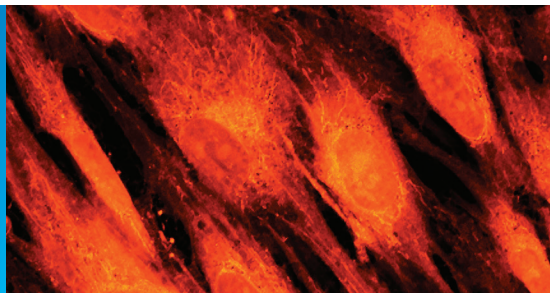
Imaging and Testing Dry and Hydrated Fixed Mouse Lung Endothelial Cells Using a Nanoindenter



Introduction

Scanning and mechanical testing under fluid is of particular interest in the areas of life science and biology. In this article, a new nanoindenter test method is used to scan and indent dry and hydrated fixed mouse lung endothelial cells. The cell scans were important to identify areas of the nucleus for indentation tests that possessed little topography change. First, the dry cells were scanned and tested, then the cells were hydrated for two hours before the cells were rescanned and retested. Results of the indentation tests showed a large difference in the elastic modulus of the hydrated cells tested under fluid versus that of the dry cells.

Samples

The samples were mouse lung endothelial cells that were fixed to a glass cover slip, using glutaraldehyde, and dried in air. Fixing the cells to the glass substrate in this manner cross-links the cell membranes and keeps them stationary for scanning. Figure 1 shows the dry sample mounted in a fluid puck for testing. The sample was first tested in the original dry state and then the sample was hydrated with a 0.9% sodium chloride (saline) solution for two hours prior to retesting (wet condition). Figure 2 shows the hydrated sample loaded in the Nano Indenter® G200 instrument, ready for testing.

Test Equipment

The scans in this article were performed using a Nano Indenter G200 system with the Dynamic Contact Module (DCM) transducer and NanoVision options. The DCM transducer is an ultra-low force transducer with ultra-high displacement resolution which is ideal for testing and scanning soft samples. Scanning is conducted using the NanoVision option, which consists of a high-precision piezo-actuated X-Y translation stage that allows scanning probe microscopy using the nanoindenter tip and transducer. The primary advantage of G200 scanning is high-precision targeting accuracy (20nm) for indentation placement and acquiring quantitative mechanical properties using the scanned images.

The indentation tests conducted on the cells were performed using the Continuous Stiffness Measurement (CSM) technique. Each indent was targeted from the scanned image to ensure the measurements were conducted on a region of the cell that provided a surface conducive to indentation testing; i.e., mostly flat and spacious relative to the indentation size. The scans and indentation tests were performed using a Berkovich tip.

Figure 1. Fixed mouse lung cells mounted in a fluid puck for scanning and testing with the Nano Indenter G200 system.

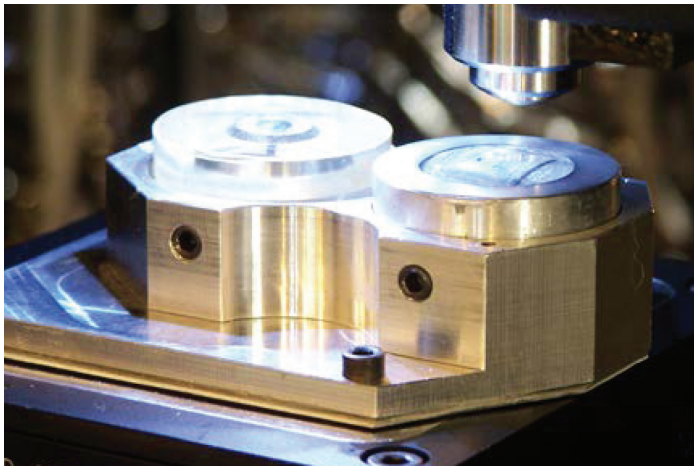


Figure 2. Hydrated fixed cells ready for scanning and testing with the Nano Indenter G200 system. The meniscus of the fluid is allowed to mound up over the sample to allow ample testing time before drying occurs.

