Coupled Mechanical Stimulation Reveals Non-Linear Adaptive Structural Response

Robert L. Steward Jr.[*,**], Philip R. LeDuc[*], Chao-Min Cheng[***]
[*]Departments of Mechanical and Biomedical Engineering and Biological Sciences, Carnegie Mellon University, Pittsburgh, PA 15213, U.S.A.
[**]Current Address: Department of Environmental Health, Harvard School of Public Health, Harvard University, Boston, MA 02115, U.S.A.
[***]Institute of Nanoengineering and Microsystems, National Tsing Hua University, Hsinchu 300, Taiwan

Summary This paper describes the effects of coupled modes of mechanical stimulation on cell behaviour using a custom-fabricated device. Using our novel method we observed a non-linear response reflecting the cell's ability to adjust to a mechanically diverse environment.

Introduction

Cells have the ability to exhibit a diverse array of responses under mechanical stimulation. These responses are integral to the proper execution of important cellular responses such as cell motility, metastasis, and differentiation.[1-3] Mammalian cells nay experience various forms of mechanical stimulation including tension, compression, or fluid shear stress. In accordance, depending on the cell's physiological location these stimuli may be experienced separately or simultaneously. Though true most studies generally focus on a single mode of mechanical stimulation and its effect on cell behaviors. In this study, we developed a mechanical stimulation technique, allowing us to examine how single cells respond to multiple modes of mechanical stimuli through the observations of the cells' internal structure.

Techniques

A custom device was used to exert uniaxial strain and/or fluid shear stress across the basal and apical surfaces of single cells, respectively.[4] An advantage of our system is that each mechanical stimuli may be induced on single cells in an orthogonal manner, allowing us to investigate the coupled effect of two modes of mechanical stimulation in competing directions. We used a pressure regulator us to induce strains ranging from 1% to 20% and a flow pump to induce fluid shear stresses ranging from 0 to 6 dynes/cm^2. Fibroblasts and endothelial cells were cultured on ecm-coated elastomeric membranes and exposed to various magnitudes and durations of uniaxial strain and fluid shear stress for up to 24 hours. Following mechanical stimulation cells were stained for f-actin and imaged using fluorescence microscopy.

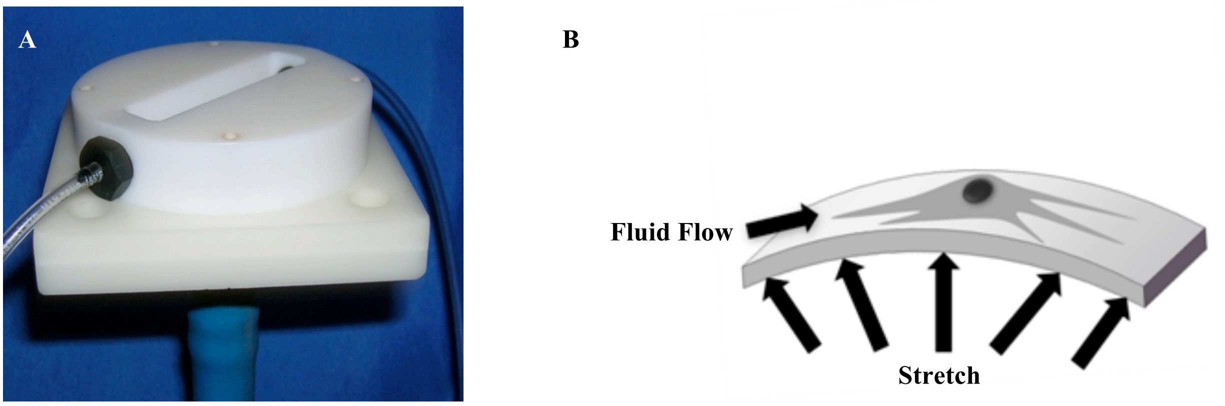

Figure 1. Optical image of custom fabricated device used to mechanically stimulate cells (**A**) and schematic of single cells experience multiple modes of mechanical stimulation (**B**).

Results and Discussion

Analysis of actin cytoskeleton reorganization reorganization after inducing both modes of mechanical stimulation revealed an orientation that deviated away from the orientations found when both modes were applied independently. We were able to induce horizontal or vertical alignment by lowering and/or raising the magnitude of shear stress or stretch. This response was observed to behave in a nonlinear manner, implying that cellular response to multiple mechanical stimuli (ex. fluid shear stress and mechanical strain) is not a simple, linear relationship, but in fact nonlinear in nature.

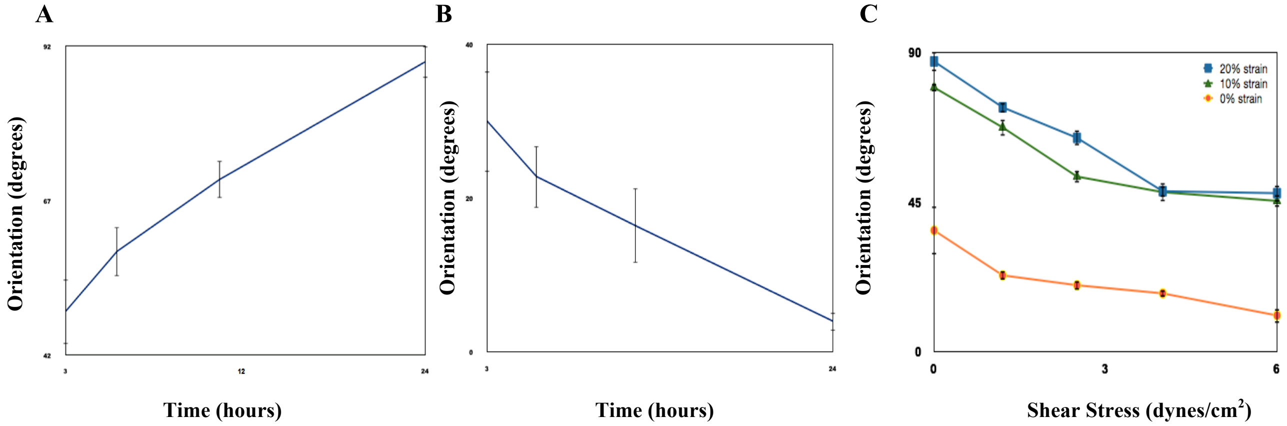

Figure 1. Cell structural response of fibroblasts to 20% uniaxial strain (A) and fluid shear stress of 0.6 Pa (B) from 3 – 24 hours. Cell structural response of fibroblasts after exposure to constant strain of 20%, 10%, and 5% while varying fluid shear stress from 0 – 6 dynes/cm^2.

Conclusions

The structural response of cells exposed to coupled modes of mechanical stimulation were found to have opposing responses when these various modes of mechanical stimulation were imposed separately. For example single cells exposed to a constant uniaxial stretch were observed to behave in a nonlinear manner in comparison to single cells exposed to fluid shear stress and uniaxial stretch, which were observed to behave in a nonlinear fashion. In fact, we observed a wide range of cellular orientations that were non-linear combinations of the mechanical stimuli imposed on them. This study, we believe, would be useful in multiple fields including systems biophysics, mechanotransduction, and cellular physiology.

References

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