Real-time Measurement of Intercellular Stresses in Cells Grown on Micropatterns

Kevin Suffoletto, Nannan Ye and Susan Z. Hua
Department of Mechanical and Aerospace Engineering and Department of Physiology and Biophysics, SUNY-Buffalo, Buffalo, NY

Statement of Purpose: The local environment plays a key role in cell morphology, proliferation, and growth. In adherent cells, the cytoskeleton structure is altered by the mechanical constraints imposed by the substrate, resulting in a redistribution of cytoskeletal stresses within the cells.[1] Using micropatterning techniques, a variety of patterned substrates have been utilized to modify the cell morphology, however the cytoskeletal stresses leading to the ultimate configuration have yet to be fully understood. Here we have used stress sensitive FRET sensors to measure the cytoskeleton stresses in cells that are subjected to a variety of well defined mechanical constraints imposed by patterned substrates. The results provide insight on molecular basis of force transduction mechanisms in cells.

Methods: The standard microprinting method is used to pattern extracellular matrix protein, fibronectin, on glass substrates. Briefly, a glass substrate was silanized with dimethyldichlorosilane. Fibronectin protein on the PDMS stamp was printed onto the silanized glass substrate to make the pattern. To directly monitor the stresses inside the cells, the FRET sensor, was genetically inserted in α-actinin, a cytoskeleton cross-linking protein, and transfected into human embryonic kidney (HEK) cells.[2] Cells were seeded on the patterned surface 24 hrs after transfection. A series of FRET images were acquired using a dual-view optical system that simultaneously acquires images from both donor (CFP) and acceptor (YFP) channels. The acceptor to donor ratio was calculated using Image-J as described previously.[2]

Results: We have printed fibronectin on glass substrates with four different patterns, including narrow stripes, T-shape, microarrays, and hexagonal as control. Cells were able to adhere and spread on the patterned substrates within 30 min. The actin cytoskeleton and cell morphology are well constrained by the pattern geometries in the period of 12 hrs.

Using actinin-sstFRET, we have measured the distribution of sub-cellular stresses in α-actinin in cells grown on patterned surfaces 2hrs after seeding. Cells demonstrated a non-uniform distribution of actinin stress on each pattern. On a rectangular pattern of 6 μm wide, cells show a higher stress (lower FRET ratio) in the nucleus region that is often extended beyond the pattern, and a lower stress at the two elongated regions attached to the pattern. This result suggests that the tension is released due to the cell attachment. On a T-shape pattern, cells spread along all three arms to form a triangle shape. An increase in density of stress fibers was observed along the non-adhesion edges (Fig. 1a-c). Analysis of time dependent FRET ratio in these regions shows that the stress increases at bridging edges during the cell spreading on the pattern. Cells stabilize with higher tension at bridge edges and lower tension at attached edge (Fig. 1). In comparison, a uniform stress distribution is shown along all edges in a cell on a solid hexagon.

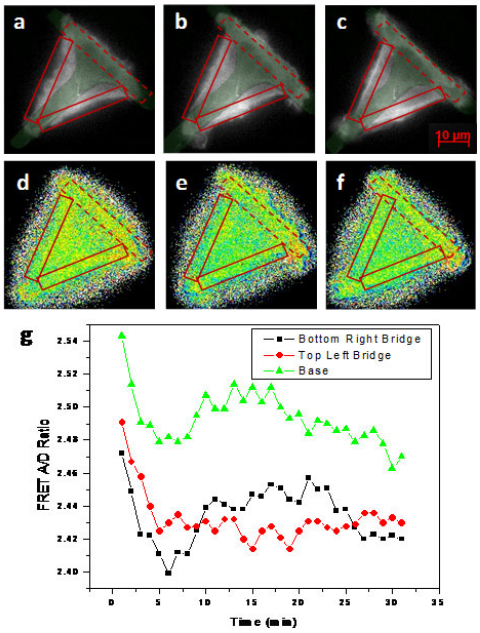

Figure 1. Stress distribution in HEK cells grown on T-shape pattern. (**a-c**) fluorescence images (CFP) of sstFRET-actinin transfected cells taken at 1 , 20, and 30 min, respectively. (**d-e**) corresponding FRET ratio showing changes in stress distribution. (**g**) time-dependent stress changes in selected bridge regions (solid outlines in **a-f**) and attached region (dashed outlines in **a-f**).

To examine the role of Myosin-II mediated contractility in the measured cytoskeletal stresses, a Rho kinase inhibitor, Y27632, was applied to the cells for 30 minutes. Regions of the cell that bridged over inhibited regions are shown to retract towards patterned regions, and this coincides with a reduction of cytoskeletal stress.

Conclusions: Using FRET stress sensors we have measured the cytoskeleton stresses and reorganization of actin cytoskeleton in real time in cells grown on patterned substrates. We show that adherent cells are prestressed and that attachment to the substrate releases the internal tension. Furthermore, an increase in stress coincides with increases in actin filament density when a cell extends over inhibited regions, suggesting the cytoskeletal stress may stimulate the cytoskeleton reorganization. These results enable a better understanding of how a cell accommodates changes within its local environment.

References:

1. Q. Tseng and M. Théry, *PNAS,* 2012, ***109 (5)***, 1506-1511.
2. F. Meng and F. Sachs, *Journal of cell science*, 2011, **124**, 261-269.