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Short communication

Probing cell structure by controlling the mechanical environment with cell–substrate interactions

Chao-Min Cheng [1], Robert L. Steward Jr.[1], Philip R. LeDuc *

Departments of Mechanical and Biomedical Engineering and Biological Sciences, Carnegie Mellon University, Scaife Hall, 5000 Forbes Avenue, Pittsburgh, PA 15213, USA

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ABSTRACT

Recent results demonstrate the exquisite sensitivity of cell morphology and structure to mechanical stimulation. Mechanical stimulation is often coupled with cell–substrate interactions that can, in turn, influence molecular response and determine cellular fates including apoptosis, proliferation, and differentiation. To understand these effects as they specifically relate to compressive mechanical stimulation and topographic control, we developed a microfabricated system to grow cells on polydimethylsiloxane (PDMS) microchannel surfaces where we maintained compression stimulation. We also probed cellular response following compressive mechanical stimulation to PDMS substrates of varying stiffness. In these instances, we examined cytoskeletal and morphologic changes in living cells attached to our substrate following the application of localized compressive stimulation. We found that the overall morphology and cell structure, including the actin cytoskeleton, oriented in the direction of the compressive strain applied and along the topographic microchannels. Furthermore by comparing topographic response to material stiffness, we found a 40% increase in cell area for cells cultured on the microchannels versus softer PDMS as well as a decreased cell area of 30% when using softer PDMS over unmodified PDMS. These findings have implications for research in a diversity of fields including cell–material interactions, mechanotransduction, and tissue engineering.

1. Introduction

Cells have the ability to sense and respond to various external forces. One such response known as mechanotransduction occurs when mechanisms convert mechanical input into a biochemical response, which is related to a variety of responses including the reorientation of cell shape (Buck, 1980; Wang et al., 1995), actin cytoskeleton remodeling (Dartsch and Hammerle, 1986; Wang, 2000) and the synthesis of extracellular matrix proteins (Carver et al., 1991). These mechanically induced adaptations are notable aspects of many disease states including heart disease, cancer, and osteoporosis (LeDuc and Bellin, 2006). The forces that cells experience from various modes of stimulations such as stretching, compression, or shear stresses, are dependent on the cells' physiological environment and location within the body. To date, there have been many studies (Carano and Siciliani, 1996; Kubicek et al., 2004; Owatverot et al., 2005; Wang et al., 2004) that examine the response of cells to various modes and types of mechanical stimulation. For example, when cells are exposed to shear stress, the actin filaments are often observed to redistribute and align themselves along the direction of shear flow (Osborn et al., 2006). When uniaxial stresses are imposed on living cells though, their actin cytoskeleton can orient perpendicular to the direction of externally applied stress (Kaunas et al., 2005). There are other modes of mechanical stimulation as well including compression. Compression, although not as well studied as tension or shear stress, also induces interesting cell morphology responses including cell alignment perpendicular to the direction of compression (Girton et al., 2002).

In concert with the mechanical environment, the substrate topography (i.e. the microchannel patterns at the surface of the polydimethylsiloxane (PDMS)) can also affect cell response including cell orientation and differentiation. Various small-scale topographical features including grooves, ridges, pores, wells, and mesas have been incorporated into cell culture systems to assay cellular response for a diversity of cell types including fibroblasts (Cheng and LeDuc, 2006; Wojciak-Stothard et al., 1995), BHK cells (Britland et al., 1996), neuronal cells (Nagata et al., 1993; Rajnicek et al., 1997), epithelial cells (Andersson, 2003), endothelial cells, and smooth muscle cells (Jiang et al., 2002; Thapa et al., 2003). These studies have provided a range of findings including the fact that BHK cells and MDCK cells interact in a distinct manner with topographies including photolithographically fabricated grooved

* Corresponding author. Tel.: +1 412 268 2504; fax: +1 412 268 3348.
E-mail address: prl@andrew.cmu.edu (P.R. LeDuc).
[1] These authors contributed equally.

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substrata (Clark et al., 1987, 1990). Furthermore, examinations of the effects of substrate stiffness in such mechano-sensitive systems are critical to promote the understanding of associated cell response. The nature of the physical attachment of cells, via their basal domains, to their substrate allows for the transmission of mechanical force to the attached, living cells. These concepts, when examined with a focus on substrate stiffness, have been shown to induce a range of cell responses including those affecting cell proliferation and motility (Lo et al., 2000).

In an effort to understand the coordinated effects of mechanics and substrate interactions and to probe cell morphology and structural responses, we developed a system to grow cells on PDMS microchannel surfaces that allows us to make localized compression changes and observe resulting cellular response. First, we studied the morphology and actin cytoskeleton remodeling of fibroblasts subjected to compression. We then probed the impact of topography and substrate stiffness, which are directly related to the mechanics of the cell through the cell–material interface, and examined cell behavior by observing and recording molecular and morphological responses. Our efforts will shed light on key aspects of complex cell–substrate interactions and provide a valuable model to mimic the mechanotransductive response of cells in the physiological milieu.

