Solutions) into the hole. The device was placed in a 60^{o}C oven (Isotemp Oven – Fisher Scientific) for ten minutes, so the sealant would harden and block the channel. While the device was in the oven, students were asked to predict how the bead velocity in each channel would change and if there would be redirection of flow. This exercise allowed them to qualitatively think of the flow in the network and its inter-connectiveness. In the report they were expected to discuss their early predictions in light of their experimental results.

The velocity of the beads in the channels, in both the unblocked and blocked case, was measured using ImageJ. The videos were opened in Image J and the Manual Tracking plugin was used to find the average velocity of a bead measured over 10-20 frames. The tracking was done by clicking on the position of each bead as it moved through the frames. To get the average velocity in a channel, a minimum of 15 beads were chosen over the full width of the channel and the velocities averaged. The calculations of average velocity were done using Microsoft Excel.

The students also worked on a theoretical model of the network using MATLAB® to predict pressure, volumetric flow, and velocity in each channel and compared them with the experimental results. Since the 1st year students were just learning MATLAB®, a MATLAB® code was given to them. The MATLAB® code used Poiseuille's equation for a rectangular channel, $\Delta P = 12\mu QL/wh^3$ where μ = viscosity, Q = volumetric flowrate, L = length of channel, w = width of channel and h = height of channel and a global nodal and channel matrix based on the number of channels and nodes. They were expected to follow the logic of the code. The code calculated the individual channel flow rates and pressure in each of the channels in the microdevice. Students had to input: (1) inlet pressure, (2) outlet pressure, and the (3) diameter and (4) length of each channel. The students were given the dimensions of each channel, they were expected to know the outlet pressure was ambient pressure. The inlet pressure was calculated based on flow rate in the microdevice and the total calculated resistance of the channel network using $\Delta P = QR_{eff}$ where R_{eff} is the effective resistance of the whole network, calculated using the individual resistances of each channel. Students calculated the effective resistance of the network considering which channels were in series and parallel in a manner analogous to an electrical circuit of resistors.

Results and Discussion

Student groups determined the average bead velocities in each channel before and after blocking, and calculated the resistance of the network before and after blocking. Then using MATLAB® they obtained theoretical calculations of pressure and volumetric flow in each channel and compared them with the experimentally obtained velocity and volumetric flowrates. They were asked to present their findings in the form of a paper with the following sections - Abstract, Introduction, Materials and Methods, Results and Discussion. The Discussion prompt was open ended and asked students to compare unblocked and blocked, theoretical and experimental, and discuss how the theoretical model and experimental model can be used in different aspects of biomedical engineering and how both of these models can be used to study the cardiovascular system.

Typical data from a student group is shown in the following tables and figures.

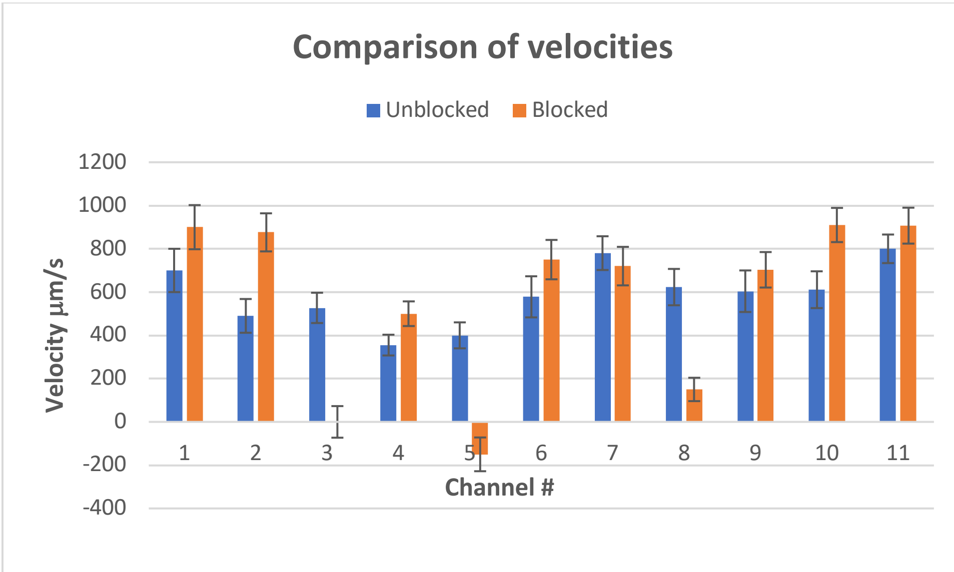

Figure 2: Bar graph of bead velocities before and after blocking Channel 3.

