"STUDENT LAB"-ON-A-CHIP:
Integrating Low-Cost Microfluidics Into Undergraduate Teaching Labs to Study Multiphase Flow Phenomena in Small Vessels

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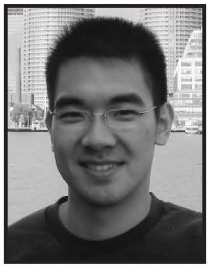
Blood is a complex fluid composed of cells and other biomolecules suspended in plasma. Its main function is to carry oxygen and nutrients to organs and tissues in the body, while also serving as a transport mechanism for elements of the immune system. Because of its composition, blood is a non-Newtonian, shear-thinning fluid that becomes less viscous at higher shear rates, and flows only after overcoming a yield stress that induces rouleaux breakup.[1] Rheological properties of blood are altered under certain pathological conditions, such as sickle cell anemia where abnormalities in red blood cell (RBC) morphology and stiffness result in cell clumping, lower RBC levels, and ultimately higher effective viscosity.[2] Knowledge of blood rheology is therefore fundamental not only to physiologists and biologists, but also to engineers who wish to design biomedical devices, engineer replacement blood vessels, or model blood flow patterns in vivo.

Courses in transport phenomena are core to most chemical engineering programs. Increasingly, interest in biomedical applications of transport and chemical engineering principles has led to the introduction of courses in biotransport and cardiovascular fluid mechanics in chemical and biomedical engineering curricula. At the University of Toronto, topics covered in these courses include blood rheology, steady and unsteady blood flow in large blood vessels, and blood flow in small vessels. The latter topic is interesting because non-intuitive microscale phenomena occur when blood flows in small vessels like arterioles, capillaries, and venules. For blood flowing at a specific shear rate in vessels less than 250 microns in diameter: 1) blood has lower effective viscosity in smaller vessels; and 2) blood hematocrit (*i.e.*, volume fraction of RBCs in the blood) is lower as vessel diameter is reduced.[3, 4] These two phenomena are collectively known as the Fahraeus-Lindqvist (F-L) effect, named after the two scientists who discovered the phenomena in a series of experiments involving the flow of ox blood in fine glass capillaries.[5] This effect can be explained by the concept of the plasma skimming layer, discussed in detail in Ethier and Simmons.[1] Briefly, RBCs concentrate in the core of small blood vessels, away from the walls where RBCs are depleted and where only a thin layer of plasma is present. In smaller vessels, this thin plasma layer occupies a larger fraction of the cross-sectional area compared to the plasma layer in larger vessels, resulting in lower RBC density (*i.e.*, decreased hematocrit) within the vessel and lower viscosity. From this basic explanation, it is clear that the F-L effect is a simple yet useful illustration of the non-Newtonian behavior of blood, and furthermore, is a textbook example of fluid-particle interactions in multiphase flows.

To enhance the students' understanding of the F-L effect and its origin, we developed a low-cost, practical, and feasible laboratory procedure that demonstrates key features of the



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original experiments performed by Fahraeus and Lindqvist. The experiment, which can be performed by the students, uses microchannels fabricated by soft lithography, a popular and widely available technique used for microfluidics research for myriad engineering applications.[6] The use of microfluidics and "lab-on-a-chip" technologies in engineering courses is a growing trend.[7, 8] In this lab, cells in suspension were forced through microchannels of varying widths and heights to mimic blood flow through small vessels. Images taken by light microscopy were used to determine cell density (*i.e.*, equivalent of tube hematocrit in blood) by cell counting, flow rate of the suspension by particle streak velocimetry, and effective viscosity as functions of channel dimensions. Here, we present the methods and results from our F-L experiment, discuss the pedagogical details related to the course and the potential usefulness of the laboratory procedure, and provide recommendations to those who may be interested in developing their own microfluidics laboratory experiment for demonstrating the F-L effect.

MATERIALS

For microchannel fabrication by soft lithography, SU-8-25 negative photoresist and SU-8 developer were acquired from Microchem Corporation (Newton, MA). Sylgard-184 poly(dimethylsiloxane) (PDMS) (Dow-Corning, Midland, MI) was obtained from Paisley Products of Canada, Inc. (Toronto, ON). Glass microscope slides for microchannel device assembly and Intramedic polyethylene tubing (PE60 and PE190) were from VWR International (Mississauga, ON). All slides were cleaned with piranha solution, prepared as a 3:1 (v/v) mixture of sulfuric acid and hydrogen peroxide. Concentrated sulfuric acid and hydrogen peroxide (30%) were from Fisher Scientific Canada (Ottawa, ON). Becton Dickinson Luer-Lok syringes and Precision Glide needles were also purchased from Fisher Scientific Canada. For cell culture, DMEM, penicillin-streptomycin (P/S), and 0.25% trypsin with EDTA were from Sigma-Aldrich Canada (Oakville, ON, Canada). Fetal bovine serum (FBS) was purchased from Hyclone (South Logan, UT, USA). T-75 and T-225 tissue-culture-treated flasks were from Fisher Scientific Canada (Ottawa, ON).

