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Low-cost microcontroller platform for studying lymphatic biomechanics *in vitro*

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Abstract

The pumping innate to collecting lymphatic vessels routinely exposes the endothelium to oscillatory wall shear stress and other dynamic forces. However, studying the mechanical sensitivity of the lymphatic endothelium remains a difficult task due to limitations of commercial or custom systems to apply a variety of time-varying stresses *in vitro*. Current biomechanical *in vitro* testing devices are very expensive, limited in capability, or highly complex; rendering them largely inaccessible to the endothelial cell biology community. To address these short-comings, the authors propose a reliable, low-cost platform for augmenting the capabilities of commercially available pumps to produce a wide variety of flow rate waveforms. In particular, the Arduino Uno, a microcontroller development board, is used to provide open-loop control of a digital peristaltic pump using precisely-timed serial commands. In addition, the flexibility of this platform is further demonstrated through its support of a custom-built cell-straining device capable of producing oscillatory strains with varying amplitudes and frequencies. Hence, this microcontroller development board is shown to be an inexpensive, precise, and easy-to-use tool for supplementing *in vitro* assays to quantify the effects of biomechanical forces on lymphatic endothelial cells.

Keywords

wall shear stress; peristaltic pump; lymphatic endothelial cells; Arduino; microcontroller platform

1. Introduction

Most tissues in the body are supported by the lymphatic system for a variety of functions, including the regulation of fluid balance, the removal of particulate matter from the interstitium, as well as the transport of fat from the intestine to the blood, among others. Collecting lymphatic vessels contract much like the heart to promote flow, with transport occurring through the coordinated intrinsic pumping of individual vessel segments known as lymphangions (Zawieja, 2009; Nipper and Dixon, 2011). These contractions continuously expose luminal lymphatic endothelial cells (LECs) to oscillatory wall shear stress (with frequencies typically below 1 Hz) (Dixon et al., 2006) as well as other forces, all of which have been shown to affect vessel pumping (Hargens and Zweifach, 1977; Gashev et al., 2002; Davis et al., 2009). In addition, several extrinsic factors also work to promote lymph

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Conflict of interest statement

None.

flow such as skeletal muscle contraction, respiration, and interstitial fluid formation. Because of the numerous mechanisms that affect lymph transport *in vivo*, flow rates can vary quite drastically under physiologic conditions; accordingly, the intrinsic lymphatic pump must actively respond to these varying *in vivo* loads. Many of these responses are, in part, endothelium mediated, and utilize similar mechanisms that have been known to regulate vasoactive responses in the blood vasculature (Gashev et al., 2002; Kawai et al., 2010; Bohlen et al., 2009). However, the magnitudes of these forces experienced by LECs *in vivo* are significantly less than those in similar-sized blood microvessels [Table 1] (Hargens and Zweifach, 1977; Lipowsky, 2005; Dixon et al., 2006), and recent evidence suggests that their unique biomechanical environment is essential in guiding lymphatic development (Chen et al., 2012; Sabine et al., 2012). Moreover, the ability to apply custom time-varying shear stresses is useful in the context of elucidating the functional response of LECs, which experience rapid and often irregular changes in the mechanical environment *in vivo*.

To study the specific effects of fluid shear stress on LECs *in vitro*, investigators have developed an assortment of devices for exposing endothelial cells (ECs) to dynamic shear stress waveforms (Helmlinger et al., 1991; Peng et al., 2000; Estrada et al., 2011). However, these systems are custom-built and fairly complex, rendering them largely inaccessible to the broader EC community. Commercial systems are available for applying fluid shear stress waveforms (e.g. Flexcell®, Ibidi, Vacu-Cell™); however, these commercial systems are expensive and have limited flexibility for applying various dynamic flow rates. In particular, these systems are incapable of producing anything other than small subset of pulsatile or oscillatory waveforms (e.g. a square wave or sine wave). Thus, the authors report a technique for enhancing the capabilities of commercially-available pumps to generate a wide variety of flow waveforms, including those seen in lymphatics *in vivo*. The proposed system is a reliable, inexpensive, and easy-to-use platform for augmenting a digital peristaltic pump with an open-loop control system to produce time-varying flow rates. Additionally, the authors further demonstrate the flexibility of this platform by using it to support a custom-built cell-straining device capable of producing oscillatory strains with a multitude of amplitudes and frequencies.

