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A Low-Cost Mechanical Stretching Device for Uniaxial Strain of Cells: A Platform for Pedagogy in Mechanobiology

Mechanical cues including stretch, compression, and shear stress play a critical role in regulating the behavior of many cell types, particularly those that experience substantial mechanical stress within tissues. Devices that impart mechanical stimulation to cells in vitro have been instrumental in helping to develop a better understanding of how cells respond to mechanical forces. However, these devices often have constraints, such as cost and limited functional capabilities, that restrict their use in research or educational environments. Here, we describe a low-cost method to fabricate a uniaxial cell stretcher that would enable widespread use and facilitate engineering design and mechanobiology education for undergraduate students. The device is capable of producing consistent and reliable strain profiles through the use of a servomotor, gear, and gear rack system. The servomotor can be programmed to output various waveforms at specific frequencies and stretch amplitudes by controlling the degree of rotation, speed, and acceleration of the servogear. In addition, the stretchable membranes are easy to fabricate and can be customized, allowing for greater flexibility in culture well size. We used the custom-built stretching device to uniaxially strain macrophages and cardiomyocytes, and found that both cell types displayed functional and cell shape changes that were consistent with the previous studies using commercially available systems. Overall, this uniaxial cell stretcher provides a more cost-effective alternative to study the effects of mechanical stretch on cells, and can therefore, be widely used in research and educational environments to broaden the study and pedagogy of cell mechanobiology.
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Introduction

Studies of how mechanical forces influence cell function, or mechanobiology, have been one of the major contributions of biomedical engineers to cell biology and physiology. Through the use of in vitro mechanical stimulation devices, it has become well-appreciated that physical cues influence cellular form and function, and that deviations in normal physical stimulation are often drivers of pathology [1–4]. While the complexity of the in vivo environment is difficult to fully recapitulate, mechanical stimulation devices allow for the simulation of more physiologically relevant environments through application of physical stimuli to cells in culture. These devices have been instrumental in developing a better understanding of how physical forces affect a number of different cell types [5–7]. Although such tools have aided in many discoveries, their use has been limited to specialized research environments, and widespread adoption remains a challenge. Furthermore, while mechanobiology has become an integral component of undergraduate curricula in biomedical engineering, education remains largely in the classroom. In this study, we describe the design, fabrication, and use of a mechanical stretching system for widespread research and educational purposes, and propose an experimental platform for mechanobiology education at the undergraduate level.

Cell stretching devices are available commercially and can also be fabricated in-house, but current devices have several constraints that limit their widespread use in research or educational environments. Commercially available cell stretchers are costly to purchase and maintain, ranging in cost from thousands to tens of thousands of dollars, and also require an additional recurring cost of device-specific stretchable culture well plates [8–11]. Several less expensive alternatives have been developed, but these devices often still rely on expensive equipment for fabrication or require customized stretchable membranes available exclusively from commercial sources [12,13]. As such, most devices are unable to adapt to experiments that require modifying the stretchable membranes, for example, the inclusion of multiple wells for high throughput analysis [14,15]. Therefore, a low-cost and reliable cell stretcher is required to increase accessibility of such devices and, ultimately, further the study of cell mechanobiology.

Project-based methods have been introduced in many pedagogical environments and are generally recognized as enhancing development of critical thinking skills [16–19]. In addition, undergraduate students who participate in research projects gain valuable hands-on skills and knowledge and are thought to play important roles in career decisions. Previous studies have also suggested that project-based and experiential learning techniques create better student motivation, which results in significant enhancement in learning [20–22]. Although mechanobiology has become a component of many undergraduate biomedical engineering curricula in recent years, project-based and experiential learning modules in this area remain limited given the high cost of the necessary equipment.

Here, we describe the design and validation of a cost-efficient uniaxial cell stretcher that can be integrated with undergraduate mechanobiology education. This project was carried out predominantly by undergraduate students with the involvement of high school students. The device is composed of durable, yet inexpensive aluminum parts, and the mechanical motions are controlled through the use of a computer-programmed servomotor, gear, and gear rack system. Stretchable membranes were made of silicone materials, which can be used in different sizes and shapes allowing for greater flexibility in performing different experimental assays. All components used to fabricate this cell stretcher are readily available and the device itself is, therefore, inexpensive to create and maintain. As proof-of-principle, the device was used to mechanically stimulate macrophages and cardiomyocytes. The changes in cell morphology and function were consistent with previous findings [23–26]. We propose that this cell stretching device may be integrated with undergraduate mechanobiology education to provide students experiential learning in design and fabrication of mechanical devices that can be directly utilized in cutting edge research. The students can also get in-depth exposure to the application of such devices in the study of cell physiology applicable to many physiological systems. We demonstrate that these projects allow the students to learn both hard and soft skills, and gain experience that aids in more informed career decisions.

Materials and Methods

Pedagogical Design. The process of designing, building, and validating a cell stretching platform and using this device to study the effect of mechanical stimulation on different cell types requires the development of several skills, which formed the basis of the learning outcomes required for the successful completion of the project. Through participating in this project, undergraduate students should have developed knowledge and skills in engineering design, basic cell biology, and experimental design. This required students to gain experience in programming, using computer aided design (CAD)-related software, such as SOLIDWORKS, and cell culture. Students were also encouraged to apply for either individual or project related funding through the Edwards Lifesciences Summer Undergraduate Research Program (E-SURP) and Undergraduate Research Opportunities Program (UROP) at the University of California, Irvine (UCI), respectively. Through these programs, students gained experience not only in reading and writing scientific articles, but also presentation of research findings in formal settings including at the annual Undergraduate Research Symposium at UCI. The learning outcomes associated with this project were similar to those of other experiential learning modules as key knowledge and skills that promote learning and individual development are gained through student involvement [16,27,28].