METHODS

Microchannel Fabrication

Microchannels were formed from PDMS and glass using the rapid prototyping technique (Figure 1).[9] Briefly, straight channel patterns were drawn in AutoCAD and printed at high resolution on a transparent photomask. Masters were fabricated by spin-coating SU-8-25 negative photoresist on glass slides that had been cleaned in piranha solution (30 min). After pre-baking, exposure, and post-exposure baking (according to SU-8 manufacturer specifications), the photoresist layer was developed by gentle agitation in SU-8 developer. PDMS in a 10:1 base-to-curing agent ratio was poured over the masters, exposed to vacuum to remove air bubbles, and cured at 70 °C for at least four hours. A piranha-washed glass slide and a PDMS cast of the microchannel pattern were both rinsed in isopropyl alcohol, surface-treated for 90 seconds in a plasma cleaner (Harrick Plasma, Ithaca, NY, USA), and then assembled with polyethylene tubing as inlet and outlet ports. Microchannels fabricated in this manner were either used immediately following inlet and outlet assembly, or stored indefinitely for future use.

Cell Culture

A mouse fibroblast cell line (L929) was obtained from the American Type Culture Collection (ATCC), and used as the model cell type for studying the F-L effect. Cells were seeded at ~20,000 cells/cm^2 in tissue-culture-treated polystyrene flasks, and cultured in DMEM supplemented with 10% FBS and 1% P/S. Media was changed every two days, and cells were passaged every four to five days, depending on confluency. To prepare for the F-L experiment, cells were detached from the flasks with 0.05% trypsin with 40 µg/mL EDTA, centrifuged at 284 × g for 7 min, resuspended in supplemented media at 20 million cells/mL, and kept on ice for the duration of the experiment.

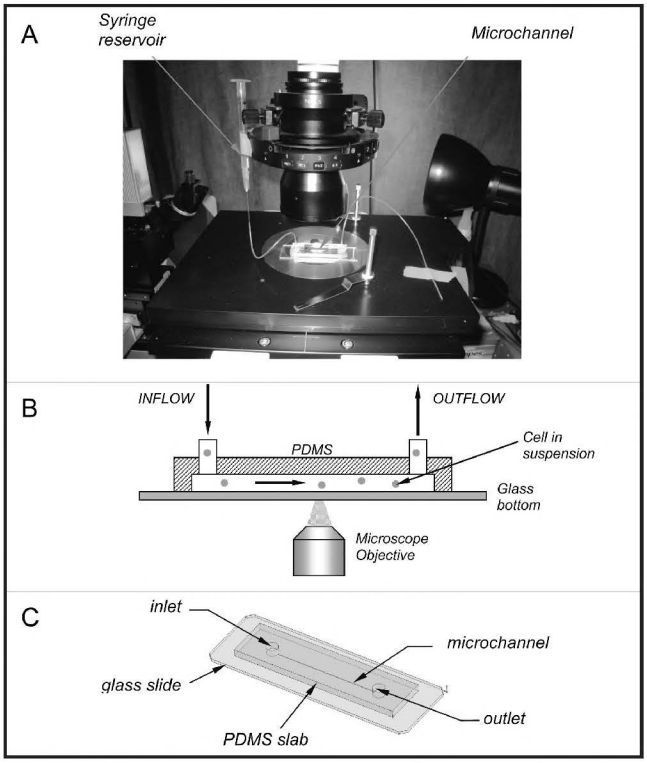

Figure 1. *Microfluidic experimental setup. (A) Gravity-driven flow is generated in the microchannel by securing the syringe containing the cell suspension to the microscope. (B) Side view of cell suspension flowing through microchannel and detected by objective of inverted microscope. (C) Construction of microchannel slide used in the laboratory session.*

Experimental Setup

To observe the F-L effect, an optical microscopy-based method was used (Figure 1). Microchannel slides were mounted on the microscope stage of an optical phase contrast microscope (Olympus IX-71), and connected via polyethylene tubing to an open syringe-needle assembly. The syringe-needle assembly was secured to the microscope at a height of ~10-15 cm above the microchannel. Cells suspended in media at 20 million cells/mL were dispensed into the syringe barrel and allowed to flow into the microchannel by gravity. Phase contrast images of the flowing cell suspension were captured with a CCD camera (QImaging Retiga, Surrey, BC) connected to the microscope, and analyzed using ImageJ software (NIH).

Particle Streak Velocimetry

Phase contrast images of the flowing cell suspension were used to determine the flow rate within the microchannels by particle streak velocimetry.[10] Suspended particles traveling at a steady velocity U generate a streakline in flow of length l over time t. Measuring lengths of streaklines for an image taken with a given exposure time yields velocity U = l/t. Particles residing on different streamlines of flow produce streaklines with varying lengths depending on the particles' location. The longest streaklines are found on the horizontal midplane, near the center of the microchannel, and correspond to maximum velocity in the microchannel.

Thus, the mean velocity, flow rate, and ultimately the effective viscosity can be calculated from measurements of the longest streakline in each image and formulae for the theoretical velocity profile in a rectangular microchannel. Figure 2A shows a typical particle streakline image obtained using fluorescent microbeads seeded into a rectangular microchannel, while Figures 2B and 2C are similar images from flowing cells.