2. Methods and Results

2.1. Arduino Uno Development Board

The Arduino Uno development board is based on the Atmel ATmega328, an 8-bit, 16 MHz microcontroller with 14 digital input/output (I/O) pins, 6 of which are capable of pulse-width modulation (PWM), as well as a 6-channel, 10-bit analog-to-digital converter. Digital communication capabilities include UART TTL serial, SPI serial, and two-wire interface serial (I²C). The Arduino development platform features a cross-platform, Java-based IDE as well as a C/C++ library which offers high-level access to hardware functions.

2.2. Programmable Fluid Shear Stress Via Peristaltic Pump

Figure 1 shows the flow system used to generate time-varying fluid shear stresses on an LEC monolayer grown in a parallel-plate flow chamber using an Ismatec REGLO Digital MS-4/12 peristaltic pump (IDEX Health and Science, Glattbrugg, Switzerland). After entering a desired periodic shear stress function and experimental duration via a custom Python script (Supplemental Appendix), the required peristaltic pump speed commands are sent serially to the Arduino Uno. The Arduino Uno stores these commands on its local EEPROM, which allows experiments to be conducted entirely with the Arduino Uno development board. Once running, the Arduino Uno recreates the desired waveform by sending each of the locally-stored pump commands at specific time increments to the peristaltic pump via a TTL to RS232 level shifter (SparkFun Electronics, Boulder, CO). The

experiment progress is displayed on an LCD, which is operated with Arduino's LiquidCrystal library.

In order to mimic the user-desired waveform, the Python script generates a certain number of discrete pump commands based on a suitable time delay for the pump. In this way, the Arduino Uno will avoid sending commands too fast for the peristaltic pump to implement. Upon initialization of the experiment, the Arduino Uno will automatically refine this delay time using a self-calibration routine that takes extraneous pump commands (*e.g.* `start` and `change direction`) into account (Supplemental Appendix). In this way, the overall period of the desired waveform is ensured to be accurate. Figure 2a–d shows several examples of desired flow rate waveforms compared with the measured flow rate waveforms on the peristaltic pump. The measured flow rate is obtained by acquiring the speed of the pump rollers via an internal pump encoder which returns a square wave whose frequency is proportional to the speed. Accordingly, because a peristaltic pump is a positive displacement pump, the velocity of the rollers is linearly proportional the mean flow rate through the corresponding tube (according to its internal diameter). Overall, good agreement is achieved between the desired and measured flow rates.

2.3. Adjustable Cell Monolayer Straining

The authors have also developed a cell-straining device [Fig. S1a] capable of imposing a static or oscillatory strain of varying magnitude and frequency [Fig. 3]. The amplitude of strain may be adjusted by adjoining one end of the connecting rod to the hub at points of varying radii, while the frequency of strain may be adjusted by regulating the speed of the DC motor powering the hub. The details of this displacement may be seen through Eq. (1),

$$x(t) = R - R \cos(\omega t) + L - \sqrt{L^2 - [R \sin(\omega t)]^2} \quad (1)$$

where $x(t)$ is the displacement of the membrane, R is the radius of the crank, ω is the rotational velocity of the crank, and L is the length of the connecting rod. Velocity feedback of the hub (crank) is acquired with an infrared photo interrupter (SparkFun Electronics, Boulder, CO), which outputs a rising voltage edge upon sensing one of the twelve slots located around the outer perimeter of the hub. Time between rising edges is measured by the Arduino Uno, which can then calculate the speed for display in revolutions per minute (rpm) on the attached LCD screen (Supplemental Appendix).

3. Discussion

In order to create time-varying waveforms on a digital peristaltic pump, such as the one used in this study, one could use a personal computer (PC) to send out serial (flow rate) commands at sufficiently-fast intervals to approximate the desired waveform. However, typical PC operating systems are not adept at precise timing, and PCs can be difficult to operate near or inside of cell culture incubators. Conversely, another option would be a conventional microcontroller: the inherent simplicity of these embedded systems give them very desirable timing characteristics and thus have been useful in a wide variety of biomedical applications (Sun et al., 1992; Bhaskaran et al., 1993; Frijns et al., 1994). However, this raw simplicity in hardware comes at the expense of writing the required software, which is typically written in either assembly or C/C++ and often requires intimate knowledge of the particular hardware architecture. This complexity in writing the software makes it difficult for non-experts to pick up and use these systems without a considerable time investment. Due to these limitations, the authors have chosen to use the Arduino Uno development board (\$30, <http://arduino.cc>), which is an open-source electronics prototyping platform based on user-friendly hardware and software. Although based on an 8-bit Atmel

microcontroller, the Arduino Uno offers high-level libraries that allows programming to be more intuitive and does not require familiarity with the low-level hardware registers usually required for I/O. In fact, its ease-of-use has made the Arduino platform recently popular with artists, hobbyists, and students interested in electronic automation (Kushner, 2011).

