Cellular and Molecular Bioengineering, Vol. 3, No. 3, September 2010 *(© 2010)* pp. 319–330
DOI: 10.1007/s12195-010-0124-0

An Undergraduate Lab (on-a-Chip): Probing Single Cell Mechanics on a Microfluidic Platform

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(Received 14 February 2010; accepted 4 May 2010; published online 14 May 2010)

Associate Editor Jin-Yu Shao oversaw the review of this article.

Abstract—The study of the mechanical behavior of cells is an active area of academic research and as such, is an increasingly important component of biomedical engineering education. However, in delivering engineering courses, practical experience with experimental cell mechanics is often challenging to provide for untrained undergraduate students, as the lab work involved is expensive, delicate and usually requires substantial experimental skill. This article reports the development of a novel lab experience for senior undergraduate students, for which a microfabricated system was designed and constructed to conduct micropipette aspiration of single cells. Application of the microfabricated system to measure the elastic modulus of primary heart valve fibroblasts produced results comparable to those made with a conventional micropipette aspiration system. The relative simplicity and affordability of the system made it accessible to undergraduate students in a laboratory course, who judged the lab as a strongly positive learning experience.

Keywords—Cellular biomechanics, Engineering education, Micropipette aspiration, Microfabrication, Cell modulus.

INTRODUCTION

The internal mechanical structure of a cell is complex and dynamic. The cellular cytoskeleton consists of a network of protein filaments that mechanically support the cell membrane, and reorganizes in response to chemical signals,[28] external mechanical forces,[24] and cell–cell contacts.[4] Because the cytoskeleton plays a role as a mechanotransducer,[30] changes in organization can result in changes in gene expression, adhesion, secretion, and metabolic activity.[14,21] These cytoskeletal changes alter the mechanical properties between cell phenotypes,[6,13,25] at various stages of stem cell differentiation,[27] and may lead to alterations in normal physiological behavior,[10] indicating or even triggering pathogenic responses. Hence, the ability to evaluate single cell biomechanics is an important experimental technique, and may provide greater insight into how cells receive and integrate regulatory signals from the surrounding environment.

Evaluating the mechanical properties of a single cell can be achieved through several methods. Experimental techniques such as atomic force microscopy, magnetic microbead rheometry, compressive testing, optical microbead manipulation, and optical stretching are typically employed,[7] but are expensive, experimentally complex and require extensive operator training before use. Micropipette aspiration (MA) is a relatively robust and simple technique capable of evaluating the structural and mechanical response of single cells.[12,18] MA simply uses suction pressure to partially aspirate a cell into a small-diameter glass tube. Geometric constraints and the resulting deformation as a function of applied pressure can then be used with relevant analytical[16,23] or computational[1,35,36] models to determine the mechanical properties of a single cell.

Modern engineering problems have no clear disciplinary boundaries. Training engineers to address these problems requires an educational curriculum which integrates disciplinary knowledge and experience.[22] Undergraduate courses in biomechanics are well-suited to this task, as they can be designed to demonstrate the importance and application of traditional mechanical engineering principles to complex problems in physiology, rehabilitation, medicine, and tissue engineering. Despite the importance of hands-on experimentation

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in engineering education,[8] such courses often lack a practical, experiential component for studies in biomechanics at the cellular scale. This is primarily due to the delicate, challenging, and expensive nature of the work involved, making them impractical for a relatively short educational experience. Even in a simple MA system, students would first be required to handle extremely fragile glass micropipettes; and second, skillfully operate an expensive micromanipulator, which would require extensive user training.

To address these issues and meet the need for a practical component in cellular biomechanics education, we have developed a simple and cost-effective microfabricated system to conduct single cell MA experiments. The integrated Micropipette Aspirator chip (iMAchip) eliminates the need for students to handle micropipettes, or access and operate a micromanipulator. Using the iMAchip, we developed a cost-effective, practical and hands-on undergraduate lab experience for senior mechanical and biomedical engineering students. The lab was delivered in the fall semesters of 2008 and 2009 at the University of Toronto, and we have evaluated the impact of this experience by means of a student survey.

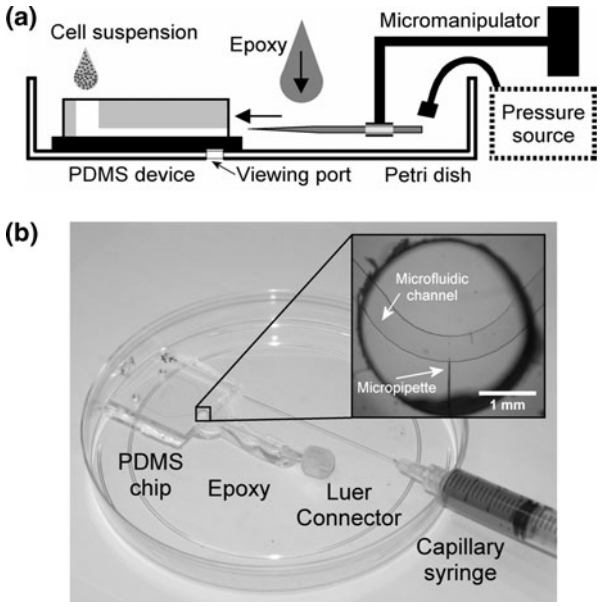

FIGURE 1. (a) Schematic side-view of the device, demonstrating fabrication and operation of the iMAchip device. The micropipette is manipulated into the PDMS device, and epoxied into place on the Petri dish. The slip-mounted micromanipulator fitting is withdrawn, and the device is primed, loaded with a cell suspension, and connected to a pressure source for cell aspiration. (b) Fabricated iMAchip system (pictured with capillary syringe used to prime the micropipette with deionized water prior to use; colored dye used for clarity).