Flow in Rectangular Microchannels

The theoretical background presented here was included in the laboratory manual presented to the students (see handout available at <www.introductorybiomechanics.com>). In the original experiments by Fahraeus and Lindqvist,[5] and in subsequent tests by Barbee and Cokelet,[4] fine glass capillaries with circular cross sections were used, and effective viscosity, μ_{eff}, was determined using Poiseuille's law:

$$Q = \frac{\pi R^4}{8\mu_{eff}} \frac{\Delta P}{L} \tag{1}$$

$$u_m = \frac{Q}{A} \frac{2D^2}{64\mu_{eff}} \frac{\Delta P}{L} \tag{2}$$

In Eqs. (1) and (2), Q is the flow rate, ΔP is the pressure drop across the capillary, L is the capillary length, R is the capillary radius, D is the capillary diameter, A is cross-sectional area, and $u_m = Q/A$ is the mean velocity in the channel. The constant β = 64 is the friction constant, equal to the product of the Reynolds number Re and the friction factor f:

$$\beta = f \cdot Re \tag{3}$$

In the current study, Poiseuille's law was modified for flow in rectangular microchannels.

Eq. (2) thus becomes:

$$u_m = \frac{2}{\beta} \frac{D_h^2}{\mu_{eff}} \frac{\Delta P}{L} \tag{4}$$

where capillary diameter D is replaced by the hydraulic diameter $D_h = 4A/P_w$, and P_w is the wetted perimeter, $P_w = 2(w + h)$. β for rectangular cross sections is governed by an empirical relationship[11] for channel aspect ratio α = h/w:

$$\beta = f \cdot Re = 96\left[1 - 1.3553\alpha + 1.9467\alpha^2 - 1.7012\alpha^3 + 0.9564\alpha^4 - 0.2537\alpha^5\right] \tag{5}$$

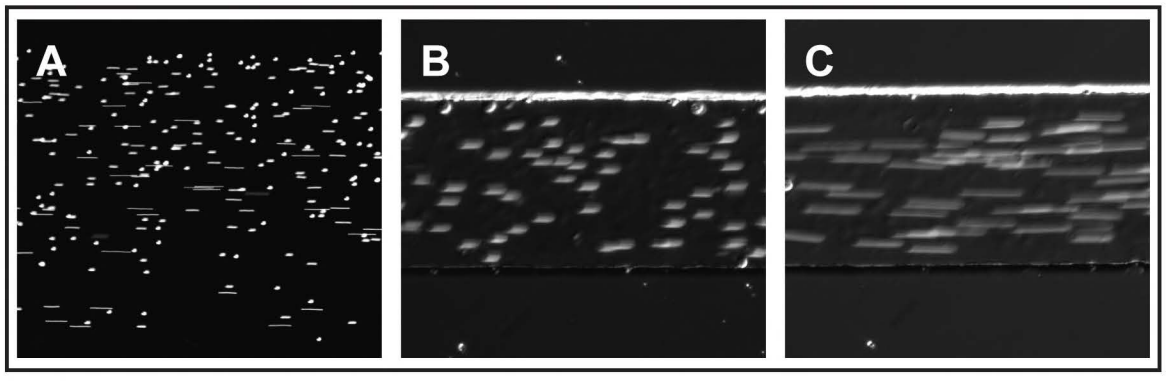

Figure 2. *Particle streak velocimetry using fluorescence microbeads or phase contrast imaging of cells. (A) Fluorescent 1-μm microbeads inside a 500-mm microchannel, using 200 ms exposure time to produce streaklines. (B and C) L929 mouse fibroblasts suspended in media at 20 million cells/mL in a 200-μm wide microchannel, using (B) 3 ms exposure time, and (C) 10 ms exposure time. The short streaklines in (B) were suitable for determining cell density within the microchannel, while the longer streaklines in (C) were suitable for determining velocity.*

For gravity-driven flow, the pressure drop across the channel is $\Delta P = \rho g H$, where H is the height difference from inlet to outlet reservoir. Thus, measurement of the mean velocity in the microchannel provides a solution to the effective viscosity using Eq. (4).

For laminar flow in rectangular channels, an approximation for the fully developed velocity profile was proposed by Purday.[11] For a microchannel of half-width a = w/2, and half-height b = h/2, the laminar velocity profile is:

$$\frac{u}{u_m} = \left(\frac{m+1}{m}\right)\left(\frac{n+1}{n}\right)\left[1-\left(\frac{y}{b}\right)^n\right]\left[1-\left(\frac{z}{a}\right)^m\right] \tag{6}$$

or

$$\frac{u_{max}}{u_m} = \left(\frac{m+1}{m}\right)\left(\frac{n+1}{n}\right) \tag{7}$$

where y is the channel height direction, z is the channel width direction, u and u_{max} are the local axial and maximum velocities, respectively, and m and n are empirical parameters found to be:

$$m = 1.7 + 0.5\alpha^{-1.4} \tag{8}$$

$$n = \begin{cases} 2 & \alpha \le 1/3 \\ 2 + 0.3(\alpha - 1/3) & \alpha > 1/3 \end{cases} \tag{9}$$

Figure 3 illustrates the velocity profile of Eq. (6). The profile is parabolic in the y-direction. The maximum velocity occurs at the midplane at y = 0. This maximum velocity is fairly constant throughout the midplane, except near the side walls where the no-slip condition reduces the velocity to zero.

