# SPATIAL CONTROL OF CELL SHAPE AND CYTOSKELETAL ORGANIZATION UNDER MECHANICAL DEFORMATION USING MICRO-/NANO-TECHNOLOGY

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## ABSTRACT

Apoptosis and proliferation in mammalian cells can be modulated through controlling the environmental conditions through chemical and mechanical regulation. There are critical factors which influence cell behavior including spatial control of extracellular matrix-cell interactions and mechanical effects on cell shape and cytoskeletal organization. Here, we explore the effects of spatially confining NIH 3T3 fibroblasts with the application of mechanical stretching on single cells. Micro- and nano-technology is utilized with a silicone elastic membrane to spatially confine individual cells in predefined geometries and then a uniform pressure is introduced on the silicone to stretch single cells equibiaxially. This allows us to investigate the complex nature of cell shape with mechanical deformation. In this we can explore the associated adaptations of the cytoskeleton including actin filaments and microtubules. These results will enhance the knowledge cellular and molecular function as well as provides insights into fields such as biomechanics, tissue engineering, and organic-inorganic interface.

## BACKGROUND

The field of mechanotransduction has been studied for many years in the activation of the MAP kinase pathways such as ERK 1/2 and JNK. The application of force has been shown to affect signaling in controlling ultimate cell fate by altering rates of apoptosis and proliferation. In this field local affects due to mechanical perturbations on the signaling pathways have been shown[1,2] as well as the development of a number of novel techniques for the field of cellular and molecular mechanics including fluid shear, BioMEMS, laser tweezers, and atomic force microscopy [3-7].

In cellular mechanics, the application of forces on cells is related to the link between the extracellular matrix (ECM), focal adhesion complexes, and the cytoskeleton[8]. Mammalian adherent cells can attach to the substrates through an extracellular matrix which form mechanical links through the focal adhesion complexes. These heterocomplexed formations consist of a series of interconnected proteins including $\alpha/\beta$ integrin, talin, vinculin, and paxillin allowing for a connection from the extracellular substrates into the intracellular cytoskeleton through linkages of these focal adhesion complexes to the actin cytoskeleton; this has been shown to be reorganized due to mechanical perturbations [9]. The formation of the interactions between these heterocomplexes and the cytoskeleton has a strong influence on the ability of the cell to attach and spread on the substrates. Thus the modulation of these interactions can affect the mechanical response of these cells through techniques including control of extracellular matrix concentration and micropatterning. Here we propose to address fundamental issues in mechanical perturbations of single cells through using the ability to spatially control the cell-ECM interactions and thus control cell shape and the cytoskeleton.

## EXPERIMENTAL METHODS

### Cell Culture

NIH 3T3 fibroblast cells were used in this experimental system. These cells were cultured with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% calf serum, glutamine (0.3 mg/ml), penicillin (100U/ml), and streptomycin (100 μg/ml), 20 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid at pH of 7.4 under 5% $CO_2$. Before seeding the cells on the flexible membranes, they were washed once with Phosphate Buffered Saline (PBS) and then exposed to trypsin ethylenediaminetetraacetate. After dissociation from the tissue culture plates, the cells were counted and then plated onto the flexible membranes. The cells were then allowed to attach and spread over the extracellular matrix coated substrates before the experiments were conducted.

### Microcontact Printing on Flexible Substrates

The size, shape, and position of the islands and intervening non-adhesive regions within the micropatterns were empirically determined through preliminary studies with each cell type. The geometric design of the pattern was created using AutoCAD software and a commercial photomask printing company. This mask was used to create a master by overlaying the photomask on the photoresist coated silicon wafer. The resulting features of the photoresist can be used as a mold to fabricate the flexible poly (dimethylsiloxane) stamp by pouring and curing the polymer over the surface of the wafer[10]. The flexible

substrates were manually stamped with the adhesive molecules. After stamping, the intervening spaces are filled with non-adhesive or blocking molecules. Cells are then plated onto the micropatterned surfaces to study the substrate-dependent control of cell shape and function under mechanical deformation.