MATERIALS AND METHODS

Unless otherwise stated, all chemicals and reagents for cell culture were purchased from Sigma-Aldrich (Oakville, ON, Canada). Fluid connectors, valves, and adapters were purchased from Cole-Parmer Canada (Montreal, QC). Other equipment and materials were obtained from Fisher Scientific Canada (Ottawa, ON).

iMAchip Overview

In order to improve the robustness and eliminate the need for an expensive micromanipulator in an MA setup, a microfabricated approach was developed (Fig. 1). The primary purpose of the microdevice is to put cells in close proximity to a fixed micropipette tip. Large pressure differentials applied to the micropipette can then be used to attract nearby cells to the tip, where well-controlled pressures can then be used to aspirate cells into the pipette. Once a measurement is made, the cell can be expelled from the micropipette; and the system flushed and reused.

The system was designed to flush a cell suspension through a microfabricated U-shaped channel, 500 μm in width. A straight flowing channel could be used just as effectively as the shape of the channel is not a critical design parameter for device operation. A second channel narrows to 20 μm in width and connects to the apex of the U. The purpose of this channel is to allow a standard micropipette to access cells in the confined space of the U channel. The micropipette is permanently fixed into place within the channel, so that the experimental system is robust, portable and self-contained.

Mold Master Fabrication

Fabrication of microchannels was conducted using soft lithography, in which a photoresist based 'master' is used as a negative relief template for polymer casting. To create the masters, SU-8 25 (Microchem; Newton, MA, USA) was spin-coated at 2000 RPM onto a 3″ × 2″ glass slide. The glass slide was previously coated with an unpatterned SU-8 seed layer to enhance adhesion between the microstructures and the glass. A masked pattern was printed on a transparency film at 20,000 DPI (Cad/ART Services; Bandon, OR, USA). The master was soft-baked, patterned by selectively photo-crosslinking the resist by exposure through the mask, hard-baked and developed according to the manufacturer's specifications. The resulting SU-8 features were measured on a Wyko optical surface profilometer (Veeco Instruments Inc.; Woodbury, NY, USA) and found to be 30–35 μm thick. The masters

were then treated with a silanization agent, (Tridecafluoro-1,1,2,2-tetra-hydrooctyl)-1-trichlorosilane (United Chemical Technologies, Bristol, PA, USA), under vacuum conditions, to facilitate mold-release of the patterned elastomeric polymer slabs.

iMAchip Fabrication

Sylgard 184 poly(dimethylsiloxane) kits (PDMS; Dow-Corning, purchased through A.E. Blake Sales Ltd.; Toronto, ON, Canada) were obtained and the monomer and crosslinker reagents were mixed in the standard 10:1 ratio. The uncured polymer was poured over the microfabricated master, and placed under vacuum to remove any trapped air bubbles. PDMS was cured in an oven for at least 4 h at 80 °C. The resulting patterned PDMS slabs were then removed, peeled away from the master, and trimmed to size. A 1/8″ punch was used to core out the fluid inlet and outlet ports. The patterned PDMS surface and a 3″ × 1.5″ glass slide were treated in an oxygen plasma corona for 30 s each, generated by a hand-held corona discharge unit (Electrotechnic Products; Chicago, IL, USA). The substrates were then placed in conformal contact such that the base of the Y-channel was within millimeters of the glass edge. The device was heated on a hotplate at 80 °C for 20 min to permanently bond the two materials (Fig. 1).[11] A 0.2% w/v solution of Pluronics F127 surfactant was then flushed into the channel, to minimize adhesion between the channel surfaces and the cell samples. The Pluronics solution was removed and the device channels were flushed with deionized water twice, before allowing them to dry at room temperature.

A 1/8″ diameter hole was drilled into the base of a 160 mm Petri dish approximately 2 inches from the edge of the dish. A quick-drying epoxy was used to bond the device to the Petri dish, such that the nexus of the Y-channel was centered over the hole in the Petri dish.

Micropipettes of varying diameters were prepared from glass capillaries using a commercial pipette puller (Model 700C, David Kopf Instruments; Tujunga, CA, USA), and the tips were cut using a De Fonbrune microforge (Warner Instruments; Hamden, CT, USA). Pipettes with tips having an inner diameter between 6 and 10 μm were selected for use in the iMAchip setup. In order to reduce non-specific adhesion of the cells to the micropipette during device operation, Sigmacote was applied to the micropipettes prior to use. Micropipette tips were dipped into a vial of Sigmacote for 1–2 s and removed. Air was then pushed through the micropipette to clear the tip of any excess solution. Pipettes were allowed to dry for several hours in a fumehood before use.

For each iMAchip manufactured, a micropipette was manipulated and fixed within the channel, as per the schematic in Fig. 1a. A selected micropipette was mounted in a micromanipulator (Siskiyou; Mission Viejo, CA, USA), on a slip-fit tube mount aligned parallel to the microscope stage (Olympus IX71; Olympus Microscopes; Markham, ON, Canada), to which the Petri dish containing the fixed PDMS-glass microchannels was clamped. The micropipette was carefully manipulated into the base arm of the Y-channel until the pipette tip protruded ~20 μm into the main channel. Epoxy was used to bond the micropipette into the base of the Y-channel, and the system was left untouched for 20 min while the epoxy hardened. The slip-fit tube mount was then withdrawn, leaving the micropipette embedded in the microchannel base. A section of Intramedic polyethylene tubing (Clay Adams PE190) was used as a step adapter between the micropipette and a 1/16″ ID PVC tube, which was in turn connected via a barbed adapter to a female luer lock port. Epoxy was applied to the connections to ensure there was no leakage. The completed iMAchip was then stored before use.