Figure 2 shows a bar graph of bead velocities in each channel before and after blockage in Channel 3 (refer to Fig.1 for Channel numbers). This figure shows when unblocked how the velocities changed in each channel as their widths changes and as they split. When the microfluidic device was blocked at Channel 3, velocities changed in most channels and flow changed direction in Channel 5. During the lab students were asked to hypothesize what would qualitatively happen to the bead velocities on blocking of Channel 3. Most students were able to intuitively figure out that when Channel 3 was blocked the velocity of the beads increased in Channels 2, 4, 6, and 10. There is a decease in bead velocity for Channels 5,8, and 9, while in Channels 7 and 11 bead velocities remain the same.

Students were asked to calculate the total resistance of the network using Poiseuille’s equation. First, they grouped the resistances of the channels in units that could be added in series or parallel. Figures 3 and 4 shows schematics for resistance in blocked and unblocked case.

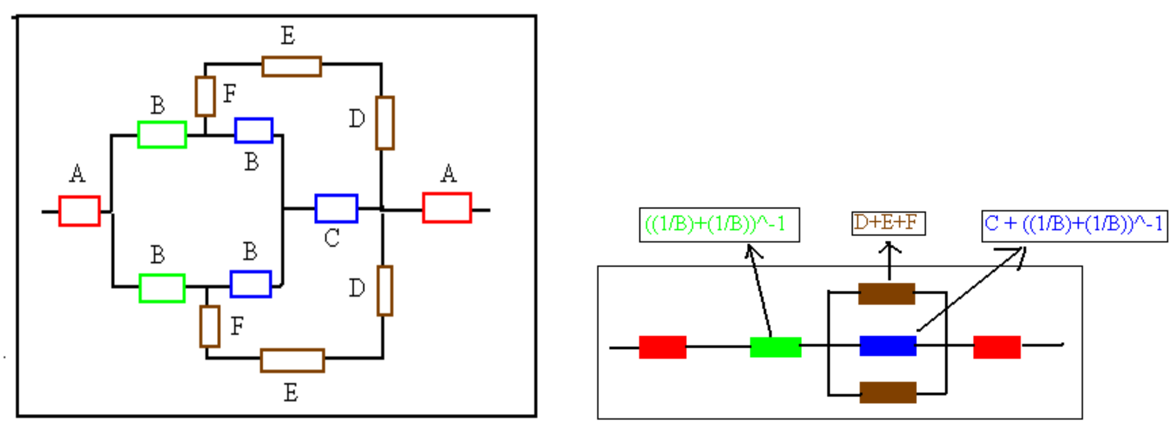

Figure 3: The resistances of the unblocked microfluidic network represented as color coded units

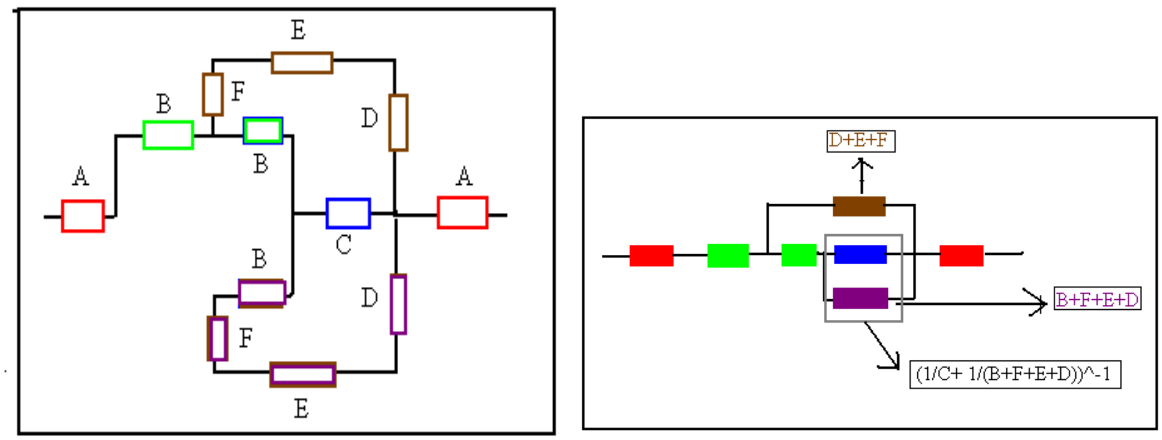

Figure 4: The resistances of the blocked microfluidic network represented as color coded units

Table 1 shows the calculated resistance for each channel as well as the whole network blocked and unblocked.