**Experimental Set-up for Equibiaxial Stretching**

A novel system for creating equibiaxial stretching was developed which used flexible silicone membranes. The membrane was clamped in the custom made device centered above a circular opening. Cells were cultured on the upward facing side of the membrane allowing media coverage. After the attachment and spreading of the cells, the membranes were deformed by the addition of defined pressures on the silicon. This pressure coupled with the constraints on the edges of the membranes created a defined equibiaxial stretch on the membranes.

**Visualization of Cell Morphology and Cytoskeleton**

Indirect immunofluorescence localization of the actin cytoskeleton and microtubules is achieved through fluorescently labeled phalloidin and also specific primary antibodies for β tubulin. Secondary antibodies were utilized for mouse conjugated fluorescein or texas red. For examination of cytoskeletal proteins, these cells were fixed in 4% paraformaldehyde in PBS then washed with PBS three times. 0.5% Triton X-100 was used which removes the membranes and soluble cytoplasmic components. Digital microscopy and morphometry is performed using the IPLab and NIH Image analysis.

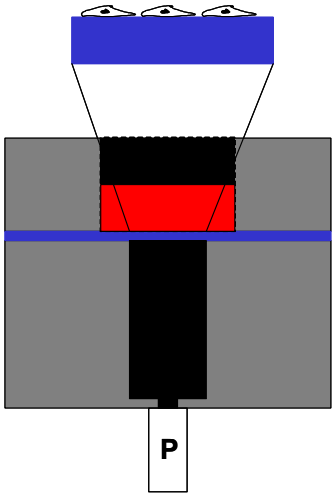


**Figure 1. Equibiaxial stretching apparatus. A pressure is used to stretch the membrane and the associated adherent cells.**

**RESULTS AND DISCUSSION**

3T3 fibroblasts were first cultured on flexible substrates coated with varying concentrations of fibronectin to determine the optimal conditions for attachment and spreading. This concentration was used as a gauge for micropatterning specific geometric shapes to control cell shape. Initially we patterned cells on silicone membranes in the shape of squares with side of 5, 10, 20, 30, and 40 μm per side. These cells were fixed, permeablized, and labeled to visualize the cytoskeleton.

Cells on these silicone flexible substrates were then introduced into a custom fabricated cell stretching apparatus. This equibiaxial stretching device operates through confining the perimeter of the flexible membrane with circular clamps. A differential pressure is applied on the flexible membrane which is physically separated from the membrane-cell surface. This force induces an associated deformation of the flexible membrane; the cells which are attached to the substrate through the extracellular matrix also are stretched. The fibroblasts are subjected to strain of 0 to 20% for time periods of up to one hour. The cells are then fixed and stained to examine the actin

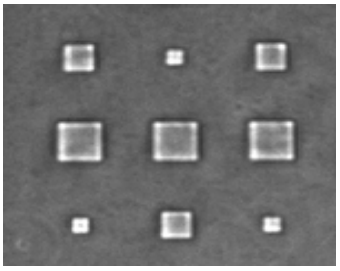

**Figure 2. Microfabricated substrates coated with extracellular matrix for spatial control of cell shape in square configurations.**

filaments and microtubules organization. The cells are also subjected to oscillating strain conditions as this has been implicated in unique signatures of cytoskeletal reorganization.

The application of mechanical deformation on NIH 3T3 cells reveals the localized effects of mechanical stimulation when spatially controlling cell shape in structure. When physically constraining cells to square morphologies, the corners of the cells exhibit distinct characteristics compared to the sides of the same cells. Further the ability of the cell to attach and spread through confining the size of the squares produces local differences in the actin cytoskeleton. This could not be accomplished previously as cells would assume morphologies in random orientations and configurations. For these types of experiments, there are a multitude of future directions to pursue including the examination of modulating the spatial distribution of the focal adhesion complexes, investigating novel transmembrane heterocomplexes in mechanotransduction, and developing the ability to analyze these spatiotemporal characteristics with a novel real time apparatus. Through this set of experiments though, we have been able to make significant advances as we are able to create a novel system to examine local effects of cell shape, cytoskeleton and mechanics to understand the complex behavior of mammalian cells.

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