The peristaltic pump system demonstrated in this study is an example of a successful augmentation with the Arduino Uno. Although the minimum delay time between commands (~30–50 ms) required by this pump prohibits waveforms with high temporal resolutions to be used, this system can adequately reproduce the oscillatory flow functions seen in the lymphatics [Fig. 2c–d]. However, this limitation is unique to digital pumps such as these; pumps with an analog input would be limited only by the speed of their electromechanical dynamics, which could allow various vascular waveforms to be produced. Analog-capable pumps could make use of the Arduino Uno's PWM output and an inexpensive H-bridge circuit to produce time-varying flows.

In addition to augmenting pump-based systems, the authors further demonstrate the flexibility of this platform by supporting a custom-built cell-straining device for mimicking the strain amplitudes and frequencies experienced by LECs within collecting lymphatics. The device can produce strains between 10–50% and frequencies exceeding 1 Hz, which is within the observed range for the lymphatics (Hargens and Zweifach, 1977; Dixon et al., 2006; Davis et al., 2009). The Arduino Uno's precise timing capabilities (D'Ausilio, 2011), coupled with its ability to use hardware interrupts, allows the system to measure the strain frequency and display it on an attached LCD for user feedback. Thus, the authors propose the Arduino Uno as an inexpensive, flexible, and adaptable platform for the development of *in vitro* assays to quantify the effects of biomechanical forces on the lymphatic endothelium.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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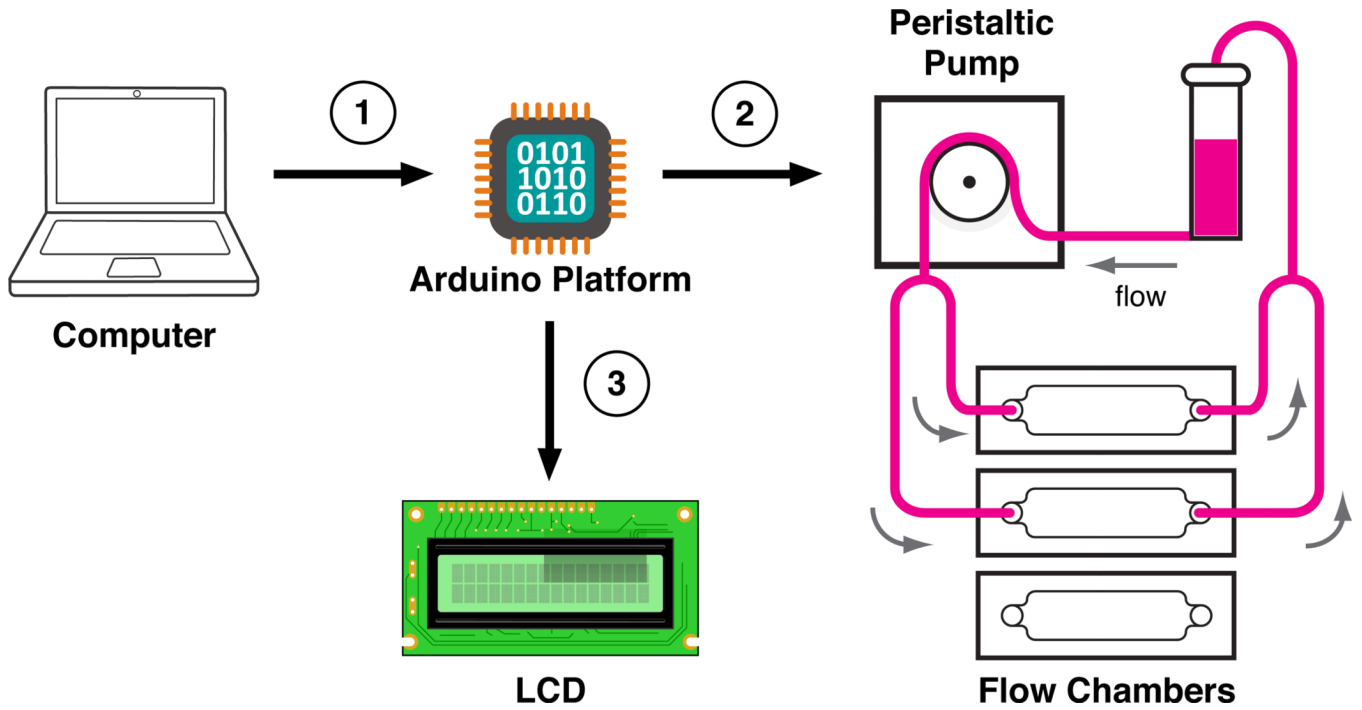