Normalized Cell Density

To determine volume cell density within each of the four microchannels, short-exposure-time images were captured, and the number of cells in each image was counted. The total cell volume in the image was equal to the product of the number of cells and the volume of one cell, estimated by assuming that each cell was spherical with average diameter 16.5 μm (determined using the Vi-CELL Analyzer (Beckman Coulter, Mississauga, ON)). Dividing the total cell volume by the volume of the channel section in the viewfield yielded the volume cell density. Finally, the volume cell density was normalized by dividing it by the known suspension cell density in the reservoir. This normalized value was equivalent to the relative tube hematocrit reported in the classical F-L experiments.

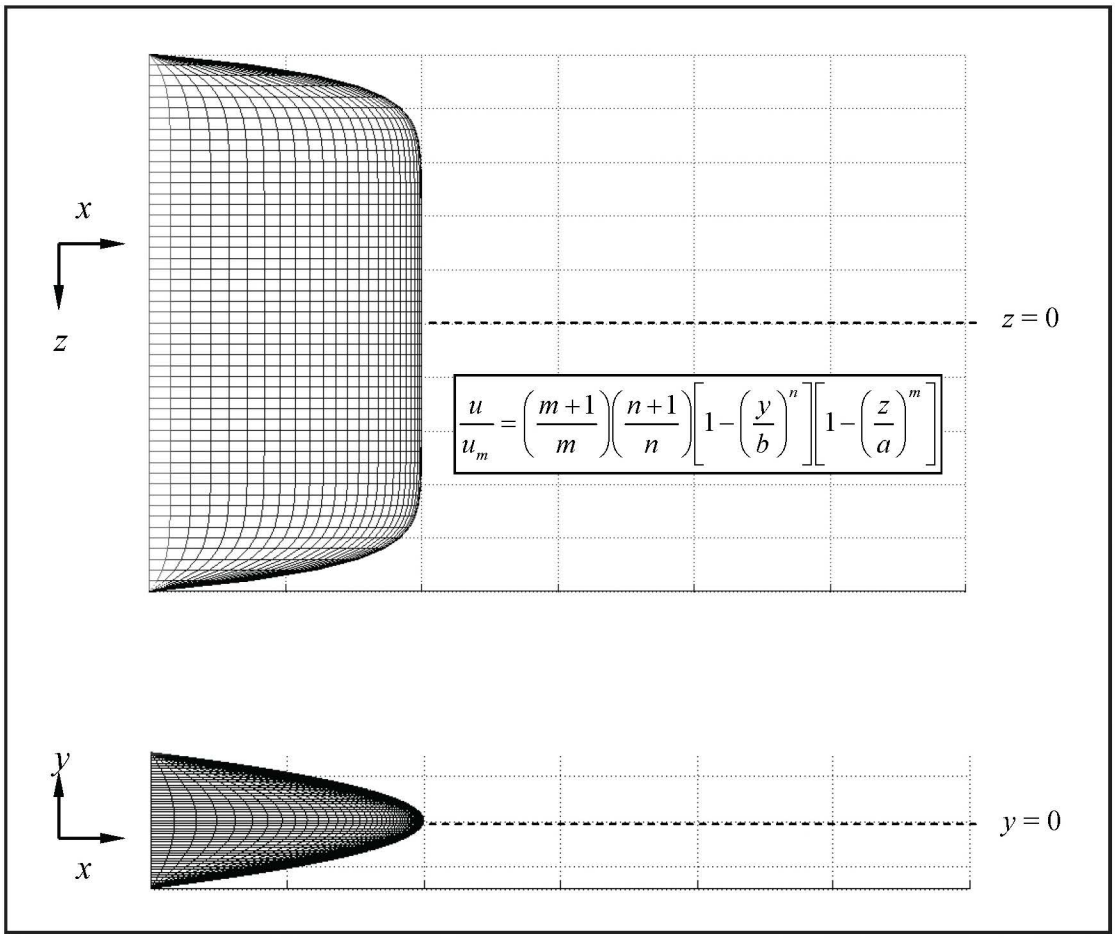

Figure 3. *Laminar velocity profile in microchannel of rectangular cross-section. The profile in the vertical x-y plane is parabolic for most of the channel width, except near the side walls where the velocity decreases to zero because of the no-slip condition.*

RESULTS OF THE EXPERIMENTS

Experimental trials of the above methods were tested for four microchannels of varying cross-sectional dimensions to demonstrate changes in effective viscosity (Table 1). For each microchannel, the column height of the cell suspension above the microchannel was measured, and 10 images each of short and long exposure time (Figure 2B and 2C) were captured. Short-exposure-time (3 milliseconds in our case) images were used to determine cell density in the microchannels, and long-exposure-time (10 milliseconds in our case) images were used to determine flow rates by particle streak velocimetry.