Immediately prior to running the aspiration experiments, the channels were primed with liquid media. To do this, a long capillary (MF34G-5 MicroFil capillaries; World Precision Instruments; Sarasota, FL, USA) was used to inject deionized water into the micropipette via the luer port connection (Fig. 1b). The capillary is carefully inserted as far down the pipette as possible, and deionized water is injected slowly as the capillary is drawn out of the micropipette.

Pressure Source Peripherals

Two ranges of fluid pressures are required to operate the iMAchip. In order to apply well-regulated, small changes in pressure for cell aspiration, the height of liquid in a fixed reservoir (at atmospheric pressure) was adjusted. A ruler was clamped vertically in a retort stand beside the microscope stage. The open reservoir, which consisted of a 60 mL syringe with the plunger removed, was mounted directly to the ruler using elastic bands, at approximately the level of the microscope stage. During the experiment, the level of fluid was adjusted by carefully adding to or removing water from the reservoir.

In order to attract cells toward the pipette tip and clear the pipette of aspirated cells, larger pressures are required. These are manually applied using a closed, filled syringe. Switching between these two pressure sources required a three-way valve with luer attachments. The three-way valve was connected to the open fluid reservoir, a closed 5 mL syringe and to the

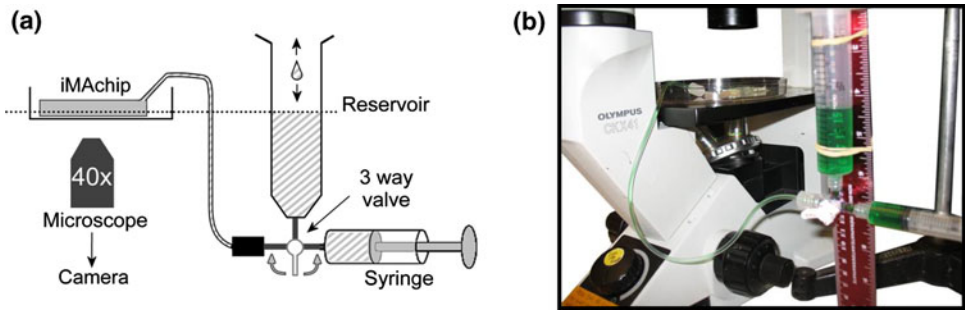

FIGURE 2. (a) Schematic diagram of pressure system. The three-way valve is used to switch between applying large, uncontrolled pressures via the syringe, and applying small well controlled pressures via the liquid column in the reservoir. Adding or removing liquid to the reservoir changes the pressure applied. (b) Experimental setup for iMAchip peripherals (green dye used for this demonstration).

iMAchip device, via tubing and a male luer-to-barb connector (Fig. 2).

Immediately prior to the experiment, the pressure sources were filled with water. Approximately 40 mL of water was dispensed into the reservoir. The valve was set to isolate the iMAchip from the reservoir and syringe, and water was drawn into the syringe. The valve was then used to isolate the syringe, and water was allowed to fill the tubing between the iMAchip and the reservoir. Once filled, the tubing was connected to the primed iMAchip device, using a droplet merging technique to prevent air bubbles being trapped in the pressure line.[33] Briefly, a small excess of liquid was added to the primed iMAchip device, such that a droplet bulged over the luer-lock connector edge. Liquid in the tubing was controlled to form a similar droplet at the connector. The two droplets were merged, and the connectors locked together, eliminating air bubble trapping during this tube connection process.

Cell Culture

Primary porcine aortic valvular interstitial cells (PAVICs) were isolated by enzymatic digestion as previously described,[32] expanded in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum, and frozen at passage five, prior to use. Immediately prior to running the lab session, frozen cells were thawed, mixed with 10 mL of DMEM, centrifuged and resuspended in 1 mL of fresh supplemented DMEM. Cell suspensions were stored on ice during the lab, and replaced with freshly thawed cells every 45 min.

Experimental Procedures

At the beginning of the experimental session, primed and filled iMAchip devices were placed on the microscope stage, and connected to the pressure source as previously described. The microscope and camera images were first calibrated to a standard length scaling factor, using a hemacytomer (Fisher Scientific Canada; Ottawa, ON).

Cells were flushed into the large channels by applying a small pressure via a standard Pasteur pipette bulb.[20] Ten microliters of well mixed cell suspension were pipetted into the inlet port of the device, and a pipette bulb was placed around the port. Gently squeezing the pipette bulb resulted in a pressure differential across the channel, and the cell suspension was flushed into the channel (Fig. 3). 7–8 μL of cell suspension was then deposited into the outlet port, to minimize the pressure differential created due to the height difference of fluid in the ports, so that the system reached equilibrium more quickly.

