

Cellular Mechanical Stress Gradient Regulates Cell Proliferation and Differentiation Patterns

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1 Introduction

How do tissues acquire their specialized form and function? If deciphered, this critical and yet unresolved question will greatly impact tissue engineering of human organs and tissues and ultimately regenerative medicine. In recent years, mechanical stresses have been proposed as a dominating morphogenetic regulator [1, 2]. However, the role of mechanical forces in tissue pattern formation, or mechano-morphogenesis, remains poorly understood. This study was designed to determine the relationship between mechanical stress gradient and cell proliferation and differentiation patterns using theoretical modeling, cell traction force microscopy (CTFM) [3, 4], and immuno-histochemistry.

2 Materials and Methods

Finite element method (FEM) modeling. FEM was used to determine the distribution of mechanical stress generated by contraction of a confluent cell monolayer of distinct shapes (**Fig. 1**). A two-layer system, a contractile top layer and a fixed bottom layer, was used to model the cell monolayer on top of a solid substrate. The Young's modulus of the top layer was 500 Pa, whereas that of the bottom layer was 100 Pa. The Poisson's ratio for both layers was assumed to be the same, 0.499

CTFM for determining traction forces of cell monolayers. CTFM was also used to determine cell traction force distributions. The detailed methods for application of this technology is described previously [4].

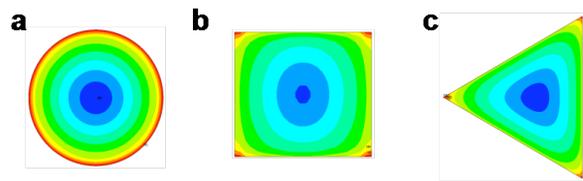


Figure 1 : Mechanical stress distributions of cell monolayers on three islands. **a)** circular; **b)** square; and **c)** triangular. Significantly higher cellular stresses were present along the edge than that in the inner region for all three islands. Also, stress concentrations occurred at the corners of the square and triangle islands.

Measuring proliferation and differentiation of cell monolayers. NIH 3T3 fibroblasts were synchronized by 2-day incubation in 1% bovine calf serum-supplemented medium after they reached confluent. Then after trypsinization 200 μ l cell suspension (3×10^5 cells/ml) were pipetted to each patterned island coated with fibronectin. After cells were maintained in culture for 2 days, BrdU incorporation, followed by immunohistochemistry, was used to measure cell proliferation of cell monolayers on circular, square, and triangular islands. In addition, immunohistochemistry was used to detect α -SMA expression of cells in these three islands.

3 Results

FEM analysis shows that there were higher mechanical stresses along the edges of three islands compared to those of inner regions for all three islands. In addition, stress concentrations occurred at the corners of the square and triangle islands (**Fig.1**). CTFM also shows that cell traction forces are highest along the edges and corners of these islands (**Fig. 2**). The traction force distributions matched well with mechanical stress distributions.

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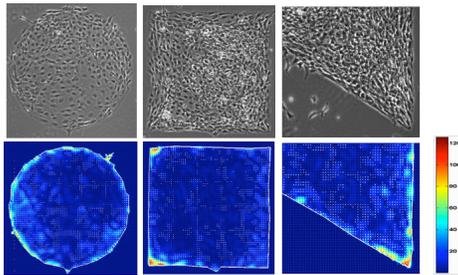


Figure 2 : Cell monolayers of circular, square and triangular shapes on polyacrylamide gel (PAG) (**top row**) and corresponding CTF distribution maps determined by CTFM (**bottom row**). The levels of CTFs were color-coded, with red representing high CTF and blue for low CTF, as indicated in the color bar on the right.

Furthermore, cells around the perimeter of all patterned monolayers showed much stronger staining signals, indicating that these cells were in active proliferation. However, cells in the inner regions of monolayers on all three islands exhibited weak signals, indication that the cells were in a quiescent state (**Fig. 3**). The stress patterns (**Fig. 1**) corresponded well to traction force patterns (**Fig. 2**).

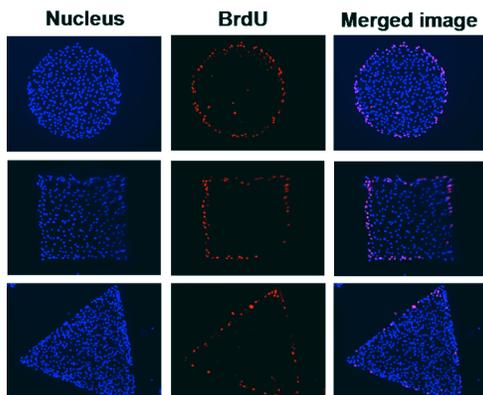


Figure 3 : Proliferation patterns of cell monolayers on three islands.

Finally, we found that for all three types of patterned monolayers, the cells around the perimeter expressed markedly higher α -SMA levels than those in the inner regions (**Fig. 4**). As α -SMA is a specific marker for myofibroblasts, this means that cells became differentiated into myofibroblasts. The distribution of α -SMA expression corresponds to the distribution of mechanical stress for all three cell

monolayers, indicating a correlation between α -SMA expression and cellular mechanical stress.

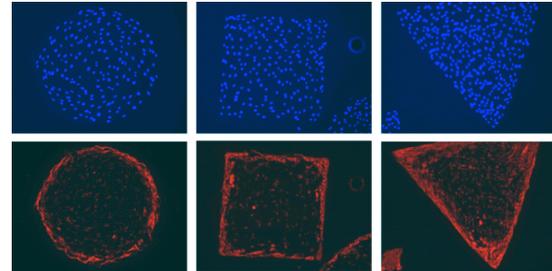


Figure 4 : α -SMA expression patterns of cell monolayers on three islands.

4 Discussion

Our results indicated that the “location” of cells in a monolayer is an important regulator of cell proliferation and differentiation for NIH 3T3 fibroblasts. The results are independent of the shape of cell monolayers since cell monolayers on the circular, square, and triangular islands exhibited similar patterns of cell proliferation and differentiation. The “location cue” appears to be mechanical stress gradients, as the highest stress regions correspond to those of the cell proliferation and differentiation. Further studies are underway to determine whether cell proliferation and differentiation patterns change once cell mechanical stress distributions are altered. This seems so as our preliminary data show that increasing cell mechanical stresses by application of TGF- β resulted in expanded inner regions of cell monolayers that underwent cell proliferation, and reducing mechanical stresses by BDM treatment eliminated cell proliferation pattern (data not shown). Taken together, the results of this study suggest that mechanical stress gradients generated by cell aggregates act as a morphogenetic cue to induce spatial patterning of cell proliferation and differentiation.

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