Figure 4 shows results for effective viscosity and normalized cell density from one representative trial. Effective viscosity was calculated using Eqs. (6) and (7) to determine mean microchannel velocity from measured streaklines, and then using Eq. (4) to solve for μ_{eff}. Effective viscosity decreased monotonically as the hydraulic diameter of the microchannel was reduced. Normalized cell density also decreased with decreasing hydraulic diameter, although the results for the 116- and 176-μm-wide microchannels deviated substantially from the general trend. The results for effective viscosity, and the general trend for normalized cell density, were consistent with the classical observations by Fahraeus and Lindqvist.

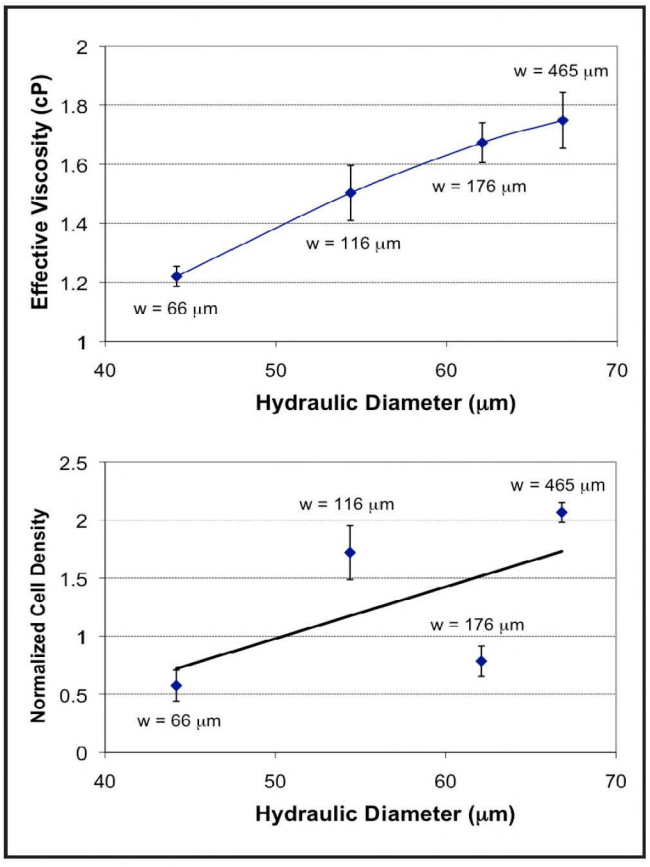

Figure 4. *(A) Effective viscosity vs. hydraulic diameter. Effective viscosity decreases monotonically with decreasing hydraulic diameter, as expected from the Fahraeus-Lindqvist effect. (B) Normalized cell density vs. hydraulic diameter. The general trend of decreasing normalized cell density with decreasing hydraulic diameter is apparent.*

DISCUSSION OF EXPERIMENTAL RESULTS

Fahraeus and Lindqvist observed that the effective viscosity and relative tube hematocrit of flowing blood in glass capillaries less than 250 μm in diameter both decreased as tube diameter decreased.[5] These phenomena were confirmed by Barbee and Cokelet,[3, 4] and are now frequently cited as textbook examples of the non-Newtonian behavior of blood. To enhance student understanding of this concept, we designed a laboratory session to allow students to observe the F-L effect firsthand. Four microchannels with hydraulic diameters ranging from ~ 40 to 70 μm were fabricated by soft lithography. Gravity-driven flow through the channels demonstrated that the effective viscosity and tube hematocrit decreased for smaller channels, consistent with the F-L effect reported in the literature.

Development of this laboratory session was made possible by the advances in microfluidics technology, and the continuing trend for less expensive and more accessible fabrication techniques. Microfabrication facilities and resources for producing chips by soft lithography are available at many universities, and increasingly so. If these facilities or materials for the production of SU-8 masters are not available or are too costly, alternative fabrication methods may be used, including recently reported techniques that employ Shrinky-Dink thermoplastics,[12] or rapid felt-tip marker masking.[13] While these techniques generally result in microchannels with dimensions that are difficult to characterize accurately due to greater surface roughness and less uniformity along the channel length, they are attractive because of their extremely low cost, and would likely be adequate for demonstration of the F-L phenomenon.

The laboratory procedure involved flowing a concentrated suspension of cells (20 million cells/mL of mouse L929 fibroblasts) through the microfluidic channels. This cell suspension is considerably different from a normal blood sample since there are typically ~5×10^9 RBCs/mL in blood, and RBCs (~ 8 μm) are biconcave disks that are much smaller than the spherical fibroblasts in suspension (~ 16 μm diameter). Using a non-blood sample has several advantages, however. First, the cell concentration can be tailored to produce images that have appropriate lengths of streaklines for easier analysis. A blood sample was used during preliminary lab testing, but the high density of RBCs generated overlapping streaklines, and thus was not well-suited for velocimetry. Secondly, from a biosafety standpoint, the mouse fibroblasts are an established cell line that requires facilities to be biosafety-certified to Containment Level 1 standards.[14] In contrast, human blood samples require Containment Level 2 safety. Since the L929

cells demonstrated the F-L effect in an effective manner, these two advantages made the cell line an attractive alternative to blood. We note that commercial microparticles can be used as an alternative to cells if cell culture facilities are not available, but we suggest that they be avoided if possible since they lack important cellular properties, such as deformability and the propensity for aggregation, that provide students with a more useful learning experience.