When the flow of cells within the microchannel had subsided, the equipment was ready for aspiration experiments. The water reservoir was adjusted such that the water level was a few millimeters below the experimental equipment. This height difference was recorded as the 'tare pressure height', and serves to apply a small suction pressure to the cells, forming a seal between the cell and the micropipette rim. The valve system on the pressure sources was then adjusted to isolate the reservoir, such that the syringe-based pressure source could be used to attract and repel cells from the micropipette tip. This required some experimental skill and practice, but was achievable by students in a lab session. A steady application of pressure was used to draw cells close to the pipette tip. Immediately on contact, the pressure source was switched to the reservoir system, where the preset tare pressure formed a seal between the cell and the pipette tip. After 60 s, an image was taken of the cell, slightly aspirated into the pipette. Liquid was then removed from the reservoir with a pipette, creating larger suction pressures at the micropipette tip. The height difference between the water level and the microscope stage was again recorded as the 'aspiration pressure height'.

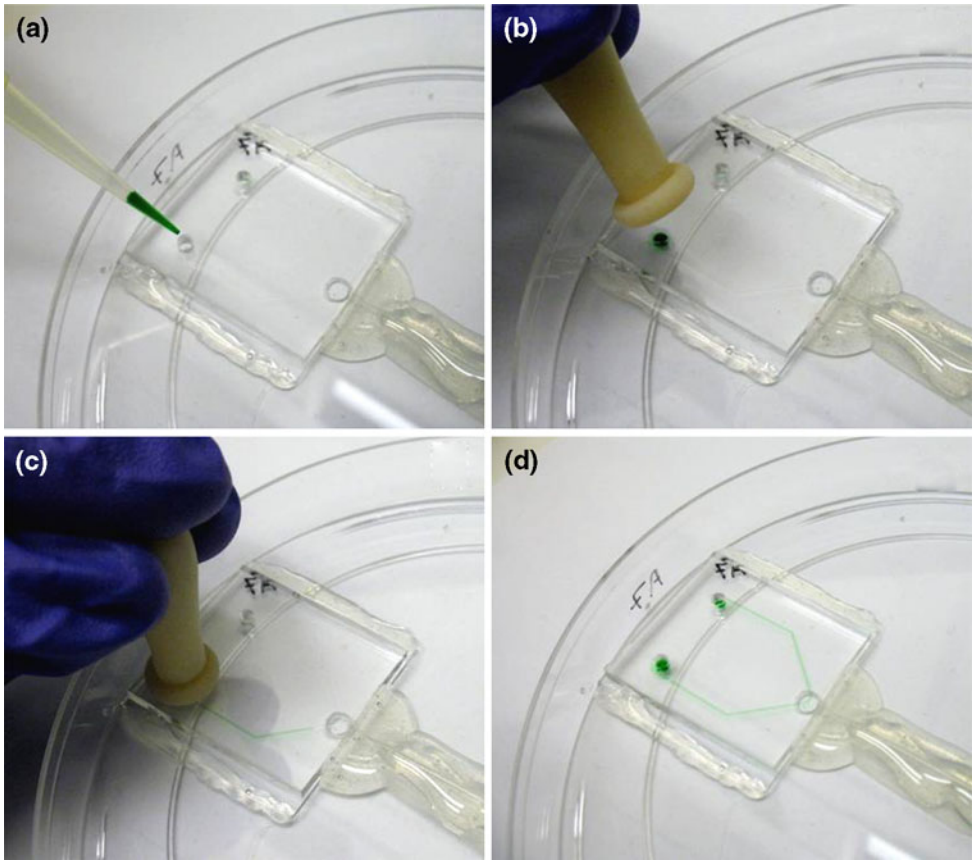

FIGURE 3. Loading cell samples (green dye used for clarity) into the iMAchip devices. (a) Cell suspension is pipetted into the microchannel access port. (b) A Pasteur pipette bulb is placed over the port and (c) gently squeezed, (d) driving fluid flow through the channel.

Cells were allowed to deform for at least 60 s, until they reach their equilibrium aspiration length, before a picture was taken.

Analytical Model for Micropipette Aspiration

The most common mechanical model for MA, the half-space model, approximates the cell as an incompressible elastic half-space, where aspiration length is proportional to the applied suction pressure and inversely proportional to the elastic modulus.[26] Despite a number of simplifying assumptions, this half-space model is widely used in current MA studies.[3,15,26] The elastic modulus is given as:

$$E = \frac{3\Phi_p \cdot \Delta P \cdot R_p}{2\pi \cdot L_{asp}}$$

where E is the Young's modulus, L_{asp} is the difference between the tare and final aspiration lengths, R_p is the pipette radius, ΔP is the difference between the tare and final aspiration pressures, and Φ_p is a dimensionless constant dependent on the ratio between pipette wall thickness and pipette radius. The L_{asp} and R_p parameters were measured directly from the images of aspirated cells, using image analysis software (ImageJ; NIH). A schematic diagram of an aspirated cell (Fig. 4) is provided to indicate the required measurements. The value of Φ_p is taken as 2.1 for most experiments.[26] ΔP is calculated using the equation:

$$\Delta P = \rho \cdot g \cdot \Delta h$$

where ρ is the density of the water column (1 g/mL), g is the acceleration due to gravity (9.8 m/s^2), and Δh is the difference between the tare and aspiration heights of the liquid column.

