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Individually programmable cell stretching microwell arrays actuated by a Braille display

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Abstract

Cell culture systems are often static and are therefore nonphysiological. *In vivo*, many cells are exposed to dynamic surroundings that stimulate cellular responses in a process known as mechanotransduction. To recreate this environment, stretchable cell culture substrate systems have been developed, however, these systems are limited by being macroscopic and low throughput. We have developed a device consisting of 24 miniature cell stretching chambers with flexible bottom membranes that are deformed using the computer-controlled, piezoelectrically actuated pins of a Braille display. We have also developed efficient image capture and analysis protocols to quantify morphological responses of the cells to applied strain. Human dermal microvascular endothelial cells (HDMECs) were found to show increasing degrees of alignment and elongation perpendicular to the radial strain in response to cyclic stretch at increasing frequencies of 0.2, 1, and 5 Hz, after 2, 4, and 12 h. Mouse myogenic C2C12 cells were also found to align in response to the stretch, while A549 human lung adenocarcinoma epithelial cells did not respond to stretch.

Keywords: Cell stretching; Polydimethylsiloxane; Cell morphology; Endothelial cell; Epithelial cell; Lung

1. Introduction

In the body, cells are continuously exposed to mechanical deformation originating from processes such as muscle movement, respiration, and the pulsatile nature of blood flow. The ability of cells to sense and respond to mechanical strain is important in many tissues including the vasculature, lung alveoli, and skeletal muscle [1–3]. The process by which cells convert mechanical signals into biochemical responses is known as mechanotransduction. Extracellular forces are transduced across the cell membrane to effect intracellular biochemical events such as proliferation, differentiation, and alignment. To study such events, cell culture systems designed to replicate *in vivo* situations, where cells are exposed to mechanical stimuli, are useful to better represent physiological conditions.

To apply mechanical strain to cells *in vitro*, a variety of stretchable cell culture substrate systems have been developed. The majority of devices consist of cells cultured on a membrane (either a circular one held about its periphery, or a rectangular membrane held at opposite ends) and stretch applied either multiaxially, where there is a nonuniform strain through two axes (radial and circumferential) [4], or uniaxially where there is a single axis of uniform tensile strain with a small magnitude of compression [2]. Multiaxial strain can be applied to circular membranes by injection of air or liquid into a chamber beneath the membrane [5–7] or direct displacement with an indentor [8,9]. Uniaxial strain is often applied by fixing one end of a rectangular membrane while the other end is attached to a motor-driven movable frame [2,10].

These devices have demonstrated the value of *in vitro* stretching systems for a wide range of cellular studies. Existing

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cell stretching systems, however, are macroscopic and low throughput. Typically one cell type is exposed to one stretching condition per device. Commonly used devices have between 6 and 24 cell stretching wells but expose all wells to the same stretching condition [9]. What would enhance many *in vitro* studies and cellular screening assays would be the ability to test multiple conditions and cell types in parallel. Additionally, as the number of cellular samples that are exposed to different stretching conditions increases, the use of fewer cells and reagents, as well as the development of software algorithms to automate analysis of cellular responses to stretch in these array based systems would be required. A readily observed cellular response to mechanical strain is cell alignment. For example, upon cyclic stretching certain cells orient perpendicular to the direction of stretch including endothelial cells [10–13], fibroblasts [14], smooth muscle cells [15,16], cardiac myocytes [17], avian skeletal muscle cells [9], and mesangial cells [18].

Here, we describe a device with an array of miniature cell stretching chambers that enables efficient study of the effects of mechanical strain *in vitro* in a parallel manner amenable for higher throughput screening. The system uses microwells with flexible bottom membranes that are placed over the computer-controlled, piezoelectrically actuated pins of a refreshable Braille display (Fig. 1a). Each pin is independently controlled according to a computer program to push against the flexible bottom membrane of the microwells and apply a cyclical radial strain to cells cultured on the membrane (Fig. 1a). Computer control allows modification of parameters such as the frequency and duration of stretch, while an alteration in fabrication allows modification of the magnitude of stretch. Advantages of this device include the ability to create a microscale environment for cell culture and to run multiple experiments in parallel. The Braille device contains multiple independent pins, each of which can be actuated at varying frequencies. Commercially available Braille displays typically have between 320 and 1536 pins. We constructed a custom setup [19] that is more compact and has the potential to test up to 48 conditions with one display. We also developed image capture and automated image processing protocols to enable efficient analysis of morphological cellular responses across the multiple microwells. For biological validation we tested the response of three different cell types expected to behave differently in terms of cell alignment to mechanical stretch: (i) human dermal microvascular endothelial cells (HDMECs) (Fig. 1b,c), (ii) A549 human lung adenocarcinoma epithelial cells, and (iii) mouse myogenic C2C12 cells.