Table 1: Individual channel and overall resistance in the microfluidic network

Vessel Type	Width (um)	Height (um)	Length(um)	Resistance (kg/mm^4-s)
A	200	100	5000	0.27
B	100	100	5000	0.53
C	50	100	500	0.11
D	200	100	4300	0.23
E	200	100	4300	0.23
F	50	100	500	0.11
Total Resistance Unblocked				**0.96**
Total Resistance Blocked (kg/mm^4.s)				**1.37E+00**

The experimental model using a PDMS microfluidic device allowed students to physically manipulate the device and take part in data acquisition and trouble-shooting, providing them with hands on skills in microscopy, measurements, and image analysis. Comparing the experimental data with a mathematical model allowed students to see how well their experimental data matched with predictions and discuss any differences.

Figure 5 shows a schematic of microfluidic network with nodes and elements (Channels) used in the MATLAB® program. The code was given to the students. At any node n the sum of all the internal flows into channels connected at that node must be equal to the externally imposed volume flowrates Q. A global element matrix and a global nodal matrix was assembled with the input of pressures (P_1 and P_9), lengths, channel height and widths, with this information P_is and Q_is were solved.

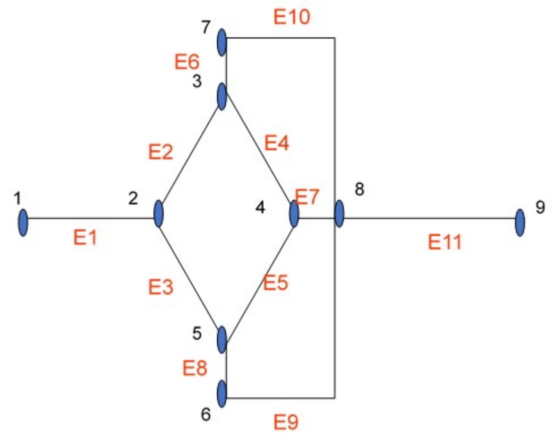

Figure 5: Schematic of the Nodes 1-9 and channels E1 – E11.

Figures 6 shows the MATLAB® prediction of volumetric flow in the channels in the unblocked and blocked cases. Figures 7 and 8 compares theory and experimental flowrates in the unblocked and blocked cases.

