Concurrent Visualization and Characterization of Single Cell Mechanical Properties

Scott T. Wood, Sandra Deitch, Delphine Dean, PhD.
Department of Bioengineering, Clemson University, Clemson, South Carolina.

Statement of Purpose: Fibroblasts are the most common cell type in the heart and are constantly under dynamic load in normal healthy conditions. In response to injurious mechanical loading, fibroblasts can undergo significant cytoskeletal remodeling through differentiation to a myofibroblast phenotype, leading to changes in mechanical properties that may eventually contribute to heart failure [1]. The ability to predict the behavior of cells from their nanoscale structures could elucidate the mechanisms behind many tissue mechanical properties [2]. One of the major limitations of most methods for characterizing cell mechanical properties is that they cannot easily be adapted for cells inside 3D constructs. Therefore, in this study we utilized concurrent Atomic Force Microscopy (AFM) and confocal microscopy to generate force maps of fibroblasts inside thin collagen matrices overlaid on fluorescence images of the same cells before, during, and after AFM nanoindentation.

Methods: Fibroblasts were cultured in 3D type I collagen gels on glass coverslips. The cells were allowed to grow for 3 days inside the 1mg/ml collagen gels in a solution of 95 parts DMEM, 5 parts fetal bovine serum, and 1 part antibiotic/antimycotic.

We then used AFM nanoindentation (Asylum MFP 3D) to generate a force map of a cell while at the same time using confocal microscopy (Olympus IX81 Disc Spinning Confocal Unit [DSCU]) to image the plasma membrane (red, Cell Mask Deep Red plasma membrane stain from Invitrogen), and nucleus (blue, DAPI) of the exact same live cell. Force measurements were performed every 2.5 µm using a 5 µm diameter spherical tip in order to satisfy the Nyquist requirements. Because generation of a force map of an entire cell at such resolution can take more than an hour, measurements were taken on fibroblasts in media within a heated fluid chamber maintained at 37 °C and images were taken before, during, and after AFM nanoindentation. The confocal image and AFM force map of each cell were then overlaid to show the stiffness of that particular cell at each point within the cell (Fig. 1).

In a second set of experiments, we also observed changes in the fibroblasts during stress relaxation. We indented and held the AFM tip 1 µm into a fibroblast (again in media within a heated fluid chamber maintained at 37 °C) and held it in place for 120 seconds (long enough for the stresses within the cell to dissipate). At multiple points during this process, a 3D image of the same cell was generated using a stack of confocal image slices with the AFM tip in place. This allowed us to generate a time-lapse series of 3D confocal images of the cell before, during, and after indentation to watch how the cell shape changes during an AFM stress relaxation experiment.

This series of experiments was performed on untreated fibroblasts, as well as fibroblasts treated for 1 hr with 1 µM cytochalasin D to depolymerize actin filaments and 20 µM nocodazole to destabilize microtubules.

Results: Our technique was able to both resolve cell shape and structure in a collagen matrix as well as assess its mechanical properties (Figure 1). The advantage of this technique is that the optical images and AFM force maps can be overlaid so that mechanical property dependence on intra-cell location can be easily identified. In 2D culture, fibroblasts are more spread and the thinner periphery appears stiffer than the central region. In contrast, our present study found that the nuclear region of fibroblasts in collagen gels were significantly stiffer than the outlying regions. The rest of the cell body had a modulus closer to the collagen matrix. The cell was more viscous than the surrounding collagen matrix and showed significant relaxation and hysteresis.

It should be noted that during the roughly 60 minutes required to obtain the force map in Figure 1, the cell migrated in the gel. With concurrent time-lapsed optical and AFM techniques, the motion of the cell can be tracked as the mechanical measurements are captured. In addition, the cell remained alive and did not show evidence of damage even though DAPI was used to visualize the nucleus.

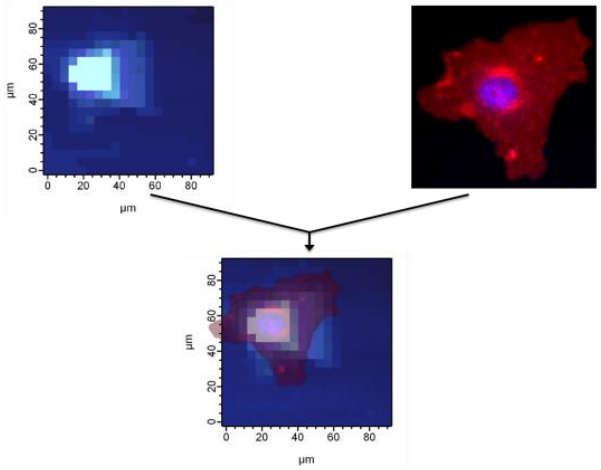

Figure 1. Force map of a fibroblast overlaid on an image of the same cell generated using concurrent AFM nanoindentation and confocal microscopy

Conclusions: The concurrent use of AFM nanoindentation with confocal microscopy provides a useful novel method for the visualization of the effects of AFM nanoindentation on cell shape and structure. This technique is particularly interesting due to its ability to observe these effects in live cells that actively respond to AFM nanoindentation as images could be taken in real time if so desired. In future studies, the force maps and confocal images generated in these experiments will be incorporated into a 3D structure-based finite element model in order to provide a unique and compelling method of validation for our model.

References: 1. Porter K. J Pharm Thera. 2009;123(2):255-278. 2. Ingber DE. J Cell Sci. 2003;116(7):1157-1173.).