Figure 1. Outline of the flow system: (1) the Arduino platform is programmed by a personal computer (PC) over USB to create a desired fluid shear stress (flow rate) waveform for a certain duration of time; (2) once programmed, the Arduino can independently control the peristaltic pump via an RS232 serial connection with precise timing in an open-loop fashion; (3) the experimental progress is displayed on a connected LCD screen for the experimenter to monitor.

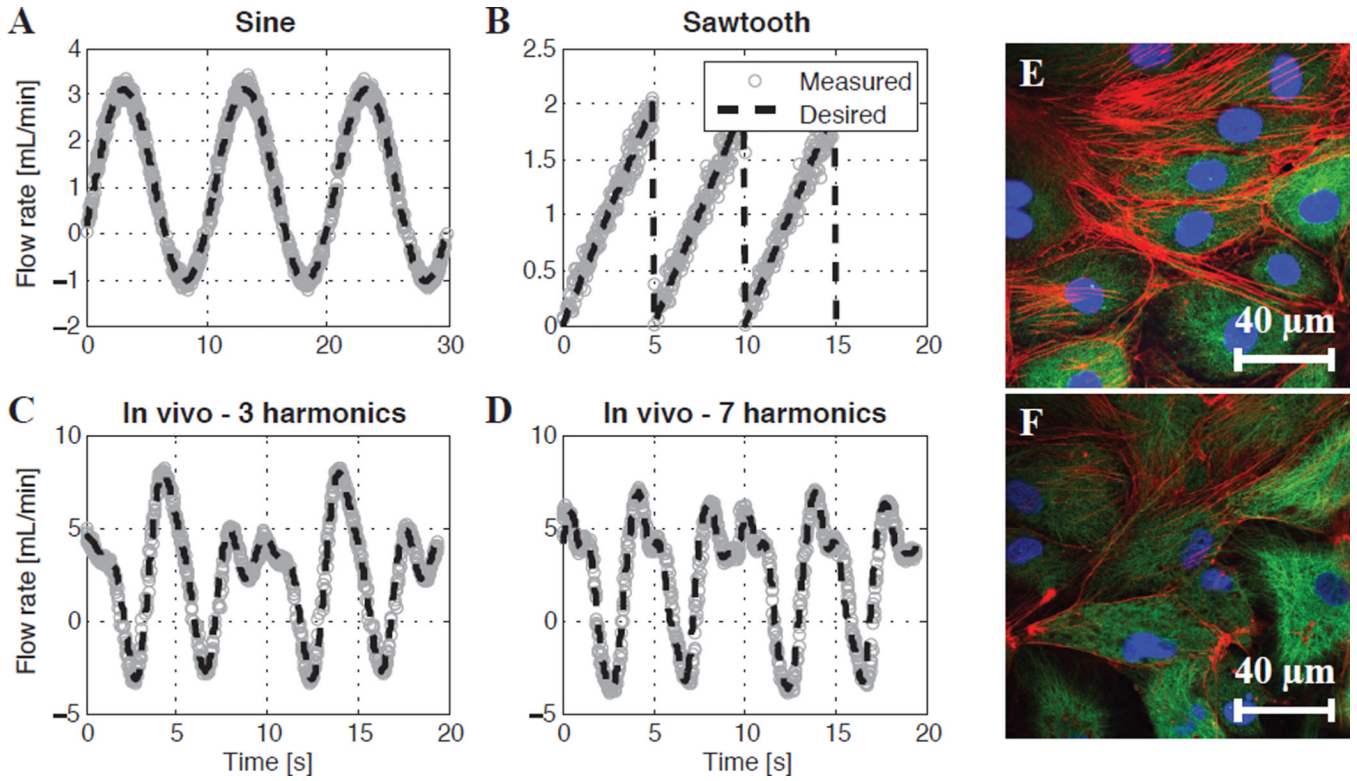


Figure 2. Measured vs. desired flow rates for the digital peristaltic pump being controlled by the Arduino Uno (a–d). The pump system accurately produces several desired waveforms: (a) a sine wave, (b) a sawtooth wave, (c) a waveform with the top three harmonics of a rat lymphatic waveform measured *in vivo*, and (d) a waveform with the top seven harmonics of a rat lymphatic waveform measured *in vivo* (Kassis et al., 2012). (e) Controlled LECs cultured under a static, no-flow condition. (f) LECs exposed to the sine wave found in (a) for 24 hours. For the false-colored images: the red channel is F-actin (phalloidin), the green channel is tubulin (- tubulin), and the blue channel is the cell nucleus (DAPI).