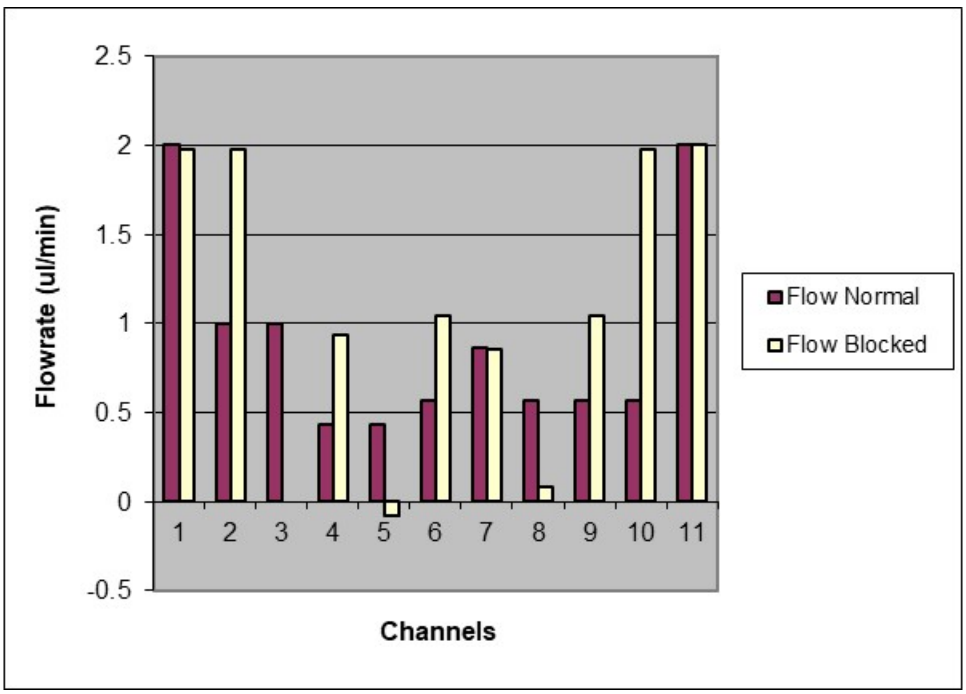

Figure 6: MATLAB® model prediction of flow in Channel when unblocked and blocked

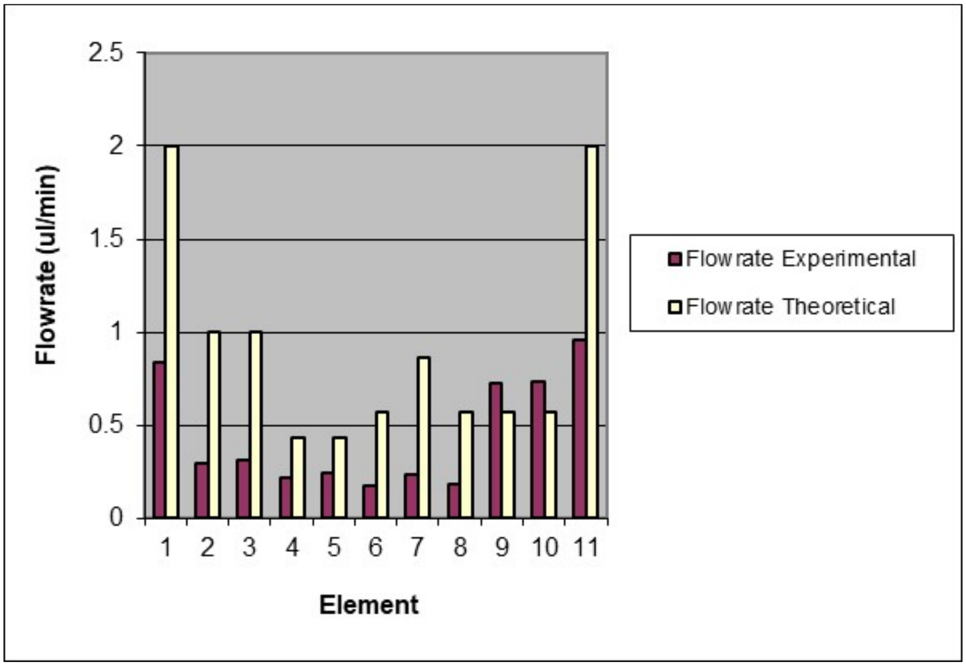

Figure 7: Comparison of experiment and model data for Unblocked microfluidic device