The use of the cells themselves as tracer particles was convenient, but the relatively large cell size compared to typical tracer particles meant that the cells likely interacted hydrodynamically with the surrounding fluid, and did not accurately represent the true channel velocity, as when 1-μm particles are used to generate streaklines (Figure 2A). This discrepancy is likely more important for wider microchannels where the cell density is greater, and particle-fluid interactions are therefore greater than in narrower microchannels. For the purposes of this lab, however, it was found that the use of cells did not adversely affect the ultimate outcome and that the F-L effect is clearly noticeable under the proposed experimental conditions.

Results for normalized cell density in the microchannels followed the expected trend as predicted by the F-L effect. There were inconsistencies with some of the results, however. First, the normalized cell densities for the 116- and 465-μm-wide microchannels were larger than unity when normalized cell densities were expected to be always less than unity for conduits having hydraulic diameters less than 250 μm. Second, the 176-μm microchannel had a considerably lower cell density compared to the 116-μm microchannel. These two anomalies may be attributable to two important differences between the experimental setup described here vs. those of the classical experiments: 1) the microchannel cross-section is rectangular, which likely impacts the effective surface area available for a plasma skimming layer to form; and 2) the syringe-needle assembly and microchannel reservoir geometry likely concentrated the cell suspension prior to its entrance into the microchannel, leading to cell densities higher than the density predicted for the reservoir cell suspension. This latter issue may be avoided by re-designing the microchannel geometry at the inlet port to reduce the amount of cell accumulation.

COURSE BACKGROUND, LABORATORY IMPLEMENTATION, PEDAGOGY, AND FEEDBACK

Course background

The lab has been conducted the past two years as part of MIE439-Biomechanics, a one-semester senior-level course offered by the Department of Mechanical & Industrial Engineering at the University of Toronto. The course serves as a capstone elective primarily for students in the bioengineering streams of mechanical and chemical engineering, and those in the biomedical engineering program of the Division of Engineering Science. This course provides a broad survey of topics within biomechanics, ranging from cell biomechanics to human locomotion, with emphasis on solving physiological problems using basic engineering principles. The course is popular, with typical enrollment of approximately 40-60 senior engineering students each semester. The course consists of three one-hour lectures per week, biweekly tutorial sessions, three laboratories per semester, and a semester-long group project. Evaluation is based on mid-term and final examinations, laboratory reports, homework assignments, and final class presentation and written technical report of the group project. There are no formal prerequisites, but the nature of the curricula ensures that all students have basic understanding of elementary dynamics, application of the Navier-Stokes equations, the concept of viscosity, and the difference between Newtonian and non-Newtonian fluids; these concepts are also reviewed during lecture. Indeed, it is the application of these principles and the synthesis of fundamental concepts from lower-level courses to solve complex biological problems that make this course unique from other electives.

Laboratory Logistics and Personnel

The laboratory was held in the undergraduate teaching laboratory of the Institute for Biomaterials and Biomedical Engineering (IBBME) at the University of Toronto. The IBBME teaching facility has biosafety level 1 (BSL-1) designation and has basic equipment for sterile cell culture work, as well as six phase contrast microscopes equipped with video cameras and basic imaging software.

Due to practical issues of course scheduling and the limited capacity of the teaching lab, the lab has been run in three one-hour sessions the past two years. In each section, students were further divided into groups of three to four students, with each group stationed at one microscope with one set of microchannels to obtain a shared set of data between all team members. Because of these logistics, the lab assignment was designed for completion within 50-60 minutes and preparations were made to attempt smooth transition between the three sections of students, such that as one section completed their work and the next was ready to begin.

TABLE 1
Measured Microchannel Dimensions

Channel	Height (μm)	Width (μm)	Cross Sectional Area (10^3 sq. μm)	Hydraulic Diameter (μm)
1	33.2	66	2.2	44.2
2	35.5	116	4.1	54.4
3	37.7	176	6.6	62.1
4	36	465	16.7	66.8

One week prior to the laboratory session, the students were divided into their groups and informed of the logistics. In the week leading up to the lab, various preparations were made. A laboratory manual was posted on the course Web site for students to download (available at <www.introductorybiomechanics.com>). The relevant theoretical concepts were presented in the regular lectures prior to the lab so that the lab served as reinforcement of the lecture material. Also during the week before the lab, cells were maintained and expanded in the teaching facility by a teaching assistant and lab technician to obtain sufficient quantities for running the lab. On lab day, the instructor, teaching assistant, and lab technician were present for the entire three-hour session to provide basic background materials, assist the students in setup, monitor their progress during the assignment, and provide formative feedback. Because dedicated hands-on training could not be provided due to limited resources, student groups relied on help from the staff and, in some cases, team members who had cell-handling and lab-bench experience from other bioengineering courses. Students were also given detailed instructions in the lab handout on how to operate the microscope and use the software package, and they were expected to come to the lab having read the material.