EXPERIMENTAL RESULTS AND DISCUSSION

In order to validate the iMAchip setup, we repeated a characterization experiment previously performed by our group using a standard MA system,[35] on primary sub-cultured PAVIC cells. MA measurements could be influenced by cell type, operating temperature, analysis procedure, applied pressure levels, micropipette diameter, and micropipette wall chamfer radius,[35] all of

which were kept constant between the two studies. Aspiration measurements were made on eight separate PAVICs, using two iMAchip setups. Sample images of a cell being aspirated are shown in Fig. 5. The results, shown in Fig. 5c, indicate a mean elastic modulus of 927 ± 256 Pa (standard deviation). The values for cell modulus obtained in this experiment were not significantly different from those reported by Zhao *et al.*[35] (738 ± 222; $p = 0.052$ by *t*-test), who used a standard MA system and the half-space model. The slight differences observed could have been due to heterogeneity in the cell populations measured[5]; the use of passaged cells, which stiffens PAVICs[35]; or differing extent of valvular disease between the animals from which the cells were harvested. To date, no other group has conducted MA experiments on porcine valve interstitial cells. However, Merryman *et al.*, have used this technique to characterize ovine valve interstitial cells.[17] Their results (346 ± 142 Pa) are significantly different from our studies ($p < 0.001$ by *t*-test), likely due to differences in the animal model, culture conditions, and experimental parameters, which can impact modulus measurements.[35]

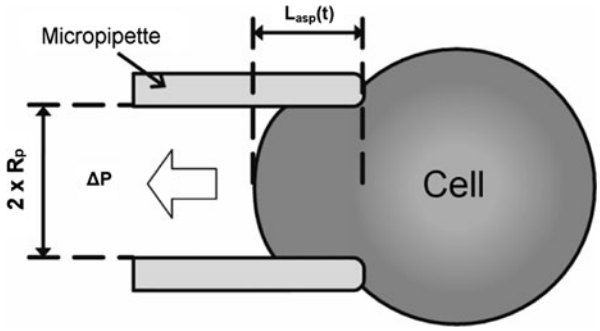

FIGURE 4. Cell aspiration schematic indicating key parameters in the elastic half space analytical model.

For this validation experiment, these data points were obtained by an operator familiar with using a standard micropipette aspiration system. Qualitatively, the measurements were made in approximately the same amount of time required using a standard MA setup (~7–8 min per cell). Untrained undergraduate students were not as quick in data collection. Due to their limited time in the lab and the learning curve necessary to understand and correctly operate the equipment, students were only able to generate data for 1–2 cells per group. Though qualitatively similar to the data collected in our validation studies, student-generated data is not reported here, as comparison with these limited datasets would be ineffective.

Besides eliminating the cost and complexity of the micromanipulator from an MA system, there are other advantages to this microfluidic platform. The operator appreciated the fact that the exclusion of the micromanipulator from this setup eliminated vibration of the pipette tip, which made it easier to form an adequate tare pressure seal around the cell for aspiration. The operator, previously inexperienced with microfluidics, was also able to quickly learn how to manipulate fluids within the system, using the Pasteur pipette

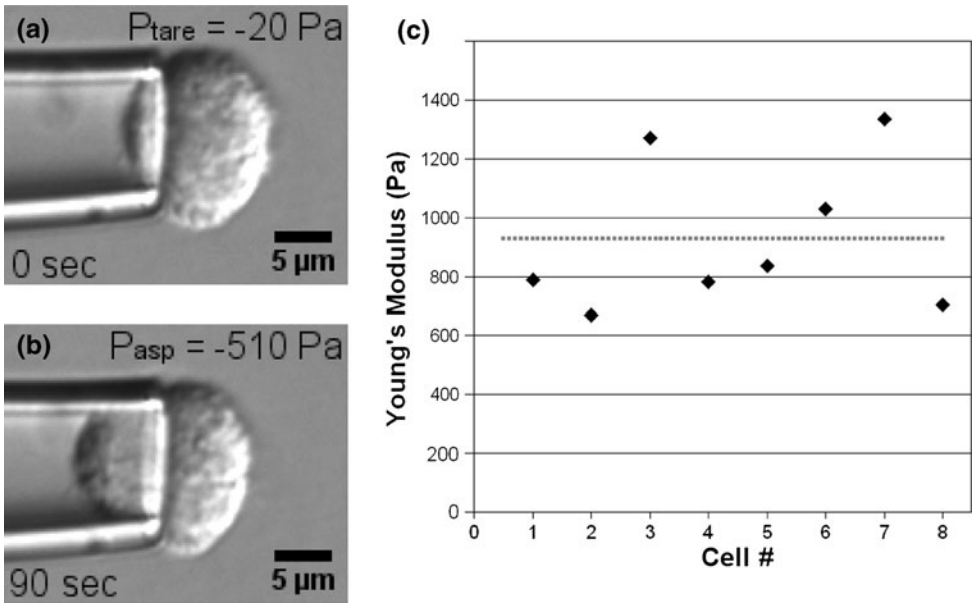

FIGURE 5. (a, b) Sample images of cells being aspirated at a tare pressure and at a higher aspiration pressure. (c) Young's modulus for eight individual single cells, calculated using the elastic half-space model. Mean modulus value represented as a dotted line.

bulb. In contrast with previous attempts to conduct MA experiments in microfluidic channels with rectangular cross sections,[19] the ability to use circular micropipettes in this system was a distinct advantage, as it allowed the use of well-established analytical models to extract the stiffness of the cell from the MA parameters. For these experiments, we used the half-space model to determine cell modulus, because it was covered in the course curriculum and has been shown to be reasonably accurate for this cell type.[35] This does not preclude the use of other more complex models,[12] or the use of a video capture camera to determine the time-dependent deformation (viscoelasticity) of single cells, by analytical or inverse finite element approaches.[35] However, measurements of viscoelasticity will require careful interpretation, due to the potentially confounding effects of the large initial suction pressure used to attract cells to the pipette tip. This should not be a concern for the reported equilibrium measurements, as the final aspiration length is independent of the rate of pressure application for this cell type.[35]

Though it was not utilized or controlled for in this lab, the use of a microfluidic system could have additional advantages in controlling temperature of the system. Because the liquid volumes in the channel are much smaller than those in standard MA systems, the temperature of the system can be controlled quite precisely using a heated stage. Temperature has been shown to play a role in cytoskeletal biomechanics,[31] and though it was not controlled for in these experiments, could be an additional parameter to include in this lab experience.

