ADHESION PROTEINS FOR MECHANOTRANSDUCTION ASSAYS
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ABSTRACT
Mechanical forces play a key role in the regulation of biological and molecular processes such as cell adhesion and cell differentiation. We have developed MEMS devices to create in vitro cellular micromechanical environments and for the study of mechanosensing in cell-cell and cell-matrix contacts. However, cells must be well adhered to study their response to mechanical stimulation. Here, we present both calibration of a high-throughput uniaxial strain array device and screening experiments of adhesion protein coatings for mechanical strain experiments on C2C12 skeletal myoblasts. Calibration results show applied strains range from 1-12% in the array. Laminin showed the best adhesion properties for C2C12 alignment studies. These devices with appropriate surface treatments will enable high throughput experiments to evaluate strain thresholds for cell realignment, remodeling and cell-signaling studies.

KEY WORDS: cell adhesion, mechanotransduction, strain array, C2C12 skeletal myoblasts

INTRODUCTION
Mechanotransduction is known to play a key role in cell behavior by interacting with biochemical signaling pathways [1]. To elucidate the underlying mechanisms, we recreate the cellular micromechanical environment in vitro and apply external loads similar to those seen in native tissue [2]. Microfabricated integrated strain arrays (ISA) [3] enable high-throughput testing of cells at multiple strain levels as they generate a distinct strain profile in each well. In this study, the ISA provides 6 replicates of a no strain control and 4 uniaxial strain levels by stretching the culture membrane across elliptical posts of varying dimensions (1mm, 1.5mm, 2mm, and 2.5 mm) as in Fig. 1. In this study, we calibrated the ISA to verify the distinct uniaxial strain levels across the wells and evaluated performance of extracellular matrix proteins on C2C12 skeletal myoblast alignment under mechanical strain. C2C12s were chosen for their excellent viability during chronic studies and their realignment properties in response to uniaxial cyclic strain [4].

EXPERIMENTAL METHODS
Device and calibration
The ISA reported by Bachtold et al. was modified using elliptical posts to provide uniaxial strain to adhered cells [3]. This high-throughput device consists of a polydimethylsiloxane (PDMS) culture chamber, an acrylic pneumatic chamber, and a microcontroller that applies strain conditions (Fig 1). The cell culture chamber has 30 wells (6 columns and 5 rows) in a 96-well plate format created by bonding a 250 µm thick PDMS membrane to a PDMS well layer. The PDMS membrane is pulled over various sized acrylic posts in the pneumatic chamber by applying negative pressure to the bottom of the membrane. IDGlide lubricant enables smooth operation for several hours. Applied pressure is controlled by a microcontroller which specifies constant or cyclic pressure at a user-defined magnitude and frequency. The device was imaged during operation with an upright microscope (Fig 2). Calibration of the strain array device was done using fluorescent bead markers and a micro-PIV algorithm [5].

Adhesion protein coating and cell culture
PDMS membranes were O2 plasma treated and wet autoclaved prior to laminin (Invitrogen, 2µg/cm²), fibronectin (Invitrogen, 2µg/cm²), gelatin (Sigma, 0.1 wt%), and growth factor reduced Matrigel™ (BD Biosciences, 1:50 dilution with DMEM) deposition. The remaining two columns were coated with RGD elastin-like protein [6] (0.1 wt%) or left blank after allowing the PDMS to return to a hydrophobic state. Coated devices were incubated overnight and rinsed with PBS to remove excess protein material prior to cell culture.

C2C12 skeletal myoblast cells were cultured in DMEM medium supplemented with 20% fetal bovine serum at 37°C with 5% CO2. Cells were seeded at 10⁴ cells/well and cultured for 12 hours prior to stretching. During this period, time-lapse bright field images of cell adhesion were taken every hour (not shown). Uniaxial strain was applied to cells in the range of 1-12% at 1Hz for 6 hours. After stretching, cells were fixed with 4% PFA, blocked with BSA, and stained for m-cadherin (Invitrogen), f-actin (rhodamin-phalloidin, Invitrogen), and counterstained with DAPI for nuclei visualization. The DAPI-labeled images were used to determine how many cells remained adhered after strain.

RESULTS AND DISCUSSION
Calibration results (Figure 3) show expected strain increase as the vacuum pressure increases and as post size decreases. We obtain a reasonable range of strain levels similar to those found in native muscle tissue. The results of the cell adhesion protein coating screen are shown in Figure 4. Laminin (LN) had the highest adhesion under strain and the cells realigned perpendicular to the uniaxial strain field as expected (Fig. 5) [4]. Matrigel™ films showed high adhesion but poor cell alignment suggesting poor strain transmission to the cells.

We are currently using this system to study the recruitment of m-cadherin to C2C12 cell-cell contacts under strain. M-cadherin is known to play a role in cell adhesion during myoblast fusion into myotubes [7], but the details of this process are still poorly understood. The uniaxial strain ISA and adhesion protein screening results will enable future mechanotransduction studies and provide a powerful tool for a wide variety of biological applications.
Figure 1: The integrated strain array device. A PDMS cell chamber is placed on top of a PMMA pneumatic chamber which can pull the membrane over various sized posts using vacuum. The device has a no strain control row and four different post sizes in subsequent rows. This gives six replicates of four strain levels and a control.

Figure 2: Schematic of the full experimental set-up. The integrated strain array device is placed on the microscope stage and the pressure profiles are controlled by GUI linked to the serial port of a microcontroller.

Figure 3: Uniaxial strain calibration for the integrated strain array. Calibration was done for one device with n=3 for each strain level plotted. Strain increase with increasing vacuum pressure and decreasing post size. This plot does not include the no strain control strain which is nominally zero.

Figure 4: Number of cells adhered to the PDMS membrane after 6 hours of cyclic strain normalized by the number of cells initially seeded in the imaged area. Cells proliferated during the incubation period prior to strain. There were five wells for each coating material (n=5 for each bar). Laminin and Matrigel showed the best adhesion properties.

Figure 5: C2C12 skeletal myoblasts seeded on a Laminin coated PDMS membrane before (right) and after (left) undergoing mechanical stretch. Strain was applied for 6 hours at 1Hz. Cells realigned perpendicular to strain.

REFERENCES