After completion of the lab, students were asked to analyze the data and complete three post-lab questions listed in the laboratory manual. The questions provided students with the opportunity to re-examine the experimental design, and discuss possible sources of error in the experiment. Since the post-lab questions were given to the students before the start of the lab, students were prepared to make observations about the procedure, and discuss possible improvements for the lab.

In terms of material costs and other resources, the teaching facility provided the space and access to equipment. Device fabrication and cell maintenance and expansion totaled approximately $200 CAD. Approximately 30 hours of time from the teaching assistant were devoted to design, development, and validation of the lab procedure prior to the pilot study. An additional 10 hours subsequent to development were devoted to preparations for operation of the actual lab, including microdevice fabrication, cell maintenance and expansion, student interaction on the day of the lab, and post-lab feedback.

Laboratory Pedagogy

The laboratory exercise served mainly to reinforce the concept of the F-L effect taught in lecture. An additional benefit of the lab, however, was that it acted as a hands-on exercise in cell handling, microscopy, and flow visualization, as well as a tool to reinforce other aspects of the bioengineering curriculum. Blood rheology and hemodynamics comprise a significant portion (approximately 25%) of the lecture material in MIE439, yet prior to this lab, the material was presented only during lectures and not through an active-learning experience. Engineering students have many different learning styles,[15] and lab exercises such as the one described here

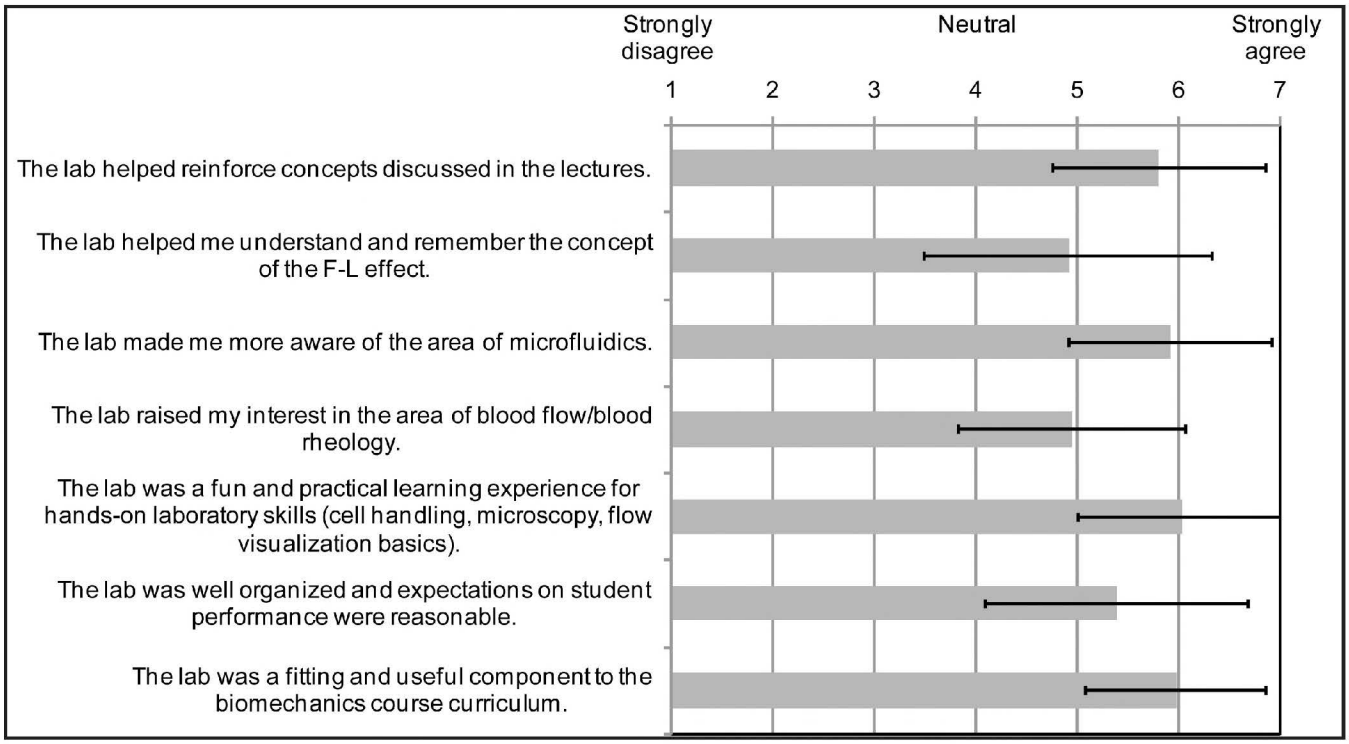

Figure 5. *Summary of student feedback from a voluntary online survey. Bars represent mean ± standard deviation for 34 to 36 responses per question.*

complement the lecture material, provide a visual representation to abstract concepts, and cater to the visual and sensory learners of the class.[16]