Two undergraduate engineering students were recruited initially to begin the design process. Over the next four years, junior undergraduate students were recruited and mentored by their senior counterparts. This approach created a continuity of knowledge over multiple years and provided the students with mentoring experiences. The undergraduate students were from biomedical and mechanical engineering programs in their sophomore or junior years. As the project progressed, some of the students wanted to continue and stayed to pursue graduate degrees and participated more directly in recruitment. After working on this project, the students were given surveys to gauge learning and to provide feedback on how the project can be improved for a better learning experience.

Fabrication of Uniaxial Cell Stretching Device. The uniaxial cell stretching device is composed of two experimental substrates housed in a 10.16 cm (4 in) × 15.24 cm (6 in) 6061-T6 aluminum channel (Fig. 1(*a*)). The substrates, made by joining silicone sheets and 2.54 cm (1.0 in) inner diameter silicone tubing, are held in place by a movable center clamp and fixed outer clamps, with top clamps and wing nuts used to apply pressure and maintain substrate tension during application of cyclic stretch. Cyclic strain is generated by using a programmable servomotor (Servo City, Winfield, KS) to move the center clamp, which is coupled to a gear and gear rack system. This center clamp slides on two 0.635 cm (0.25 in) rails and is aided by bronze bushings to reduce friction and wear. Once the device is assembled, the experimental substrate has a length of 3.81 cm (1.5 in) in the direction parallel to the uniaxial stretch. Different amplitudes of strain can be generated by programming the servomotor to rotate a circumferential distance corresponding to a fraction of the experimental substrate length. For example, 5, 10, 15, and 20% strain amplitudes can be generated through rotating the servogear 0.191, 0.381, 0.572, and 0.762 cm (0.075, 0.150, 0.225, and 0.300 in), respectively. In addition, an aluminum block is used to either extend both experimental substrates and generate static strain, or extend one experimental substrate and create a temporary static strain until the servomotor is powered resulting in equal cyclic strain in both experimental substrates (Fig. 1(*b*)). A 1 Hz cyclic stretch was used

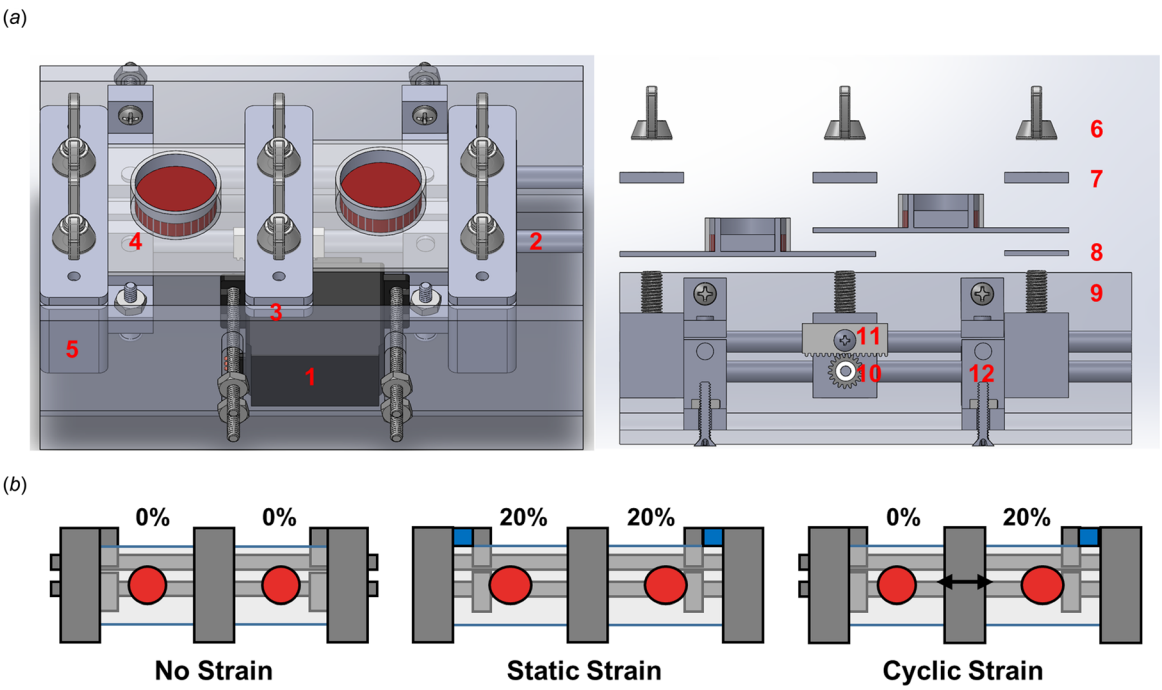

Fig. 1 Design of uniaxial cell stretching device. (*a*) Three-dimensional schematic of uniaxial cell stretching device (left) including a cross section and expanded view (right) to better display the gear and gear rack mechanism for mechanical motion as well as the clamping mechanism used to maintain substrate tension. The device consists of a 6061-T6 aluminum housing (transparent), programmable servomotor (1), 0.635 cm (0.25 in) rails (2), moveable middle clamps (3), experimental substrates (4), stationary side clamps (5), wing nuts (6), top clamps (7), a silicone sheet to balance the experimental substrates (8), 0.635 cm (0.25 in) in threaded rods (9), gear (10), gear rack (11), and 0.635 cm (0.25 in) 0.25 in shaft support (12). (*b*) Schematic of unstrained (left), static strain (middle), and cyclic strain (right) configurations of the device.