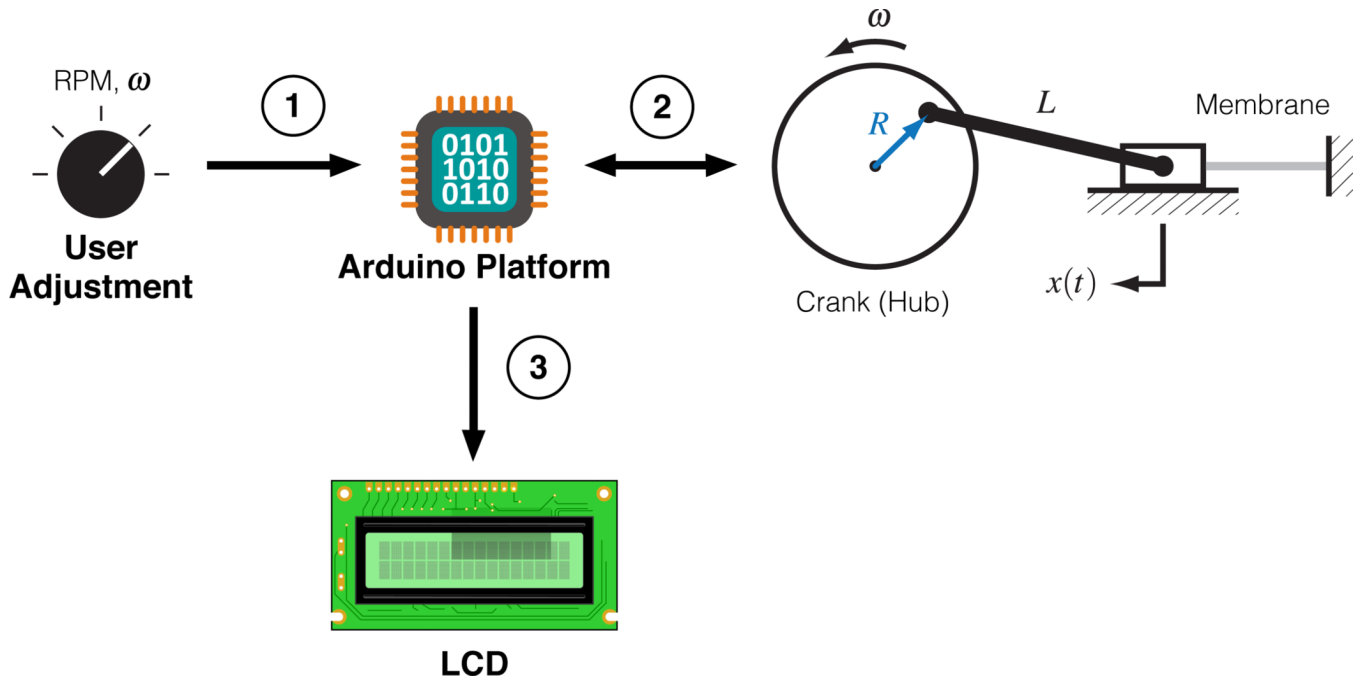


Figure 3. Outline of the cell-straining device: (1) the user adjusts a potentiometer knob that controls the PWM frequency output of a motor driver board; (2) the PWM frequency from the motor driver board controls the speed of the crank, which dictates the frequency of strain, and this measurement is fed back to the Arduino Uno via a photo interrupter; (3) the Arduino Uno uses the photo interrupter as an encoder to calculate the speed of the crank then displays it on an attached LCD.

Table 1

Typical pressure and wall shear stress (WSS) values in the microvessels of the body.

| | Arterioles | Venules | Capillaries | Collecting Lymphatics |
|--------------------------------------|------------|---------|-------------|-----------------------|
| Pressure (mmHg) | 75–100 | 20–30 | 30–40 | 3–4 |
| Average WSS (dynes/cm ²) | 50–60 | 10–30 | 20–50 | < 1 |