Other than the content described in the lab handout, students were not responsible for additional material related to microfabrication or microfluidics since these were not main topics within the course. Nonetheless, the exposure to microfluidics allows the students to learn basic aspects about this emerging field, its impact on biological and biomedical research activities, and its associations with other relevant courses in their chemical, mechanical, or biomedical engineering programs. Thus, the microfluidics aspect of the current lab assignment provides students with a clear example of the integrative nature of bioengineering as well as the importance of making connections between different science and engineering disciplines, an issue that remains an ongoing challenge in the development of core bioengineering curricula at many universities.[17]

The post-lab activities were limited to student contemplation of the questions posed in the lab handout. A formal laboratory report was not required, so as to relieve the "burden" of another report[18, 19] and to allow the students to focus on learning the concepts. To ensure the material was reviewed and the questions answered, the students were informed before the lab that a question on the final exam would be based directly on the lab exercise. As such, answers to the post-lab questions were not provided to the students. Though some may argue that a mandatory write-up of the exercise would have further improved chances of students retaining the material,[20] our guarantee of a final exam question in fact resulted in more student-staff interaction, and created a new opportunity for formative feedback because students came forward to discuss their interpretations of the post-lab questions with the teaching staff in preparation for the exam.

Logistics and resource limitations prevented the students from receiving hands-on training on the equipment prior to the lab. Therefore, to successfully complete the lab, teams had to rely on the laboratory manual and laboratory staff for assistance, but more often on their colleagues' experience and the team's ability to solve problems. Thus, an unintended benefit of the lab exercise was that it provided an opportunity for students to engage in face-to-face promotive interaction and to develop collaborative skills for future team-based projects.[21]

Student Feedback

Students in the Fall 2008 course were asked to provide feedback by completing a voluntary online survey; approximately 60% of the students responded. Feedback was generally very positive (Figure 5). The majority of students moderately or strongly agreed that the lab reinforced concepts from lecture and helped them understand and remember the F-L effect—the main objectives of the lab exercise. Many students appreciated the "hands-on experience" that was closely aligned with lecture material, such that the lab enabled them to "visualize the F-L effect," making it "very educational" and "useful for understanding the theory" from lecture. As summarized by one student: "Anyone can draw diagrams of fluid flow in capillaries and provide the equations, but it didn't really mean anything to me until I saw it happen—and this lab enabled that." Similarly, the vast majority of students moderately or strongly agreed that the lab was a fun and practical learning experience for hands-on laboratory skills that had the added benefit of making them more aware of microfluidics. The opportunity to work with "cutting-edge," "high-tech" equipment that was "simple," involved "something other than computer simulations," and allowed them to see "real cells" was mentioned frequently by the students. In total, 94% of the students agreed that the lab exercise was a useful component of the course curriculum.

An additional benefit of the lab was that it acted as a hands-on exercise in cell handling, microscopy, and flow visualization, as well as a tool to reinforce other aspects of the bioengineering curriculum.

Most students generally "appreciated being able to use the (laboratory) time to learn the concepts without the pressure or burden of having an ugly follow-up report." In contrast, a minority felt that a formal lab report would further reinforce concepts by forcing the students to answer the questions fully. Interestingly, only 56% of students agreed that the lab helped their performance on the final exam. Qualitatively, students did very well on the exam question related to the lab, but because a similar question was not asked in years prior to implementing the lab, it is not known to what extent the lab exercise was responsible for the students' performance. The majority of students reported that they were more interested in blood rheology as a result of the lab.

Criticisms and suggestions for improvement were primarily related to the logistics of the lab. Many students commented that they would have preferred more than one hour to complete the lab because they had felt rushed, and several felt that the groups should be limited to two students so that there would be more opportunity for everyone to get hands-on experience and the laboratory room would be less crowded. Laboratory and course staff had the same opinion, and these issues will be addressed in the future by having several 1.5 hour sessions over multiple days. Other criticisms were related to equipment issues (*e.g.*, a malfunctioning camera, software problems,

leaky connections in some chips), and problems with cells clogging in the channels, which delayed data collection. Clogs were readily cleared by application of positive pressure with the syringe, and—as suggested by one student—may be minimized by using other cell lines, such as nonadherent Jurkat cells (an immortalized line of T-cells).

CONCLUSIONS

Microfluidics was successfully implemented into an undergraduate teaching laboratory session to demonstrate the Fahraeus-Lindqvist effect visually through optical imaging. Effective viscosity and normalized cell density within the microchannels was calculated and compared qualitatively to expected results. Overall, the experiment produced results that were consistent with the observations made originally by Fahraeus and Lindqvist. The experimental setup was easy, affordable (assuming soft lithography equipment and biosafety-certified laboratory facilities are available), and reasonable to manage. Students learned to apply particle streak velocimetry as a technique for determining flow rate within microchannels, and were able to observe flow phenomena firsthand in a practical laboratory setting. The implementation of this lab session therefore appealed to visual and sensory learners, and generated interest in the topic on hemodynamics and blood rheology.

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