for all experiments in this study. The CAD files and drawings have been made publicly available [29]. The parts and their costs as well as detailed assembly instructions are provided in the Appendix, which is available under the "Supplemental Materials" tab for this paper on the ASME Digital Collection.

Experimental Substrate Preparation and Strain Validation. The experimental substrates were fabricated through sealing silicone tubing (McMaster-Carr, Santa Fe Springs, CA) to a 0.05 cm (0.02 in) thick silicone sheet (Stockwell Elastomerics, Philadelphia, PA) using polydimethylsiloxane (Sylgard 184), which was then cured at 60 °C. To validate the strain profiles generated by the cell stretcher, videos were captured of the servo gear rotating and the experimental substrate stretching and deforming. The videos were processed through IMAGEJ software to track either a single point of interest on the servogear or a 5×5 matrix of markers on the surface of the substrate using the MTrack2 plugin [30]. The data obtained were analyzed using a custom python code to validate the waveform output by the servo or the resulting strains parallel and perpendicular to the direction of stretch, respectively [31].

Macrophage Cell Culture. Experimental substrates were sterilized by autoclave, several 70% ethanol and phosphate buffered saline washes, and then coated with a 10 μg/mL fibronectin solution and incubated at 4 °C overnight. The substrates were further rinsed with phosphate buffered saline before cells were seeded onto the surface. Bone marrow derived macrophages were obtained by flushing the bone marrow from the femurs of 6–12 week old female C57BL/6J mice (Jackson Laboratory, Bar Harbor, ME). This was accomplished using Dulbecco's modified eagle medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% penicillin/streptomycin, 2 mM L-glutamine (all from Thermo Fisher Scientific, Carlsbad, CA), and a 10% conditioned media, produced from CMG 14–12 cells that express recombinant mouse macrophage colony stimulating factor, which differentiates bone marrow cells to macrophages. Red blood cells were removed by treating the collected bone marrow cells with a red cell lysis buffer. The cells were then centrifuged, resuspended in the culture media, and seeded onto nontissue culture treated petri dishes for 7 days, before being harvested using an enzyme-free dissociation buffer (Thermo Fisher Scientific, Carlsbad, CA) and seeded onto experimental substrates. The resulting macrophages were seeded at a density of 2×10^5 cells per substrate. Following 4 h of incubation, the media was replaced with either regular or 0.3 ng/mL interferon-gamma (IFN-γ) and 0.3 ng/mL lipopolysaccharide (LPS) containing media then cyclically stretched at a 10% stretch amplitude for a period of 18 h. Following stretch, supernatants were collected and analyzed for the presence of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) cytokine secretion using ELISA kits (BioLegend) following the manufacturer's instructions.

Cardiomyocyte Cell Culture. Neonatal rat ventricular myocytes (NRVM) were harvested from two-day-old neonatal Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), as previously described [32]. Briefly, the ventricles were extracted and homogenized in Hanks balanced salt solution before being exposed to 1 mg/mL trypsin overnight at 4 °C. The tissue was then digested with 1 mg/mL collagenase at 37 °C and the released myocytes were resuspended in M199 culture medium supplemented with 10% heat-inactivated FBS, 0.1 mM minimal essential medium nonessential amino acids, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 3.5 g/L glucose, 50 U/ml penicillin, 2 mg/L vitamin b-12, and 2 mM L-glutamine. The cells were then seeded at 1×10^6 cells per well onto fibronectin-coated substrates. The media was changed according to the following schedule:

(1) From seeding (0 h) to 24 h after seeding—10% FBS M199 culture medium

(2) At ~24 h after seeding, the media is changed to fresh 10% FBS M199 culture medium as the cells would have consumed the available nutrients
(3) At ~48 h after seeding, the media is changed to fresh 2% FBS M199 culture medium. At this point, it is standard in primary cardiac cell culture to reduce the amount of FBS in the media to limit the proliferation of any fibroblast. As the primary harvest produces 95–97% pure cardiomyocyte cultures, this is an essential step to prevent delamination of cells [33].

The cells were subject to the following stretching protocol:

(1) From seeding ($t = 0$ h) to 0.5–1 h after seeding, the cells were cultured in the wells of the stretcher with no cyclic stretch.
(2) From 0.5–1 h after seeding to 6.5–7 h after seeding, the cells were subject to cyclic stretch at a 20% stretch amplitude.
(3) From 6.5–7 h after seeding to 72 h after seeding, the cells were cultured in the wells of the stretcher with no cyclic stretch.