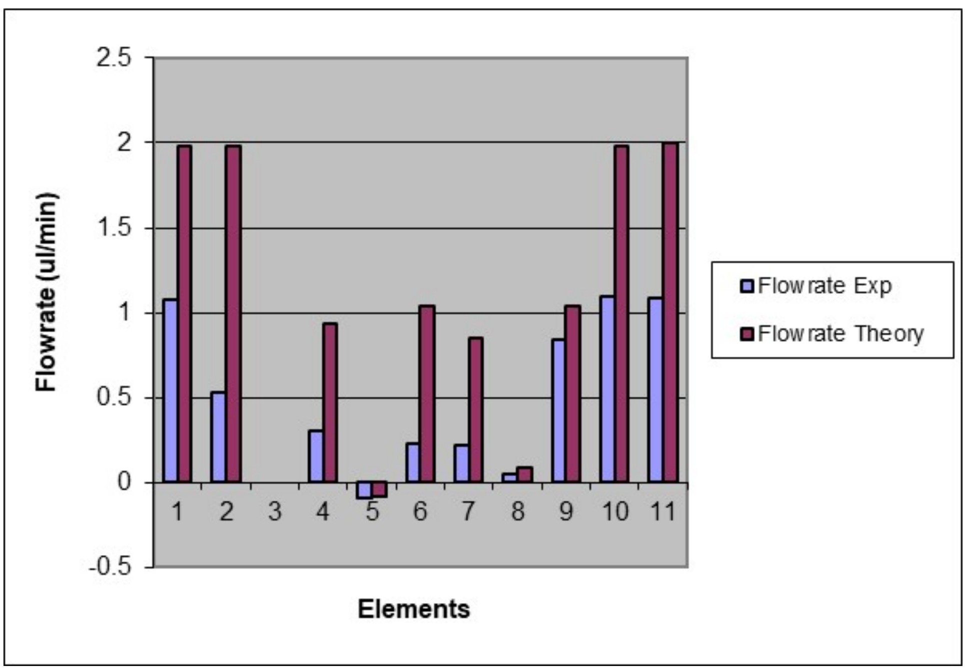

Figure 8: Comparison of experiment and model data for Blocked microfluidic device

Figures 7 and 8 consistently shows that the calculated velocity is higher than the measured velocity. Many students were uncertain of why this was the case and they attributed this to poor measurement techniques. However, when challenged on what the model was calculating and what the experiment was measuring, discussion centered around the fact that they were measuring beads not the fluid and there is drag on the beads which would result in the beads having a lower velocity relative to the fluid. Also, the position of the bead in the channel is important, the flow is fastest in the center of the channel and slowest at the edges. Beads were sampled from along the width of the channel, resulting in a wide distribution of velocities in each channel. The camera had a hard time capturing the faster beads in the center, so sampling was skewed to slower beads which would result in a lower average velocity. After analysis students also concluded that increasing the number of beads sampled would improve the statistics of the average velocity in a channel.

Before blocking the channel, students were asked to predict how bead velocity would change in all the channels. To be able to predict if the velocity would increase, decrease, or stay the same in each channel students needed to apply the knowledge that $Q = vA$, where Q is the volumetric flowrate, v is the velocity, and A is the area. 60% of the student's initial predictions correctly identified the trend in the change in velocity after blocking. 40% were unclear how the bead velocity in Channels 5, 8, and 9 would change. However, when writing the report with their experimental data in hand they were able to explain the trend of bead velocities as it moved through the network.

The success of this experiment and the supporting lectures in achieving the learning objectives was assessed by: 1) the written report which reflected the understanding of the concepts of pressure, volumetric flow and resistance; 2) Successful running of the MATLAB® code; 3) calculation of resistance; and 4) Answers to HW questions related to these concepts.

Specifically, in the Discussion section where students were asked to compare experiments to theory and to reflect on their initial prediction of flow in the channels after a blockage, 90% of the students did a good job explaining the difference between the experimental and theoretical results and why bead velocity changed as it did. Homework questions that asked students to predict velocity and pressure changes with model networks that varied parameters of input pressure, volumetric flow and blockages were solved by the students with a high rate of success.

Another objective was to have the students describe how this type of device can be used in cardiovascular research. Students successfully described how blockages can affect pressure and flowrates in vessel in the brain and circulatory system and if you have a redundant network redirection of blood flow can occur. They also postulated that a more complex theoretical model can be used to pinpoint vulnerable areas in these systems. They provided examples where microfluidics can be used to generate shear stresses, cell vascular networks and three-dimensional models of vascular tissue to study the vascular system.

Student performance indicated that the learning objectives were achieved based on both the final grade for the lab, and HW answers to pressure, volumetric flow, and resistance relationships. In addition, peer evaluations that asked students to rate themselves and each team member on time management, quality of work and contribution to the team showed that student worked well as a team. Student evaluations of this lab were quite positive. Comments included "Lab was interesting", "Interesting to learn about microfluidics", "Cool to measure bead flow", "The lab was the best part of the course: it was very interesting and helped me understand the material and gain valuable lab skills".

Conclusion

We have reported on a laboratory experiment coupled with a complementary computational exercise that introduces microfluidics to a freshman engineering class. Students used a PDMS microdevice as a model of the vasculature to characterize the resistance and flow velocities within this network. They then compared it to a theoretical model using MATLAB®. Microfluidic technology offers great possibilities to systematically study vascular cell biology that closely mimic the *in vivo* situation. Through this exercise and supporting lectures student were exposed to fluid dynamics of the vascular system, gained skills in image analysis, MATLAB®, team work and technical writing. Overall the lab was well received.

References

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