Scan and Indentation Test Methodology

A new scanning method and algorithm was used in performing the scans of the dry and hydrated cells. Traditional contact scanning methods do not provide enough flexibility in the scanning force to enable imaging of soft samples under fluid when areas of large and small topography changes are combined. The fixed endothelial cells consisted of a nucleus with a membrane that extended outward and was fixed to the substrate. While the nucleus had substantial topography change, the extending membrane did not. In traditional contact scanning modes, this requires that the scanning force for imaging be set to a value that will maintain contact with the nucleus of the cell—this is generally too large of a force for the hydrated membrane of the cell. Therefore, the new scanning technique uses the data from the previous scan line to anticipate the minimum scan force that can be used to perform the following scan line. This minimum scan force is determined by the maximum vertical velocity detected during the previous scan line. The coupling between scan force and vertical velocity is not immediately obvious; therefore, a short explanation is provided. When performing contact scanning over the surface of a material, the instrument's control rate continuously updates the applied force to compensate for any topography change. If the force was not continuously adjusted, then the leaf springs inside the transducer—the springs that are used to laterally secure the indenter shaft—would cause variations in the scan load as the topography changed. As the sample topography increased the springs would be compressed causing an increase in scan force, and as the topography lowered the springs would be relaxed causing a decrease in scan force. Therefore, the force adjustment is conducted to compensate for any force change related to the internal stiffness.

The rate at which the force can be adjusted is determined from the control rate of the instrument, in this case 500Hz.

Between two control points the scan load must be high enough to maintain contact with the sample or else the tip will come off the surface. If the tip loses contact while entering a hole then the tip will regain contact when the topography changes directions, but if loss of contact occurs at a peak with a gradient change then the tip will not regain contact. Hence, the minimum force that is required during the scan is the force that will deflect the springs in the transducer (the internal springs on the Nano Indenter G200 system have a stiffness of approximately 80N/m) by the vertical distance between two consecutive control points. The minimum scanning force for a scan line is then the maximum vertical distance between two control points for the scan line multiplied by the spring stiffness, as shown in Equation 1.

$$Scan\ Force_{min} = \Delta h_{max} * K_s$$

where Δh_{max} is the maximum displacement between two consecutive control points and Ks is the transducer spring stiffness. The maximum change in displacement is also equivalent to the maximum vertical velocity divided by the control rate, which, when substituted into Equation 1, yields Equation 2.

$$Scan\ Force_{min} = \frac{V_{max}}{R} * K_s$$

where V_{max} is the maximum velocity and R is the rate of control. Equation 2 has the intuitive advantage that when the sample is scanned at higher lateral velocities, higher vertical velocities also occur which require higher scanning forces.

The new scanning method uses the scan line data to determine the minimum scan force used in the following scan line. This allows a continuous adjustment of the scan force as new areas of the sample are encountered. For the hydrated cells, this allowed the cell membrane to be scanned at lower forces while the nucleus was scanned at a slightly higher force to maintain contact. For practical applications, the minimum scan load is increased by a multiplier to compensate for any variation in the sample topography for each successive scan line.

Each indentation was targeted using the scans to ensure that the test area was of sufficient size and flatness to make the indentation measurement. The indentation tests were conducted using the CSM technique; the test protocol is described elsewhere.[1,2]

Results and Discussion

For practicality, all of the dry samples were imaged and tested first, then the stage locations of the cells were noted for targeting after hydration. Scans of the dry samples are shown in part (a) of Figures 3 through 5. In each of these scans, the nuclei and the cell membranes appear in great detail. The areas of the membrane that have been polymerized and fixed to the substrate have thicknesses on the order of 50nm to 100nm. The nuclei are approximately 1000nm in height.

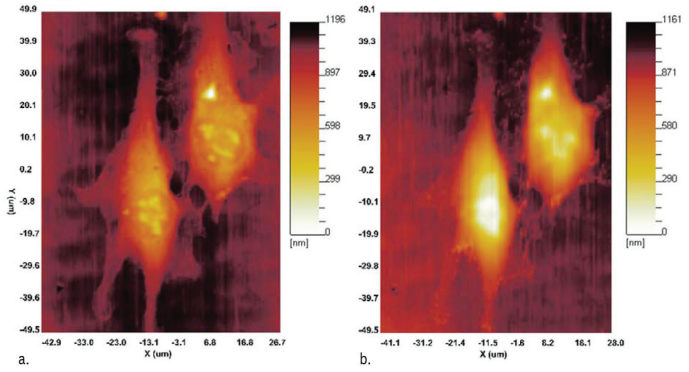


Figure 3. Scans of dry endothelial cells (a), and hydrated endothelial cells (b).

After scanning the dry cells, the sample was hydrated for two hours before rescanning the cells under fluid. The meniscus of the fluid in the sample puck was allowed to mound up and over the well containing the sample. As testing progressed, additional fluid was periodically added to the puck to maintain a meniscus. Image (b) in Figures 3 through 5 show the results of the scans conducted, under fluid, of the hydrated cells. The cells in Figures 4 and 5 show an increase in the height of the nuclei of approximately 300nm due to hydration. Images of the nuclei were softened due to the hydration, but similar characteristics of the cells remained. The softened membrane areas showed some damage due to scanning the sharp tip over the surface; however, the general structure of the cells remained. To induce less damage to the cell membrane, a different tip such as a conical shaped tip could be used for scanning—this would provide a larger elastic contact that would prevent most of the damage. However, the Berkovich tip was chosen because it has a well-defined geometry and can be accurately calibrated for indentation testing at shallow penetration depths.