At 72 h after seeding, the cells were fixed and stained as previously described [33]. Briefly, a solution of 4% paraformaldehyde (Fisher Scientific Company, Hanover Park, IL) was used to fix the cells, and the tissue was stained for actin (Alexa Fluor 488 Phalloidin, Life Technologies, Carlsbad, CA), sarcomeric α-actinin (mouse monoclonal anti-α-actinin, Sigma Aldrich, Inc., Saint Louis, MO), and nuclei (40,60-diaminodino-2-phenylinodole, Life Technologies, Carlsbad, CA). Secondary staining tetramethylrhodamine-conjugated goat anti-mouse IgG antibody (Alexa Fluor 633 Goat Anti-Mouse or Alexa Fluor 750 Goat Anti-Mouse, Life Technologies, Carlsbad, CA) was used to visualize sarcomeric z-lines.

Analysis of Macrophage and Cardiomyocyte Morphology. Following 18 h of cyclic stretch, phase contrast images of macrophage cells were taken using the EVOS cell imaging system (Thermo Fisher Scientific, Carlsbad, CA). A minimum of 150 cells from each condition were outlined and analyzed using IMAGEJ, which fits an ellipse to each outlined cell. The software was then used to compute the aspect ratio and angle relative to the direction of stretch. Aspect ratio, or the ratio of the major and minor axes of the fitted ellipse, was used to measure cell elongation. The angle of the cell was computed as the angle of the major axis relative to the horizontal or the direction of stretch. Therefore, cells aligned in the direction of the stretch will have an angle of 0 deg. The orientational order parameter (OOP) was then calculated using the obtained angle values to quantify the degree of macrophage alignment in response to cyclic stretch. Details regarding the OOP measurement have been previously described [32–34].

The cardiomyocyte tissue morphology was qualitatively evaluated by imaging actin, sarcomeric z-lines, and nuclei through the stretcher membrane with an UPLFLN 40× oil immersion objective (Olympus America, Center Valley, PA) mounted on a IX-83 inverted motorized microscope (Olympus America, Center Valley, PA) mounted with a digital CCD camera ORCA-R2 C10600-10B (Hamamatsu Photonics, Shizuoka Prefecture, Japan). To quantitatively compare the results to those previously published, the orientational order parameter of the actin was calculated.

Results

Validation of Uniaxial Cell Stretcher. The designed, low-cost, uniaxial cell stretching device was subjected to a number of tests to ensure adequate and repeatable mechanical function. For example, by tracking and analyzing the rotation of the servogear and positional markers on the experimental substrates, the waveform and the strain profiles output by the servo were computed. The servo was capable of generating sine, triangle, and square waves through fine adjustments in the rotation speed and acceleration of the servo gear (Fig. 2(*a*)). Similarly, changing the degree of rotation of the servo gear generated different strain amplitudes. The parallel and perpendicular cyclic strains generated by the device were similar to theoretical 10% and 20% stretch

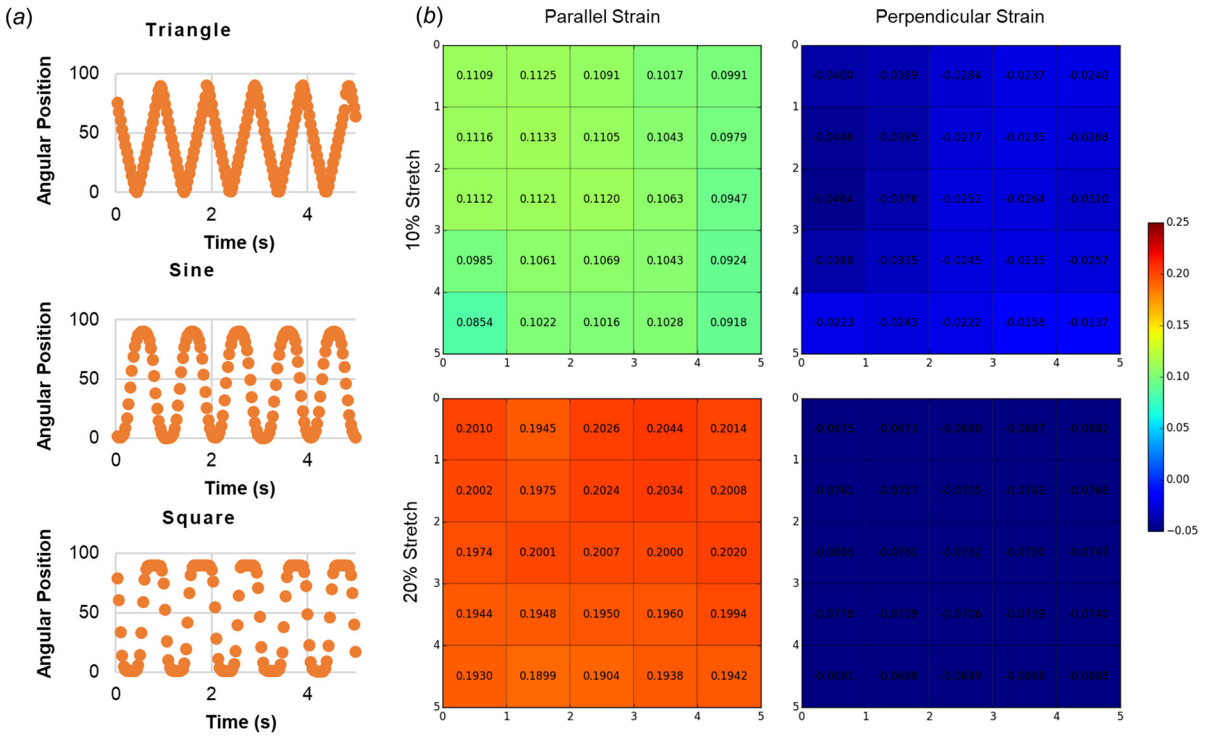

Fig. 2 Validation of uniaxial cell stretcher. (*a*) The designed cell stretcher is capable of generating a 1 Hz triangle, sine, or square wave through manipulating various speed and acceleration parameters. (*b*) Measured strains in both the directions parallel and perpendicular to stretch were quantified and were found to be uniform and consistent with the desired 10% and 20% strains.

