1999 Bioengineering Conference June 16-20, 1999 Big Sky, Montana

EFFECTS OF DEFORMING STRESS ON THE APPARENT STIFFNESS OF ALVEOLAR EPITHELIAL CELLS

Rolf D. Hubmayr, Mark A. Schroeder, Timothy A. Baker, Ben Fabry,* Bruce J. Walters

Thoracic Diseases Research Unit
Division of Pulmonary & Critical Care Medicine
Mayo Clinic
Rochester, Minnesota

* Physiology Program
Harvard School of Public Health
Boston, Massachusetts

INTRODUCTION

Magnetic twisting cytometry is a technique in which ligand-coated ferromagnetic beads are used to apply a mechanical shear stress to cells via specific transmembrane receptors. The resulting bead rotations (angular strain) can be measured and the cells' apparent stiffness computed. This stiffness is a measure of the ability of the cell to resist distortion. Using this technique, Wang et al. (1993) demonstrated that endothelial cells stiffen with increasing magnitude of imposed twisting stress when beads are coated with ligands (such as RGD peptides) that are recognized by integrin receptors. In contrast, magnetic beads coated with ligands to transmembrane receptors that do not associate with the cytoskeleton (such as bovine serum albumin) are not nearly as restricted in their rotation and yield low stiffness values. The computation of angular rotation (strain) relies on the accurate measurement of magnetic moments of beads and requires that signal changes as small as 10 pico-Tesla are resolved. The low signal-to-noise ratio mandates that at least 20,000 beads and as many cells are used to obtain a reproducible estimate of average cell behavior.

Fabry et al. (in press) have pointed out that heterogeneous bead behavior caused by loosely bound beads, and hence variability in angular strains between beads, can introduce systematic measurement errors in the average stiffness of the cell population. To quantify these errors, we compared the apparent stiffness of A549 cells (a transformed human alveolar epithelial cell line) before and after removal of loosely bound beads with centrifugation.

METHODS

A549 cells (5×10^4) were plated overnight in fibronectin-coated 96 wells. An equal number of RGD peptite® (Telios Pharmaceuticals, Inc., San Diego, CA) coated ferromagnetic beads (mean radius 2.25 μm) were added the following morning. The cells were washed twice 15 to 30 minutes later, and remanent magnetic field strength (a measure of total bead number) and apparent cell stiffness were measured. Measurements were repeated after centrifugation of the wells for ≤15 minutes at accelerations between 500 and 2000g, generating a centrifugal stress on the beads between 143 to 572 dynes/cm^2.

RESULTS & DISCUSSION

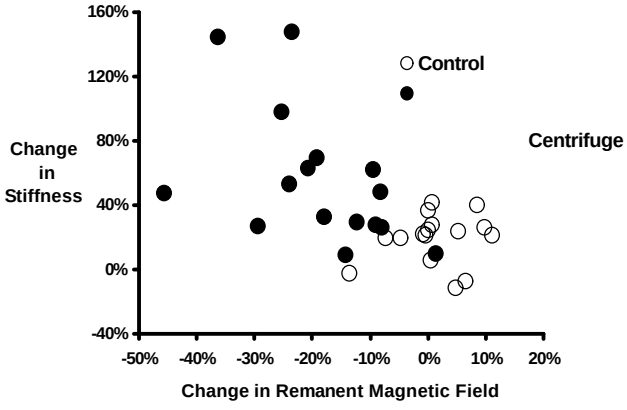

Figure 1: Relationship between the changes in remanent magnetic field and apparent stiffness in both control and centrifuge samples.

When the mechanical properties of A549 cells were measured before centrifugation by applying a magnetic twisting field of 80 Gauss (corresponding torque ranges from 100 to 180 dynes/cm^2), their stiffness averaged 163 ± 56 dynes/cm^2. Centrifugation caused a decrease in remanent magnetic field (i.e., bead loss) of 19 ± 12% and produced a 56 ± 41% increase in apparent cell stiffness (Figure 1). Bead loss and the increase in apparent stiffness were weakly correlated (slope = -1.7; r = -0.49, $p<0.05$). These results are consistent with the hypothesis that heterogeneous bead behavior can introduce systematic errors in the derivation of cell mechanical properties.

However, these data do not establish heterogeneous bead behavior as the sole cause of the apparent stiffening response. Centrifugal stress may promote focal adhesion complex formation (thereby strengthening the coupling between beads and cells) or induce a contraction of stress fibers (thereby increasing the pre-stress of the cytoskeleton). These alternative mechanisms would also be expected to raise the cells' resistance to shape change and, hence, lower bead rotations per unit applied torque. To explore these alternative mechanisms, an intermittent magnetic torque of ≤200 dynes/cm^2 was applied to cell bound beads for up to 30 minutes at a frequency of 10 cycles/minute and a duty cycle of 0.5. The resulting bead rotation (angular strain, α) was computed as

$$\alpha = \cos^{-1}(B_{ON}/B_{OFF})$$

whereby, B_{ON} and B_{OFF} refer to the beads' remanent magnetic field strengths during the final 1.5 seconds of the torque ON and torque OFF cycles, respectively. The result of one such experiment is shown in Figure 2.

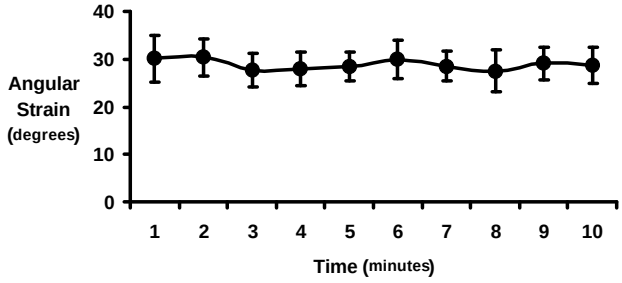

Figure 2: One minute averages and standard deviations of angular strain during the intermittent application of a torque of 179 ± 3 dyne/cm^2 in a single well of A549 cells.

After the application of intermittent stress in 13 separate experiments, the apparent stiffness of A549 cells remained 99 ± 19% of that measured at baseline.

CONCLUSION

We conclude that heterogeneous bead behavior can produce substantial bias in average cell stiffness, even when large bead torques are applied. The lack of a cell stiffening response during and after repeated twists suggests that deforming stresses do not strengthen the mechanical linkage between cells and beads and do not induce contraction of stress fibers in the A549 alveolar epithelial cell model system.

REFERENCES

Fabry B., Maksym G. N., Hubmayr, R. D., Butler, J., and Fredberg, J.J., in press, "Implications of Heterogeneous Bead Behavior on Cell Mechanical Properties With Magnetic Twisting Cytometry," *Journal of Magnetism and Magnetic Materials*

Wang, N., Butler, J. P., Ingber, D. E., 1993, "Mechanotransduction Across the Cell Surface and Through the Cytoskeleton," *Science*, Vol. 260, pp. 1124-1127.

Acknowledgments

Supported in part by NIH HL-57364