In designing the iMAchip system to be appropriate for an undergraduate lab, certain design compromises had to be made. The iMAchip system is certainly not as versatile as a standard MA system in terms of the types of experiments that can be performed. Furthermore, a key potential disadvantage is the possibility for cells to attach to and clog the micropipette, in spite of the chemical surface treatments applied to both the pipette and to the microchannels. In a traditional MA system, the micropipette would be quickly discarded and replaced with a fresh one, and this is not an option in this microfluidic version. While chemically cleaning the channels is relatively simple, it does take time, and may impact workflow, especially in a time-limited undergraduate lab situation. We made sure to produce a large number of extra devices, so that equipment could quickly be swapped out in this instance, and we found that keeping the cells on ice and replacing them every 45 min helped address this issue. We also found that using a cell line, such as the L929 or 3T3 fibroblasts substantially reduced unwanted cell adhesion, and we will use these cell lines for future labs.

IMPLEMENTATION IN AN UNDERGRADUATE LAB

Course Background

The lab has been incorporated for the past two years into the curriculum of MIE439, a single-semester senior course in Biomechanics, offered by the Department of Mechanical & Industrial Engineering at the University of Toronto. The course is a capstone design course for undergraduate students in the Biomedical Engineering Option in the Division of Engineering Science, and for those in the bioengineering streams of the Mechanical and Chemical Engineering programs. The course is primarily focused on applying principles of mechanical engineering, including solid and fluid mechanics, to living systems, and covers topics ranging from cellular and tissue biomechanics to human gait and locomotion. Course enrollment ranges from 45 to 80 undergraduate students. Course activities include three hours of lecture per week, biweekly tutorials, three lab sessions, and a capstone group design project. On-going assessment of student learning is conducted using homework assignments, lab reports, surveys and formative feedback during lectures and tutorials, and a mid-term exam. A final exam and a written technical report and presentation complete the course evaluative procedures. Though there are no formal prerequisites, the nature of the course requires students to have a basic understanding of solid and fluid mechanics, including linear stress–strain models and viscoelasticity. These concepts are reviewed in lecture, while simultaneously introducing various models of cellular mechanics.[7] The integrative synthesis of these interdisciplinary concepts is what makes this course unique and appealing, and hence is an ideal environment in which to conduct this micropipette aspiration lab.

Laboratory Logistics and Procedures

The laboratory was conducted in the Undergraduate Teaching Laboratory facilities, maintained by the Institute of Biomaterials and Biomedical Engineering (IBBME) at the University of Toronto. The teaching lab is designated Biosafety Level 2 (although this laboratory required only Biosafety Level 1), and is equipped with basic tools for sterile cell culture, and 12 microscopy stations which were used extensively during this lab. Each microscopy station consists of a phase contrast microscope equipped with three air objectives (10×, 20×, and 40×), a movable stage, digital video camera and a data collection computer, running basic image acquisition and analysis software.

Prior to lab day, students formed groups and signed up for a lab session. The finite capacities of the

teaching lab and practical considerations in course scheduling were limiting factors in the number of lab sessions we were able to conduct. In the first year of this lab, we ran three lab sessions, each one hour long. In order to address some of the concerns raised by the first group of students regarding the severely limited time available for the lab, two lab sessions, each two hours in length were arranged for the second year. In both years, group sizes were limited to four people, assigned to a single microscopy station.

During the week before the lab, students were prepared through lectures and distributed course material. Lectures covered concepts including cell mechanics, mechanical characterization techniques, and analysis methods. Traditional micropipette aspiration techniques were outlined, and a half-space model used to analyze micropipette aspiration data and extract cellular stiffness parameters was discussed in detail and included as part of a problem set assignment. A lab manual was also provided to the students (available online at www.introductorybiomechanics.com) in order to familiarize them with the new microfluidic techniques developed in this lab to facilitate micropipette aspiration, and to prepare them for the upcoming experiment. In addition to the experimental procedures provided in the lab manual, appendices detailing image acquisition and analysis procedures were provided. Simultaneously, iMAchip experimental setups were being prepared and tested by a teaching assistant. Cells were also expanded and frozen to ensure sufficient quantities for student use.

On the day of the lab, each microscope station was equipped with a primed iMAchip device and complete pressure setup. The lab manager, course instructor and teaching assistant were all present for the full duration of both lab sessions. Their roles included assisting students in equipment setup, solving technical problems, providing basic background knowledge and information, monitoring student progress, providing formative feedback, and ensuring that safe lab practices were being followed. Following a brief introductory talk in which key points of the lab equipment and procedure were highlighted, students were provided with thawed and resuspended cell solutions, and began work on the provided equipment. During the lab, students relied on help from the staff, notes taken in lecture and provided in the lab manual, and assistance from each other, depending on their individual experiences and backgrounds in either mechanical systems or cell handling techniques.