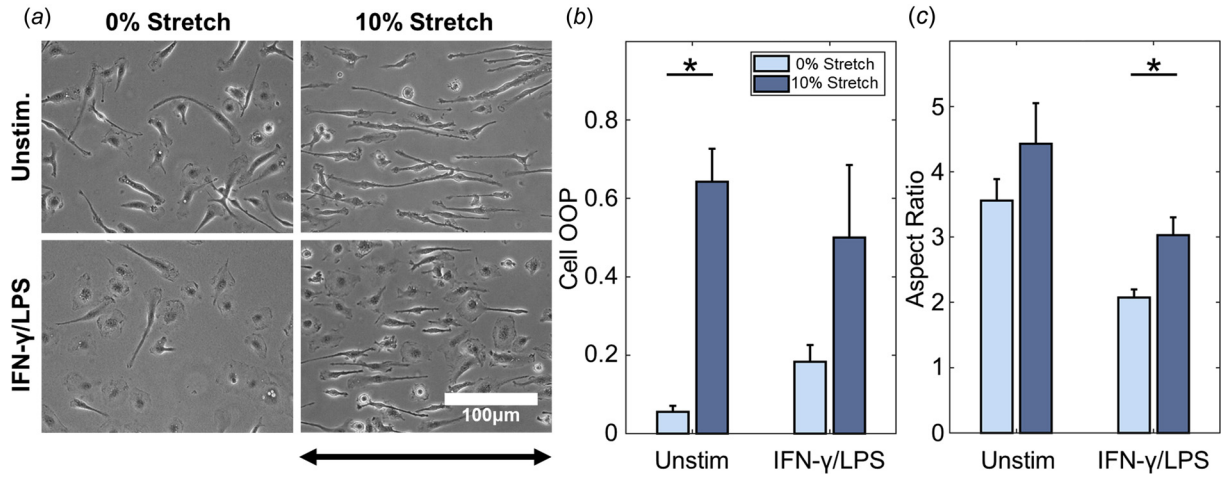

Fig. 3 Macrophages align and elongate in response to 10% cyclic strain. (*a*) Phase contrast images of unstimulated (top) and IFN-γ/LPS (bottom) stimulated macrophages cultured under either 0% (left) or 10% (right) cyclic stretch. Arrow indicates the direction of stretch. (*b*) Quantification of OOP and (*c*) aspect ratio as a measure of macrophage alignment and elongation in response to cyclic stretch, respectively. Error bars indicate standard deviation of the mean for three separate experiments and * denotes $p < 0.05$ when compared to the 0% stretch condition as determined by student's *t*-test.

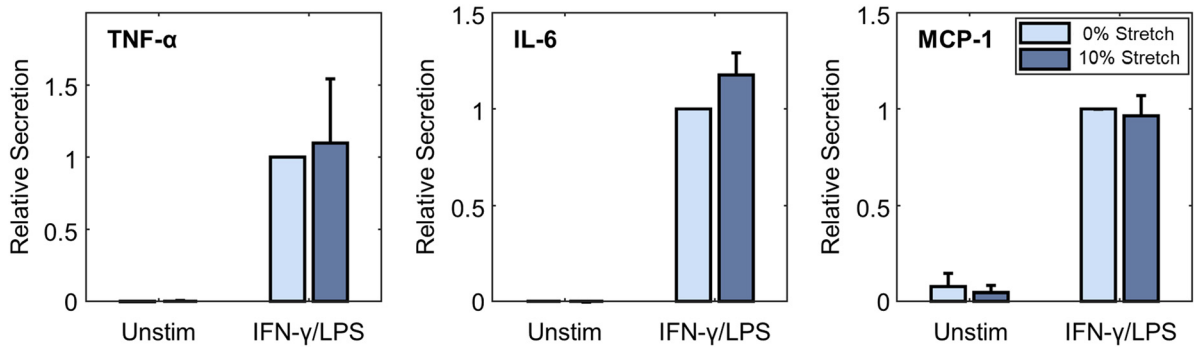

Fig. 4 Macrophage inflammatory cytokine secretion is unaffected by 10% cyclic uniaxial stretch. Secretion of TNF-α (left), IL-6 (middle), and MCP-1 (right) for unstimulated and IFN-γ/LPS stimulated macrophages subjected to either 0% or 10% cyclic uniaxial stretch. Data are normalized to IFN-γ/LPS stimulated and stretched condition. Error bars indicate standard deviation of the mean for three separate experiments.

amplitudes, respectively (Fig. 2(*b*)). This device is, therefore, able to generate uniform strain profiles in the center of the well that is comparable to other, more expensive, cell stretching systems [5,14]. However, decreases in strain were observed toward the transverse boundaries on the stretchable membrane (Fig. S1, which is available under the "Supplemental Materials" tab for this paper on the ASME Digital Collection). Once the mechanical functions were validated, the uniaxial system was used to mechanically stimulate macrophages and cardiomyocytes.

