# MEASUREMENTS OF CYTOSKELETAL DEFLECTION IN A 3D ENVIRONMENT

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## INTRODUCTION

It is now well appreciated that tissue stress causes tissue remodeling, and also that most cells are acutely responsive to mechanical stress. As a result, much work has been focused on elucidating both mechanotransduction mechanisms as well as various mechanical properties of the cell membrane and cytoskeleton. Still, there is little to no understanding of how a cell behaves to tissue stress in a natural 3-D environment, where the cell is attached to the extracellular matrix fibers in three dimensions and whose interactions with the matrix is a dynamic one. Much of the work in cell mechanics has been performed on single cells plated on a glass coverslip or coated membrane, which effectively grafts the cytoskeleton to a stiff backplate and only allows at most half of the cell's surface area to interact with the matrix and prevents the natural 3-D stress/strain behavior from being examined.

This work is focused on developing a system that will allow us to reproducibly and precisely impose an extracellular strain on a fibroblast within a 3-D matrix and observe the morphological response of the cytoskeleton in real time. This will serve as an experimental foundation for modeling cytoskeletal dynamics in a 3-D environment. Many disease states as well as important homeostatic, developmental and healing processes appear to be influenced or even controlled by physical forces which affect the cells either directly or indirectly. We hope to illuminate some of these connections by more fully understanding the coupling between the cell and its physical environment.

## METHODS

Our model was designed to impose planar shear upon a cell-populated gel matrix (e.g. fibrin, collagen, etc.) and to monitor the cytoskeletal morphology in real time. A precision-machined Delrin fixture allowed us to create gels with consistent dimensions. The fixture had a pocket for a round 18mm-diam. #1 glass coverslip, and had spacer posts which held a 22mm square upper coverslip 500 microns above the bottom coverslip. Both coverslips were cleaned by sonication in milli-Q water, acetone, and hexane, and treated with APTES (3-aminopropyltriethoxysilane). The silanized coverslips were functionalized with 0.1% w/w glutaraldehyde, and then air-dried in a sterile hood prior to use. The round coverslip was placed into the pocket of the Delrin fixture and sealed with sterile silicone glue, then cured in a dry incubator at 37°C for at least two hours.

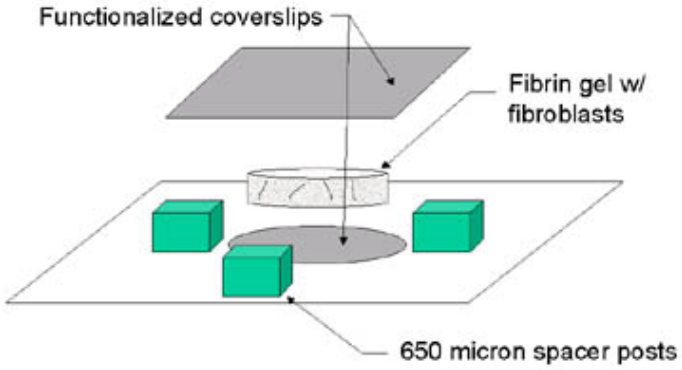


**Figure 1. Exploded schematic of *in vitro* model**

Human dermal fibroblasts (CCD1079sk, ATCC, Manassas, VA) were cultured in α-MEM (10% fetal bovine serum, 1% penicillin/streptomycin) medium and mixed with 1.5 mg/ml bovine fibrinogen at a density of $5x10^5$ cells/ml. 50 U/ml bovine thrombin was added to the mixture, and a 30-ul drop of the mixture was delivered onto the bottom coverslip. The top coverslip was placed on the spacer posts prior to the onset of gelation. The gel was allowed to polymerize in a 37°C, 5% $CO_2$ incubator for 20 minutes. The system was then incubated overnight in media containing 5 T.I.U./ml aprotinin, which prevents wholesale digestion of the gel while still allowing the cells to migrate locally and respond to mechanical cues.

We visualized the cells using a Leica Confocal Microscope. We optimized visualization conditions with cells fixed in 4% paraformaldehyde and stained with Alexa Fluor 488 phalloidin (Molecular Probes, Eugene, OR). We then carried out these studies with fibroblasts transfected with GFP-actin and GFP-vimentin. Shear strains of 5%, 10%, or 20% were delivered to the cells and cytoskeletal morphological responses were observed.

## RESULTS

Results from preliminary experiments show that we are able to clearly discern cytoskeletal features of cells suspended in a 3D gel. The morphology of the cells is quite different from the typical shapes seen on culture dishes or slides. A projection of a 3D stack of data is shown in Figure 2; f-actin stress fibers stained with AlexaFluor 488 phalloidin are clearly visible on multiple cells. The projection of this 3D data into 2D obscures the fact that the stellate projections of the large cell in the center of the figure do not lie in the same plane.

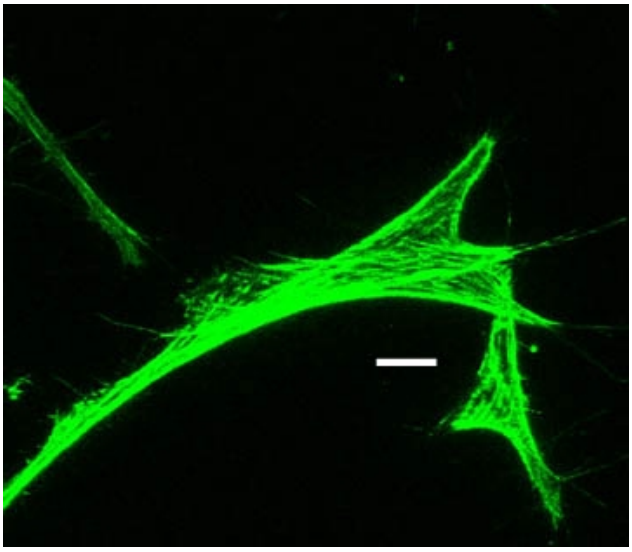

**Figure 2. Fibroblasts in a 3D gel – 2D projection of a confocal image containing 30 vertical sections across 60 microns. Scale bar is 10 microns.**

## DISCUSSION

The ability to measure and evaluate the effects of 3D forces on cells in physiologically relevant environments is crucial to understanding the mechanisms of mechanotransduction and the effects that those mechanisms have on other parts of a cell's activities. This new model, together with other recent work [1,2], will help to elucidate how cells sense their 3-D mechanical environment and subsequently remodel their immediate surroundings.

Other recent data from our laboratory [3,4] indicates a relationship between flow forces and spatial organization of fibroblasts. One possibility is that the interstitial flow forces mechanically polarize the matrix, which then transmits information to the cells. One should expect that cells respond to local forces, and cannot access global information on the state of the system. If the cells respond to fiber strain only on the fibers in its neighborhood, this would provide a method for global system information to impose orientation changes on single cells using only local inputs.


## ACKNOWLEDGEMENTS

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