After completing the lab, students were expected to analyze the data collected in their groups, using image analysis software. Sample images collected by the teaching assistant were also made accessible, for those students who were unable to obtain usable data during the limited time available for the experiment. Students were also asked to answer three post-lab discussion questions, designed to encourage reflection on the lab experience. In answering these questions, students were expected to identify and evaluate the sources of error present in this microfluidic system, and compare their impact with traditional micropipette aspiration systems. For example, cells flowing in the microfluidic channels can be at non-atmospheric pressures at the time of aspiration, which can impact pressure differentials and hence, aspiration lengths. Students were asked to quantify the impact of this effect and furthermore, to design methods and techniques to minimize this source of error. Likewise, students were asked to re-examine the experimental design to address some of other challenges and problems they encountered while performing the experiments. As these labs were considered 'experimental' (in the 'new' as well as in the 'practical' sense), no formal lab reports were required, but students were all made aware that the materials covered would be included as a component of the final exam.

Material costs for this lab were relatively small, given access to appropriate teaching laboratories, soft lithography facilities, and a pipette puller and microforge. Given the availability of these facilities, material costs for device fabrication and cell culture for 20 devices and pressure setups totaled approximately CAD $300. If a pipette puller and forge are unavailable, commercially available micropipettes can be used, but add ~$10 to the cost of each device. Once the initial design, development and characterization iterations were complete, a total of 20 h were required on the part of the teaching assistant to prepare for the actual operation of the lab. These preparations primarily covered fabrication of a batch of 20 devices; aspiration system setup; and cell culture and handling. Barring catastrophic failure of the devices, they can be reused for future labs by carefully flushing the channels and micropipette with a mild acid and rinsing thoroughly with deionized water. Minor repairs to the epoxy sealants may also need to be conducted with each use.

Laboratory Pedagogy

This lab was designed to fulfill several learning objectives. The primary course-based objectives were to reinforce the concepts of cell mechanics and micropipette aspiration delivered in lecture. Though cell mechanics comprises approximately 25% of the course material, these topics were only presented as abstract concepts in lectures without a related hands-on learning experience. The lab experience described here was designed to complement the lecture material,

and provide a visual, sensory experience of these challenging abstract concepts for those students with alternative learning style preferences.[9]

In addition to building physical intuition for the course material, the lab was more broadly designed to allow students to experience the uncertainties of experimental research. Hence, students were explicitly told about the experimental nature of this lab: using novel techniques can often result in unexpected challenges that the experimentalist needs to solve. Framing the exercise in this way resulted in some interesting innovations developed by the students themselves. For example, one of the challenges with physically lowering the water reservoir to apply small changes in pressure is that this can generate pressure waves, unless done extremely carefully and smoothly. The students identified a solution in which water was gradually removed from the reservoir with a pipette, achieving the same effect and minimizing experimental skill required. We believe that the process of experiencing, identifying and solving these practical problems is an absolutely essential learning experience for budding researchers. Furthermore, this approach forces students to develop an experimental approach to learning: overcoming the natural fears involved with trying different things when the outcome is not necessarily assured is challenging and requires these guided, yet flexible, student-defined experiences. Because of the team-based nature of the lab, this teaching approach also encouraged students to interact with each other and utilize the team members' individual experiences to solve experimental issues. This provided an opportunity for students to engage with each other and develop collaborative skills for future team-based projects.

Aside from essential details included in the lab manual, students were not explicitly taught about the microfabrication aspect of this lab, as this was not the focus of this course. However, such technologies are quickly growing in importance within biomedical engineering, and one of the key benefits of conducting this lab was to allow students to observe and experience how novel microfabricated tools can be designed to address questions and solve problems in the biological sciences. By providing a clear example of the integrative nature of biomedical engineering and other associated courses of study, we hope to motivate interest in this emerging interdisciplinary field. To this end, this learning experience was further augmented by inviting guest lecturers with expertise in both cellular biomechanics and microfabrication, and through an additional practical lab that utilizes microfluidic channels to recreate and explore the Fahraeus–Lindqvist effect, a non-intuitive microscale-related change in hematocrit that occurs when blood cells flow in small-diameter vessels like arterioles, venules, and capillaries.[34]

Following the lab, student requirements were limited to analyzing data and answering questions in the lab manual, without the requirement for a formal lab report. This was to allow students to focus on learning the core physical and experimental concepts, without being concerned about a mark for a written document.[2,29] In order to ensure student learning, however, the answers to questions in the lab manual were not explicitly provided, and a final exam question based on the lab experience was included. As a result of this approach, the course staff noticed a quantitative and qualitative improvement in staff–student interaction, as students tried to prepare for the exam by actively thinking about and questioning the lab material.

Student Feedback and Discussion

Feedback was requested from students in the Fall 2009 course, in the form of a voluntary in-class survey conducted immediately after the lab session. Eighty-seven percent of students enrolled in the class responded, and the categorical survey results are summarized in Fig. 6. Feedback was generally very positive, with 73% of the students moderately or strongly agreeing that the lab was beneficial in reinforcing the concepts discussed in lecture. Oddly enough, more than 90% of the students agreed that they had a better understanding of the details involved in performing measurements of cell mechanics, which suggests that certain aspects of the lecture material were more immediately and strongly reinforced than others. This might be a result of conducting the survey immediately after the lab, when students may not have had the time to analyze the collected data or contemplate the discussion questions included in the lab manual. Even at this early stage, however, at least 84% of the students felt that this was a fitting and useful component of a biomechanics course curriculum, and were enthusiastic about the experience.