Cyclic Stretch Orients Macrophages, But Does Not Affect Inflammatory Function. When subjected to cyclic uniaxial stretch, bone marrow derived macrophages were observed to alter their cell morphology (Fig. 3(*a*)). Following stretch, the degree of macrophage alignment was quantified by calculating the OOP. The OOP ranges from 0 for a completely isotropic arrangement to 1 for perfectly aligned organization. Significantly higher OOP values were obtained for unstimulated macrophages in response to cyclic stretch when compared to static controls (Fig. 3(*b*)). This alignment of macrophages in response to a 1 Hz uniaxial strain at a 10% amplitude was previously observed [23]. IFN-γ/LPS stimulated macrophages are also aligned in the direction of stretch and displayed significant elongation in response to cyclic stretch (Fig. 3(*c*)). However, no significant differences were observed in inflammatory cytokine secretion for IFN-γ/LPS stimulated macrophages subjected to cyclic strain at a 10% amplitude (Fig. 4), which has also been previously reported [25].

Cyclic Uniaxial Stretch Aligns Cardiac Tissues. The stretcher devices was also used to subject NRVMs to cyclic stretch. As was expected based on previously published data [35], the resultant cardiac tissues aligned with the direction of stretch (Fig. 5(*a*)). The stretched tissues were evaluated quantitatively by calculating the actin OOP. While the actin OOP of the stretched tissues was smaller than the OOPs previously reported for tissues grown on lines of patterned fibronectin [32,33], it was significantly higher than in isotropic tissues even after only 6 h of cyclic stretch (Fig. 5(*b*)).

Skills Learned and Careers Pursued. This project was initiated as a collaboration between two labs with different biological interest (macrophage and cardiomyocyte research), but a common goal of promoting undergraduate research projects. The initial group of students focused mainly on device design and manufacturing and worked under the supervision of both principal investigator's as a team. They were given a wide degree of autonomy to

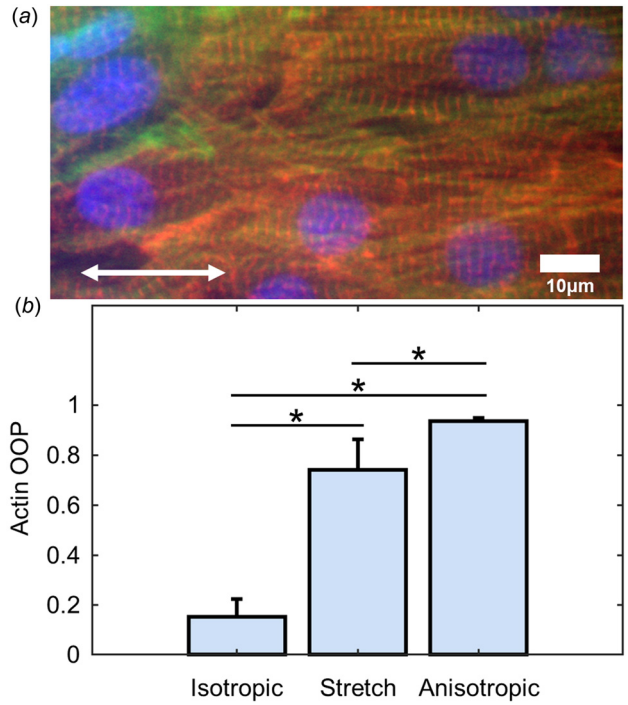

Fig. 5 Cardiomyocytes align in response to cyclic uniaxial stretch. (*a*) NRVMs after 6 h of cyclic stretching and stained for actin (green, horizontal lines), sarcomeric *z*-lines (red, vertical lines), and nuclei (blue, circular structures). There is qualitative alignment in the direction of stretch (white arrow). (*b*) The orientational order parameter of the actin fibrils from the stretch tissues compared to the previously published data [33] for isotropic and anisotropic tissues shows that 6 h of stretch produces significant alignment. Error bars indicate standard deviation about the mean for at least three separate experiments and * denotes $p < 0.01$ determined by one-way ANOVA Tukey.

research and design the stretchers. For example, it was through their independent research that the possibility of using low-cost servomotors was discovered. Beyond mentoring the newly recruited team-members, these students also mentored high-school students who were part of the center's CardioStart high-school summer program. As the project progressed, the students were more involved in the biological experiments that aligned with the research programs of each lab. However, they continued to work as a team on optimizing the device design. Thus, the students gained experience in design and manufacturing, biological experimental design, and working on an interdisciplinary team, all of which are valuable for careers in industry or academia. Overall, the students indicated that the project had a positive impact on their educational growth and helped to influence their career decisions (Table 1). Through working on this project, the students perceived that they gained valuable knowledge and skills in engineering design, experimental design, and basic cell biology. They also indicated that they gained experience in reading and writing scientific articles, programming, using CAD-related software, and cell culture. In addition, working in interdisciplinary teams and presenting their work to a wide range of audiences resulted in improved communication skills. The students were also asked to assess the impact the project had on their careers. They indicated that their direct involvement with this project helped to influence their chosen career paths and the experience also helped to develop the skills necessary for their career decisions. The first two students on the project originally intended to pursue industry careers, but both chose to also acquire master's degrees (one as part of this project and another at a different institution with his company funding the necessary tuition). Of the latter group of students, one went to industry and two are pursuing doctoral degrees.