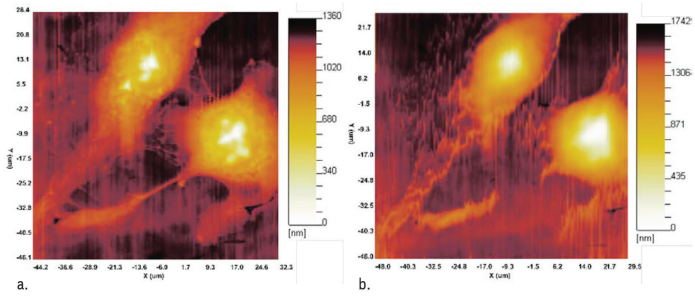


Figure 4. Scans of dry endothelial cells (a), and hydrated endothelial cells (b).

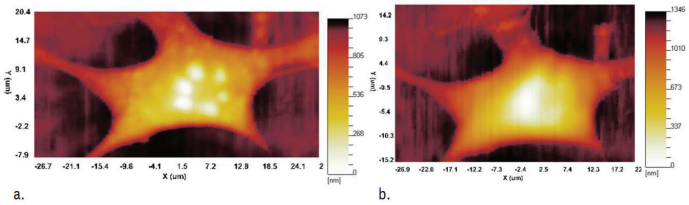


Figure 5. Scans of dry endothelial cells (a), and hydrated endothelial cells (b)

In addition to scanning, dynamic indentation tests to a depth of 300nm were conducted on the dry and hydrated cells. Using the scanned images, each cell was targeted to determine the elastic modulus of the nuclei. The averaged results of the tests performed on the dry and hydrated cells are displayed in Figure 6. Figure 6 shows elevated elastic properties at the surface of both the dry and hydrated cells, with a decreasing elastic modulus as the cells were penetrated to approximately 100nm. After 100nm of penetration, the elastic modulus of the dry and hydrated cells leveled off to an average value of 4.8GPa and 0.5GPa, respectively. The larger standard deviation in the results on the dry sample is most likely due to topography changes in the regions of the nuclei; the hydrated samples swelled like a balloon and provided less topography change over the indentation region.

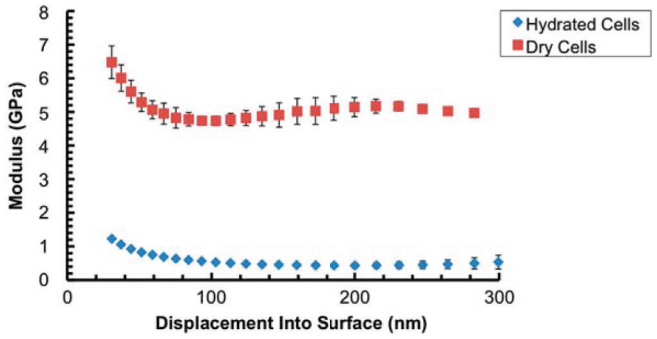


Figure 6. Nanoindentation results for the elastic modulus of the dry and hydrated fixed cells.

Conclusions

The Nano Indenter G200 system was used to scan and test dry and hydrated fixed mouse lung endothelial cells. Samples were mounted in fluid pucks which were first used to image and test the dry sample. Then, saline was used to hydrate the samples and the same cells were imaged and tested again under fluid. The scanned images of the dry and hydrated samples clearly showed cell membranes and nuclei, making it easy to identify locations acceptable for performing indentation tests. Results for the elastic modulus showed one order of magnitude difference between the dry and hydrated samples.

The new scanning method allowed the scanning forces to be automatically adjusted real-time so that the minimum scanning force could be used throughout the scan. This scanning method uses the data from the previous scan line to anticipate the minimum force that could be used to perform the following scan line. Hence, the areas of the images where little topography change were present were scanned using lighter loads than the areas containing the large topography changes associated with the nuclei of the cells.

References

1. W.C. Oliver and G.M. Pharr, An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments, J. Mater. Res., 7(6): 1564–1583 (1992).

2. G.M. Pharr, J.H. Strader, W.C. Oliver. Critical issues in making small-depth mechanical property measurements by nanoindentation with continuous stiffness measurement, J. Mater. Res. 24, 653 (2009).

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