Mechanical Properties of Primary and Immortal Fibroblasts in Cell Bi-Layers

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MECHANICAL PROPERTIES OF PRIMARY AND IMMORTAL FIBROBLASTS IN CELL BI-LAYERS

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INTRODUCTION

Immortalized cells are commonly used as analogs for primary cells in many cell mechanics, tissue engineering, and biochemical assays. However, it is not well-established whether immortal cell lines can mimic the behavior of primary cells in more physiological (three-dimensional) environments. For this project, we investigate the mechanical properties of primary cardiac fibroblasts (CFs) and 3T3 transformed fibroblasts when cultured in cell bi-layers by comparing the cells' viscoelastic properties.

Many cellular and tissue processes depends critically on the viscoelastic properties of the cell (1,2). Additionally, changes in a cell's mechanical properties are correlated with some disease states (3). Particle tracking microrheology (PTM) is an ideal technique for measuring the passive, internal viscoelastic properties of cells in more physiological constructs, since it does not require direct contact with the cells.

We found that the top layer of 3T3 cells in the bi-layer was significantly stiffer than the bottom layer, while CFs did not exhibit any significant difference between layers. These data suggest that 3T3 cells may exhibit different cytoskeletal behavior in 3D culturing scenarios compared to primary fibroblasts.

MATERIALS AND METHODS

Cell Culture

NIH 3T3 cells were cultured with high-glucose DMEM (Sigma), 10% Fetal Bovine Serum (Aleken), 50 units / mL penicillin and 50 ug / mL streptomycin at 5% CO_2.

Cardiac fibroblasts and myocytes were isolated from 1-day-old Wistar rats by a series of digestions with pancreatin and trypsin. The cells were then plated in a cell-culture flask for three hours to allow the CFs to attach to the flask surface. The myocytes were then removed with the supernatant, and the cardiac fibroblasts were cultured in the same way as the 3T3 cells.

PTM Experimental Setup and Analysis

Cells were plated on a collagen-coated glass-bottomed cell culture dish (MatTek, Ashland, MA) and seeded with fluorescent probes. After 18 hours, another batch of cells, which were also seeded with fluorescent probes, were plated on top of the confluent cell layer. The dish was then allowed to incubate for another 18 hours.

Particle tracking data was taken at the top and bottom layers of the cell bi-layer. The trajectory of each probe was tracked using the publicly available particle tracking code adapted from code by Crocker and Grier (4). The mean squared displacement (MSD) for each sample was obtained by averaging over the squared displacements of all particles at various time lags. The MSD was then used to calculate the storage and loss moduli using the generalized Stokes-Einstein relation.

Atomic Force Microscopy

Atomic Force Microscopy (AFM) was used to compare the stiffness of cells plated on glass to cells plated on a cell monolayer. An atomic force microscope (Bruker) mounted on an

inverted light microscope (Olympus IX81) was used to measure the elastic moduli of the cells. The cantilever probe used was a silicon-nitride DNP probe (Bruker) with a nominal spring constant of 0.12N/m. Each cell was indented at 1Hz in five different locations to find the average modulus for the cell. All elastic modulus data was taken at a depth of 400nm.

RESULTS

Cell Layer Comparison with PTM

In this experiment, we compared the storage and loss moduli of the bottom and top layer of a cell bi-layer at multiple frequencies. We found that the top layer of 3T3 cells has a significantly ($p < 0.001$) lower MSD than the bottom layer of cells over the entire range of time lags (Figure 1 shows this comparison at 1s), along with a significantly higher storage (20.9 ± 1.0 and 6.4 ± 0.7 Pa at 1 Hz, respectively) and loss modulus (27.8 ± 0.7 and 12.0 ± 0.6 Pa at 1 Hz, respectively). In contrast, there were no significant differences in the mechanical properties of the top and bottom layer of the CF bi-layer.

To determine whether the presence of the top layer of cells changed the properties of the bottom layer, we compared the mechanical properties of both layers of a 3T3 cell bi-layer with the properties of a 3T3 cell monolayer. We found that the properties of the monolayer closely mimic those of the bottom layer of the bi-layer. This suggests that the addition of cells to the top a monolayer does not alter the mechanical properties of the monolayer.

AFM comparison

To supplement the PTM experiments, we performed atomic force microscopy (AFM) measurements. While PTM provides information regarding the local cytoplasmic properties of engulfed beads, AFM characterizes the cortical/membrane properties.

We compared the elastic modulus of 3T3 cells and CFs plated on glass with cells plated on confluent 3T3 cell and CF layers, respectively. We found that the 3T3 cells plated on the cell monolayer were significantly stiffer ($p < 0.05$) then cells plated on glass, while CFs plated on the monolayer exhibited no significant difference with the CFs plated on glass (Figure 2).

DISCUSSION

One key finding of this study is that the top layer of 3T3 cells in a cell bi-layer is stiffer than the bottom layer, using both PTM and AFM. Other experiments have demonstrated that many types of cells will exhibit the opposite effect, with cells exhibiting elastic moduli equal to, or lower, than the moduli of the underlying substrate (5). However, some cell types become stiffer when plated on a confluent cell layer, due to an increase in cortical stress fiber density (6). This contrast between results indicates that viscoelastic properties of the cells may be dependent upon cell-cell contact as well as the stiffness of the substrate.

Interestingly, this property does not extend to primary cardiac fibroblasts, which shows no significant difference in mechanical properties as measured by both AFM and PTM. These data suggest that 3T3 cells do not mimic the behavior of primary fibroblasts in three-dimensional culturing scenarios. This difference in behavior may have manifestations starting at the cytoplasmic intra-cellular level up to the tissue structural level. It is therefore important for researchers looking at cell behavior in physiological constructs to carefully select transformed cells lines that appropriately mimic primary cells.

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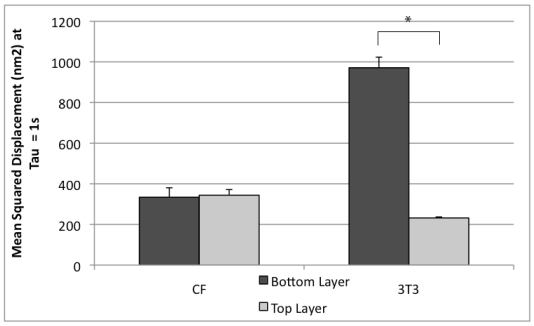

Figure 1: Comparison of MSD of top and bottom layer of CF and 3T3 cell bi-layer at τ = 1s.

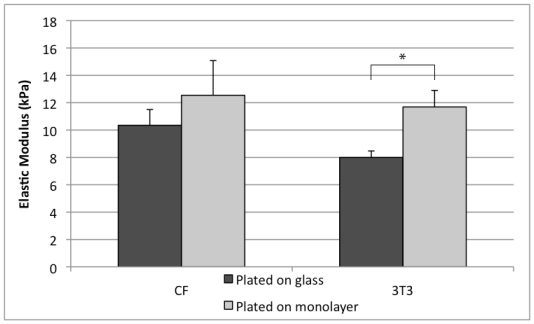

Figure 2: Average elastic modulus of CFs and 3T3 cells plated on glass and on a confluent monolayer of CFs and 3T3 cells, respectively.