Table 1 Project-based learning has a positive impact on undergraduate learning in cell mechanobiology. Summary of student respondents to surveys given after graduation. The scores shown represent the mean of the individual scores received, as indicated by the scale above.

Learning outcomes	Mean[a]
Through working on this project I:	
1. Improved my knowledge and skills in engineering design	4.6
2. Improved my knowledge and skills in basic cell biology	5
3. Improved my knowledge and skills in experimental design	4.6
4. Improved my communication skills	4.4
5. Gained knowledge and experience in cell culture techniques	4.8
6. Gained experience reading scientific articles	4.8
7. Gained experience in writing about scientific research	4.8
8. Gained experience in programming and CAD	4.4
Impact on Future	
Working on this project:	
1. Directly influenced my chosen career path	4.4
2. Helped me to develop skills useful for my chosen career path	4.8

[a]Scale: 1 = strongly disagree, 2 = somewhat disagree, 3 = neutral, 4 = somewhat agree, 5 = strongly agree.

Discussion

Device and Biological Results. This study describes a low-cost uniaxial cell stretcher that produces consistent strain profiles, similar to alternative commercially available systems. While the mechanical functions are similar, the fabrication and maintenance costs of this device are only a fraction of those required for other similar systems, thus potentially increasing the widespread availability of this apparatus. The proposed device is composed of readily available materials and utilizes a low cost servomotor to perform the mechanical motions necessary to elicit a uniaxial strain. The device is capable of multiple strain profiles with differing amplitudes and frequencies, and can maintain applied strains for a minimum of three days under continuous use, thus validating the clamping mechanism. In addition, no heating issues resulting from the continuous operation of the servomotor were observed. When compared to other commercially available stretchers, the proposed design is considerably less expensive to maintain, but has comparable functions (Table 2). However, this uniaxial cell stretcher has several limitations that can be addressed with minor modifications. For example, the servomotor used is unable to generate strains greater than a 20% amplitude. In addition, at maximum amplitude, the motor is capable of generating strains up to a frequency of 3 Hz, whereas higher frequency waves can be generated at lower amplitudes. These limitations can be addressed by substituting the given servomotor for another higher specification Hitech standard servo, which nominally increases the total cost. The standard servos typically have the same frame size, as a result, no additional modifications to the overall design of the cell stretcher should be needed.

The designed cell stretcher was used to verify the alignment of macrophages and cardiomyocytes in response to cyclic uniaxial stretch. Macrophages were subjected to a 1 Hz strain at a 10% amplitude for a period of 18 h, whereas cardiomyocytes were subjected to a 1 Hz strain at a 20% amplitude for a period of 6 h. The chosen frequencies and strain amplitudes have previously been described as within normal physiological ranges for stretch experienced by macrophages recruited to blood vessels and cardiomyocytes in the heart [36,37]. In the experiments performed, cardiomyocytes were seeded at a higher density to form a confluent monolayer that is more representative of cardiac tissues and analysis of alignment was dependent on actin organization, as determined by immunofluorescence. Macrophages were seeded more

Table 2 Comparison of proposed device to commercially available cell stretchers. Costs and specifications obtained for the commercially available Flex Cell® FX-5000™ and Cell Scale MCFX obtained from Refs. [9] and [11], respectively.

	Cell Scale MCFX	Flex Cell® FX-5000™	Proposed design
Stimulation mode	Uniaxial	Uniaxial/biaxial	Uniaxial
Cost of device	$7400	$35,490	$142.93
Number of samples/substrate	16	24	2
Cost per well	$1.64	$2.91	$0.70
Frequency range	0–5 Hz	0–5 Hz	0–3 Hz
Amplitude range	0–20%	0–33%	0–20%

sparsely to allow ample space for cell spreading in response to cyclic stretch and to distinguish between individual cells using phase contrast images, which was necessary for the analysis of alignment and elongation, since macrophages do not display actin stress fibers. The stretch duration is also unique to each cell type but is typically long enough to allow for cytoskeletal rearrangement. Although the experimental setups were somewhat different, both macrophages and cardiomyocytes displayed alignment in response to stretch.

While both macrophages and cardiomyocytes were observed to align parallel to the direction of strain, other cell types have been reported to align perpendicular to cyclic strain. For example, several studies have shown perpendicular alignment of smooth muscle cells, endothelial cells, and fibroblast when exposed to cyclic stretch [38–42]. Cardiomyocytes have also been reported to align perpendicular to stretch, but only if cells were cultured for longer periods of time prior to the initiation of stretch [26,43]. The orientation of cells in response to cyclic stretch is thought to be attributed in part to the frequency of stimulation: it is thought that low frequency strains allow time for cells to relax, and as a result, they align parallel to the applied strain. High frequency strains, on the other hand, do not allow time for relaxation, and thus, the cells align perpendicularly to minimize the force applied on them [44]. This theory, however, is limited to stationary mechanically active cells, such as muscle cells and fibroblasts, and has yet to be shown in other cell types. The response of cells to cyclic stretch, therefore, is dependent on a number of factors of which include cell type, time of culture, and frequency of applied of strain.

