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Membrane Resiliency Following Large-Scale Local Deformations Using an AFM

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It is now well known that mechanical cues influence a variety of cellular and physiological processes, however many open questions still surround the cellular response to these forces [1]. Whole-cell mechanics are derived from the combined characteristics of their subcellular components. In particular, the membrane, cytoskeleton, and extracellular matrix and their connection with each other all play pivotal roles in providing the cell with stability, and structural support [2]. Both the membrane and underlying actomyosin network must act dynamically by responding rapidly to mechanical cues [2]. Moreover the connection between the plasma membrane and actomyosin network, known as the cell cortex is thought to play a crucial role in the ability of cells to undergo shape changes [3]. The cortex must be both dynamic and resilient in order to withstand mechanical forces and allow for timely remodeling. Our objective in this study was to examine the time-dependent deformation and recovery of the membrane and underlying actin cytoskeleton in response to an externally applied load.

Combined AFM and laser scanning confocal microscopy (LSCM) was used to apply a local force to HeLa cells while simultaneously imaging the deformation of the membrane (Figure 1 A). HeLa cells transiently expressing the membrane marker, PLC-δ-EGFP, were counter stained with the nuclear dye Hoechst 33342 in order to visualize both the membrane and the cell's nucleus. A range of forces (5, 10, and 20nN) were applied above the nucleus of a particular cell within a monolayer, while simultaneous LSCM volume images were acquired. Measurements of the deformation were acquired from orthogonal images of the cell (Figure 1 B) at 1 min intervals throughout the duration of the deformation, and a ratio of deformation to initial cell height was calculated (Figure 1 C).

Cell membrane deformation was found to be dependent on the magnitude of the applied force. Creep-like behavior was observed over the 10 minute duration, with the majority of deformation occurring immediately following force application. Orthogonal views following the perturbation indicated that the majority of cells completely recovered their initial morphology. Cells that reduced their total deformation by more than 50% were considered recovered (or recovering). Approximately 80% of cells were in the recovery process even following forces of 20nN. By performing a test for membrane permeability using propidium iodide, we showed that the membrane was not punctured during these prolonged high aspect ratio deformations. Despite its connectivity with the cytoskeleton and cortex, the nucleus was not responsible for the ability of the cell to recover its morphology following large deformations, as shown by performing the experiment off-nucleus. Corresponding with on-nucleus loading, off-nucleus loading resulted in ~90% cell recovery two minutes after the load was removed.

We also performed the same experiment on cells transiently expressing both PLC-δ-EGFP (membrane) and LifeAct Ruby (actin). Results demonstrated that not only the membrane, but also the actin network recovered its initial morphology within 2 minutes. By treating cells with the known inhibitor of rho kinase, Y27632, or the actin destabilizer Cytochalsin-D (Cytd), we were able to determine the role of the actin cytoskeleton on membrane deformation and recovery. Elasticity of treated cells were significantly

decreased, as determined by AFM force curves, and the deformation observed was significantly greater than in untreated HeLa cells, a ~22% and ~44% increase for Y27632 and Cytd, respectively (Figure 1 C). Moreover the number of cells that recovered reduced drastically to only 50% and 20% for cells treated with Y27632 and Cytd, respectively.

Our results indicate that the plasma membrane can withstand high aspect ratio deformations and relatively high loading magnitudes. The majority of cells recover their initial geometry following long-durations of loading, and surprisingly the nucleus plays an insignificant role in their recovery. On the other hand an intact cytoskeleton is necessary for rapid recovery. Clearly the cell cortex plays an integral role in cell shape and deformation and future studies involving the disruption of this link may shed further light into these recovery processes.

References:

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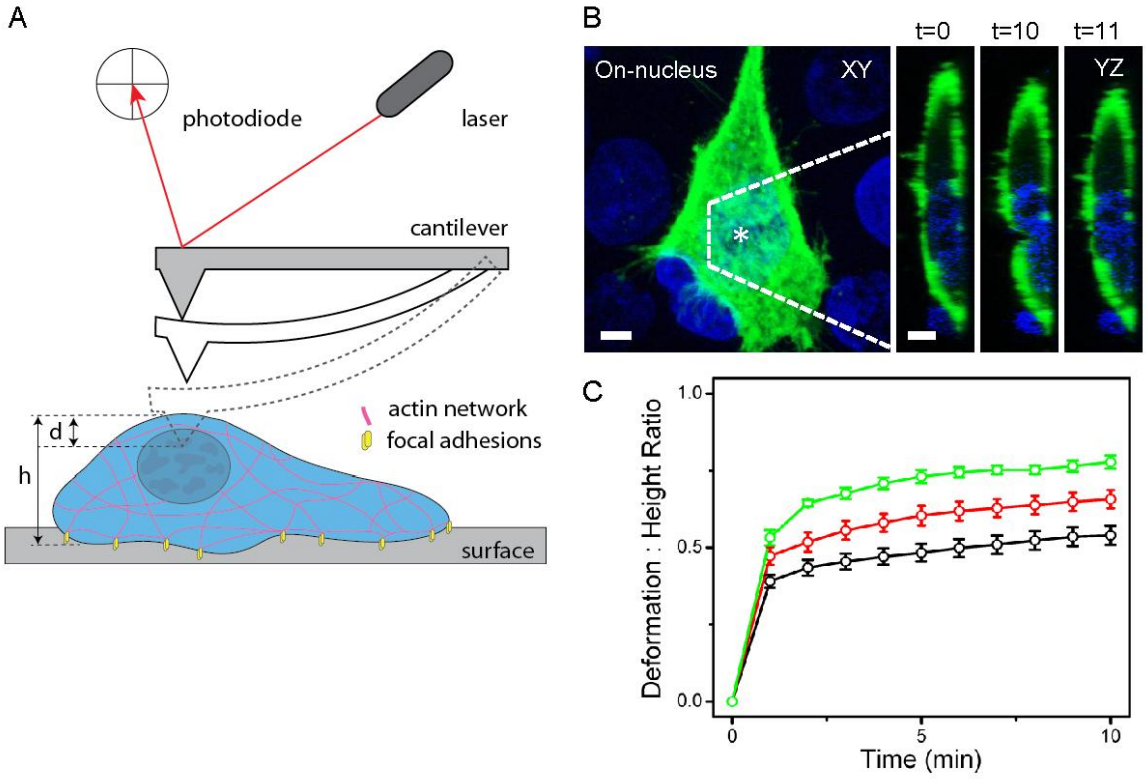

Figure 1. A) Experimental setup. B) XY and orthogonal views of a cell before, during a 10nN applied load, and 1 minute after tip removal. Green: PLC-δ-EGFP, Blue: Hoescht, Scalebars 10μm. C) Plot of deformation over time for Y27632 (red), Cytd (green), and untreated cells (black).

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