2. Materials and methods

2.1. Microchannel fabrication

Microchannels were fabricated through soft lithography (McDonald and Whitesides, 2002) to make channels that were 2 μm wide, 10 μm deep separated by 2 μm each.

2.2. Cellular microdomain compression (CMC) device

Fig. 1A is a schematic of our cell culture system with microfabricated channels, as described above, which were used as the topography substrate for static compression. To impose compression on the cells through this microchannel substrate, we fabricated a CMC device that enabled us to mechanically deform the PDMS substrate without inducing buckling in the PDMS slab, as shown in Fig. 1B. Within this device, a manual cross-roller positioning stage (Parker Hannifin Corporation, Daedal Division, Irwin, PA, USA), which has a resolution of 1 μm, is implemented to create the mechanical force needed to deform the PDMS substrate without inducing unwanted buckling. This is a significant issue for such studies that requires balancing the imposed strains and the thickness of the PDMS slabs (Singer et al., 2002).

2.3. Cell culture and mechanical stimulation

NIH 3T3 fibroblasts were cultured as previously described (Cheng et al., 2006). After dissociation from the tissue culture plates, the cells were counted and seeded on the PDMS microchannels, which were treated with

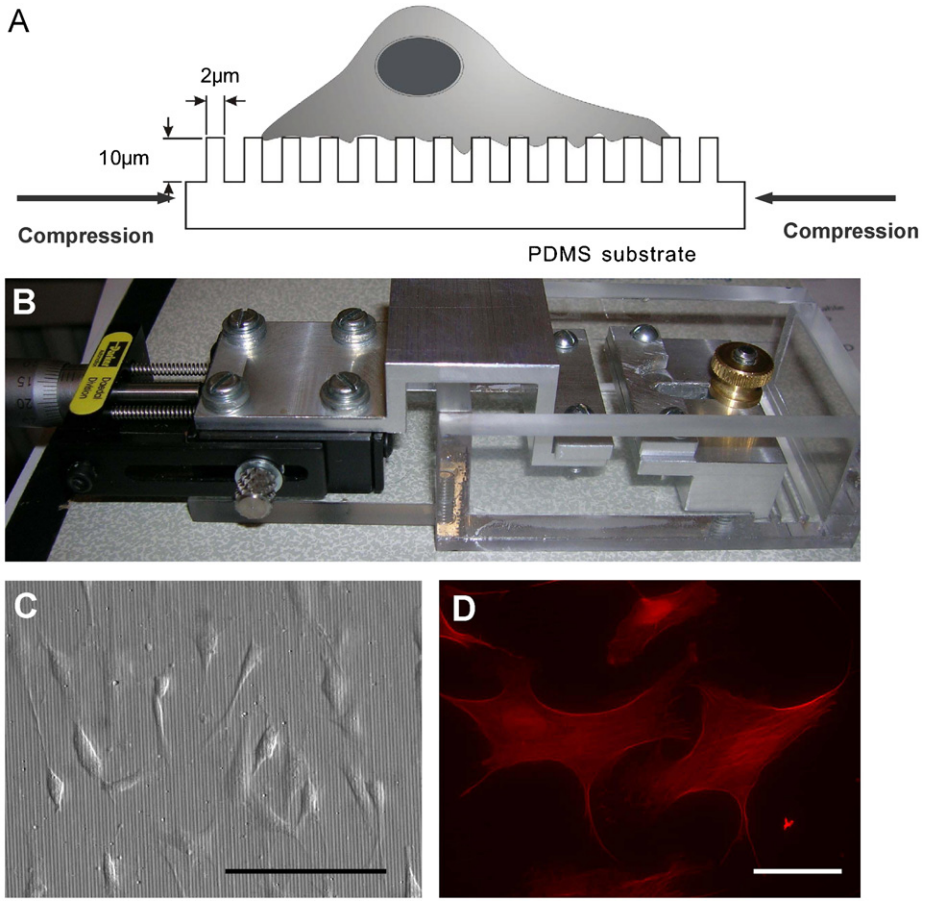

Fig. 1. (A) Schematic of the cellular microdomain compression (CMC) device with polydimethylsiloxane (PDMS) microchannels. The microchannels (2 μm in width and 10 μm in depth with 2 μm spacing) were fabricated by casting against a SU-8 mold made by soft lithography. (B) An optical image of the system that enabled us to mechanically compress the PDMS microchannel substrate. A manual cross-roller positioning stage with a resolution of 1 μm allowed us to not only impose a mechanical force to compress the PDMS substrate, but also permitted us to determine the strain on the PDMS substrate. (C) A phase contrast image of living NIH 3T3 fibroblasts that attached and spread on the PDMS microchannels, which were aligned along the vertical axis (scale bar = 100 μm) and fluorescent images of (D) actin filaments with our system. We were able to examine overall morphologies with phase contrast imaging and probe molecular response through fluorescent imaging; this allowed us to analyze cell structure at cellular and molecular levels. The actin filaments were stained using phalloidin (scale bar = 20 μm).