Pedagogical Evaluation. The design, building, and validation of the uniaxial cell stretcher, along with mechanobiological testing of the effects of cyclic stretch on different cell types, was demonstrated to have a positive impact on the learning experience of undergraduate students. Through their involvement on this project, the students perceived that they gained valuable skills and knowledge and experiences necessary to make informed career decisions. Nonetheless, the learning experience can still further be improved by implementing a few changes based on suggestions from the involved students. For example, most of the students desired additional education in mechanobiology when first starting this project, as few were aware of the impact of mechanical forces in influencing cell function. This could be implemented through classroom learning in mechanobiology, which together with the project would promote foundational understanding, and help students formulate independent research questions and interests. Students also found that working with cells as well as the stretchers and stretchable membranes was challenging at first and wanted to have more time to practice basic techniques prior to running experiments. Again, a formal course with laboratory cell culture work may provide additional opportunities for practicing basic techniques. Finally, the students involved in this project suggested further collaboration between labs, particularly for the cell experiments, as conducting research on multiple cell types may have enhanced their learning experience in cell mechanobiology.

We propose that this mechanical stretching device can serve as a platform for experiential learning for undergraduates in mechanobiology. This could be carried out as an independent research project by a group of 4–5 students, as was performed here, or could be scaled up to a laboratory course offered in conjunction with classroom learning. For the former, we believe that a few labs with long-range interest in mechanobiology of cells could collaborate to have an undergraduate student from each lab constitute a team that can mentor younger recruits. This was an extremely successful model in our experience, and provides the students with mentoring and project management experience, valued in both industry and graduate programs. Undergraduate involvement in research has previously been shown to have a positive impact on student learning and development. Students who were involved in research perceived greater enhancement of cognitive and personal skills were more likely to pursue graduate degrees [20,28,45], and were more likely to have a faculty member play an important role on their career decisions, thus highlighting the impact of faculty involvement in undergraduate growth [20]. While there are many perceived benefits of undergraduate involvement in research, there are some challenges associated with implementing the proposed project in the research environment. For example, differences in the requirements posed by each engineering curriculum could limit the time available to some students to work on this project as part of an interdisciplinary team. A potential solution to this challenge would be to provide a platform, such as through a course with both lecture and laboratory components, in which the requirements of all engineering disciplines can be satisfied.

For implementing this project in a classroom setting, we anticipate at least three modules, which could take place across several semesters or quarters over the course of one year. In the first module, students would gain experience in device design and fabrication using software such as SOLIDWORKS and basic machine shop tools. The second module would encompass device validation and basic cell culture skills needed to perform biological experiments. Students would use image analysis and software such as IMAGEJ to analyze videos and measure strains applied to stretchable membranes. In addition, students would learn aseptic cell culture technique and grow cells on culture wells fabricated on membranes. Finally, the project would culminate in the third module with hypothesis-driven experimental studies. Students would develop hypotheses based on the literature and test them experimentally using their fabricated device. Through project-based learning, students have been shown to develop critical and innovative thinking and improved learning [16–19,21]. Therefore, we propose that implementation of this project in the classroom would provide a novel experience for students in learning about cell mechanobiology.

Project-based classes are already offered at UCI, although none incorporate biological experimentation. For example, the Engineering seven series, which contains both a lecture and laboratory component, students design, build, and test a quadcopter, fitness tracker, or a microfluidic chip. The project described here can be implemented using this course framework but would likely require more than one academic quarter to complete design, fabrication, and cell-based experiments. Alternatively, the project can

be integrated directly into UCI's biomedical engineering curriculum, where undergraduate students are already required to learn CAD software and the basics of fabrication during their second year. An additional course would follow, where students learn about fundamentals in cellular mechanobiology and use their fabricated device in cell-based experiments. While a large-scale course may have broader impact and accommodate many students, the project may not have the same impact as an undergraduate research project where faculty-student interactions may be more predominant. Faculty involvement and direction in creating an experience similar to that of within the research environment would be critical in order to ensure student development and growth. Nonetheless, in any of the aforementioned formats, this project would provide students experiential learning in mechanical design and fabrication, testing and validation, and biological experimental design.

The process involved in designing, fabricating, and validating a cell stretching platform and then using the created device to study the effects of mechanical strain on different cell types provides undergraduate students with unique experiences in learning cell mechanobiology. While experiential- and project-based learning may be prevalent in other engineering disciplines, biomedical engineering has traditionally followed a theory-based instructional model, which limits practice in applying expertise and knowledge gained to new contexts [16]. This project not only offers undergraduate students experience in engineering design but also provides experience in cell and tissue culture and biological experimental design, both of which are difficult to obtain in a standard biomedical engineering curriculum. In addition, the process of working in interdisciplinary teams, presenting to different audiences, and interacting with graduate students and faculty helped undergraduate students to gain valuable knowledge and skills to improve learning and also help make informed career decisions.

Ongoing efforts to improve the device including development of multiwell substrates for testing of many different conditions, modification of the device base to allow visualization of cells by microscopy during stretch, or addition of a three-dimensional hydrogel to the substrate to render a more physiological microenvironment for cells. The described device may be used for both research and educational purposes, as a low-cost, easy to build and maintain, hands-on experience for learning of how mechanical forces regulate cellular structure and function.

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