Comments were also solicited with the survey, and these were also generally positive. Students particularly liked the "translation from lecture to practice", and the fact that the "we were expected to perform the experiments ourselves, not as a bystander watching the TA". More than 85% of survey respondents agreed that the lab was a fun and practical hands-on learning experience. Other appreciative comments included a majority of observations that the lab was "not plug-and-play", and an "individual research experience for each group". The majority of students also appreciated the chance to "look at real cells, and see how they behave mechanically". Interestingly, a small section of the class made thematically identical comments and

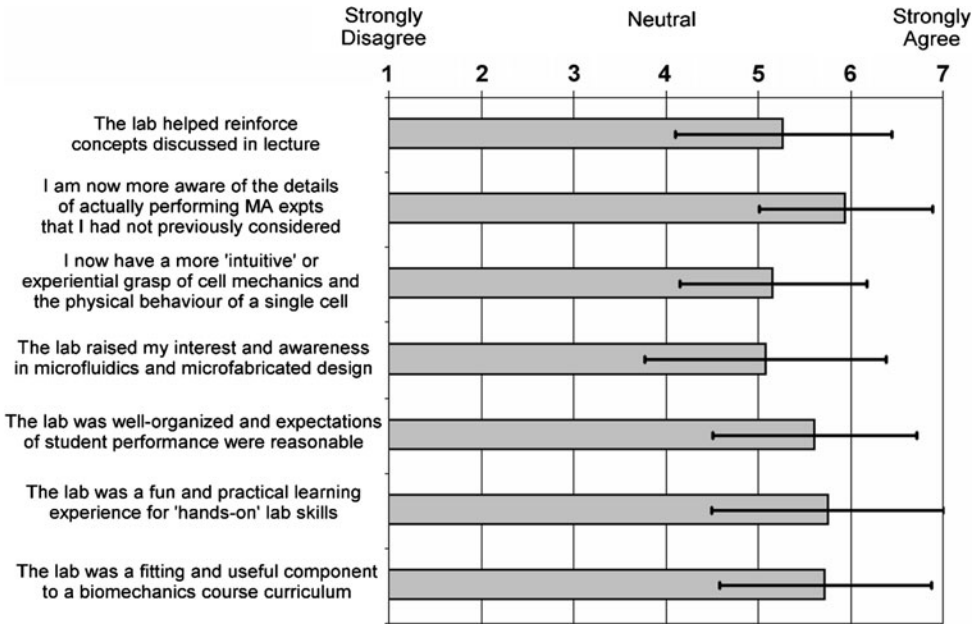

FIGURE 6. Summary of student feedback collected from a voluntary in-class survey. Bars represent means ± standard deviations for 61 student responses per question.

considered these negative experiences: ''looking at real cells is frustrating as they die easily'', and that ''the lab manual was unclear about procedures''. One student best summarized this dichotomy with the comment: ''Liked: to get to know what it is like to do real experimental work. Disliked: that it is sometimes disappointing''. A number of students also appreciated the opportunity to work with ''cutting-edge'', ''newly developed'' microdevices to do this work, and a majority of students were more interested in microfabricated technologies for life science studies after this exercise.

A large number of survey respondents were enthusiastic about the lack of a formal report, giving them ''no pressure to perform, and freedom to experiment and learn'' with the provided systems. Students also did very well on the lab-related question included in the final exam, suggesting that this pedagogical approach works well for this group of students. However, no conclusions can be drawn as similar questions were not asked in previous years, and the direct impact of this exercise on student performance cannot be assessed.

Criticism for the lab focused mainly around the logistics of running the lab, and certain technical details as to the experiment. Most of these negative comments concerned the need for more space in the lab, as areas around each microscope station were fairly limited for four people. Many students also felt that the lab would better be conducted with two or three students per group. In spite of the increased two-hour length of the lab, several students felt that more time would have been very useful. In terms of technical details, students in the first lab session were primarily frustrated by pipettes being clogged by dead or dying cells. These pipettes were difficult to clear in a timely manner, and hence prevented some students from collecting data within the lab session. This issue was addressed for the second lab session, when the staff began keeping the suspended cells on ice through the duration of the lab, and replenishing cells with fresh supplies every 45 min. This prevented cell death in the channels and prevented pipette clogging. Other criticisms were related to equipment issues: a malfunctioning camera, and a burnt-out microscope illumination lamp.

CONCLUSIONS

A microfabricated system was developed to conduct micropipette aspiration experiments to measure the mechanical properties of individual cells. The simple and affordable system was designed for use by undergraduate students as a practical component of a course in biomechanics. As such, it eliminates the need for students to access and operate expensive and delicate equipment, while obtaining a hands-on experience in studies of cellular mechanics. Students applied the system to probe the stiffness of a primary cell population, and the results produced were consistent with observations made with a conventional system.

The usefulness of the lab as a teaching tool was evaluated by means of a student survey, which indicated a strongly positive learning experience.

ACKNOWLEDGMENTS

We acknowledge microfabrication support from the Emerging Communications Technology Institute and the Toronto Microfluidics Foundry. We thank the Departments of Mechanical and Industrial Engineering; Electrical and Computer Engineering; and the Institute of Biomaterials and Biomedical Engineering at the University of Toronto for administrative and financial support. We acknowledge financial support from the Ontario Graduate Scholarship program to CM; the University of Toronto Division of Engineering Science and the Bioengineering Minor to EB; and the Canada Research Chairs in Micro and Nano Engineering Systems to YS, and in Mechanobiology to CAS.

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