2. Materials and methods

2.1. Cells and growth media

Mouse myogenic cell line C2C12 cells and the human lung adenocarcinoma epithelial cell line A549 cells were obtained from American Tissue Type Culture Collection (ATCC, Manassas, VA). Growth medium for the C2C12 cells consisted of Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Invitrogen) and 1% antibiotic/antimycotic (Invitrogen). Growth media for the A549 cells consisted of Ham's F12K medium (ATCC) with 10% FBS (Invitrogen) and 1% antibiotic/antimycotic (Invitrogen). Human dermal microvascular endothelial cells (HDMECs) were obtained from Cambrex (East Rutherford, NJ) and cultured in microvascular endothelial growth medium-2 (EGM-2MV, Cambrex).

2.2. Cell stretching device

2.2.1. ANSYS finite element analysis (FEA)

In order to estimate the strain generated on an elastomeric PDMS membrane top surface while the membrane is deformed by Braille pin movement, we performed simulations using finite element analysis (FEA) software, ANSYS 10.0 (ANSYS Inc., Southpointe, PA). To reduce the computational time, a 2-D model with an axis symmetric boundary condition was constructed (Fig. 2a). The model was composed of a flat membrane and a curved Braille pin structure, where the pin structure was assumed to be rigid. Contact analysis was performed to simulate the interaction between the two objects. Contact and target elements, CONTA172 and TARGE169, were assigned on the Braille pin and the membrane bottom surface to simulate the surface-to-surface contact. The membrane was simulated using triangular 2-D elements, PLANE2. The axis symmetric boundary condition was assigned along the *Y*-axis, and one end of the membrane was assigned the fixed boundary condition. The displacement boundary condition, which was measured experimentally (Fig. 2b), was assigned to the Braille pin. To simulate the large deformation of the PDMS membrane, nonlinear, large deformation static analysis was performed. For all simulations, a Young's modulus of 750 kPa and a Poisson's ratio of 0.49 for the membrane were used (Dow Corning, Midland, MI). Although the 2-D finite element model dramatically reduced the time required for computations, the model cannot be utilized to estimate the membrane surface strain distribution while the membranes and Braille pins are not perfectly aligned, as may be the case in the actual experiments. Such discrepancy may cause errors in estimating the strains that the cells are exposed to during stretching.

2.2.2. Fabrication of chip and preparation for experiments

The three layer stretch chips were fabricated using the elastomer poly(dimethylsiloxane) (PDMS, Sylgard 184, Dow Corning, Midland, MI) and standard soft lithographic procedures [20]. Briefly, for the top layer, three large reservoirs of 1.5 cm diameter were punched into a cured slab of PDMS (10:1 prepolymer to curing agent) (dimensions: 7 cm × 2 cm × 0.5 cm height). For the middle layer, 24 microwells (1.7 mm diameter each) were punched into another PDMS slab of the same dimensions. The bottom layer consisted of a 100 μm thick PDMS membrane formed by spin coating prepolymer onto a 4-inch diameter silanized silicon wafer and curing for 30 min in a 120 °C oven. Strict control over the thickness of the membrane was achieved by keeping constant during the fabrication process: the ratio of PDMS to prepolymer, curing times in the oven, spin coating programs for the bottom membrane, and by sealing multiple devices onto the same spin coated PDMS membrane. This was critical to ensure reproducibility. The top two layers were sealed using a PDMS glue mixture (2:3 PDMS prepolymer:toluene ratio) and cured in a 120 °C oven for 20 min [21]. The bottom two layers were sealed irreversibly by treating both surfaces with plasma oxygen (SPI Supplies, West Chester, PA) for 30 s at 500 mTorr, and placed in a 120 °C oven for 20 min post-sealing.

After fabrication, the microwells were each filled with 10 μl of 50 μg/ml fibronectin (Invitrogen) solution in growth media and placed under UV sterilization for 30 min. The microwells were then washed twice with phosphate-buffered saline (PBS, Invitrogen). After removing the PBS, the microwells were then filled, along with each large reservoir, with 0.5 ml of cell culture media.

2.2.3. Culture of cells in PDMS microwells

At subconfluence, C2C12 and A549 cells were trypsinized using 0.25% trypsin/EDTA (Invitrogen) from 100 mm diameter tissue culture plates. The trypsin was removed using centrifugation and the supernatant aspirated leaving a pellet of cells. After resuspending the cells in 8 ml of growth media, 100 μl of the cell solutions was pipetted into each large reservoir. HDMECs were grown in T-25 culture flasks and were washed and detached using