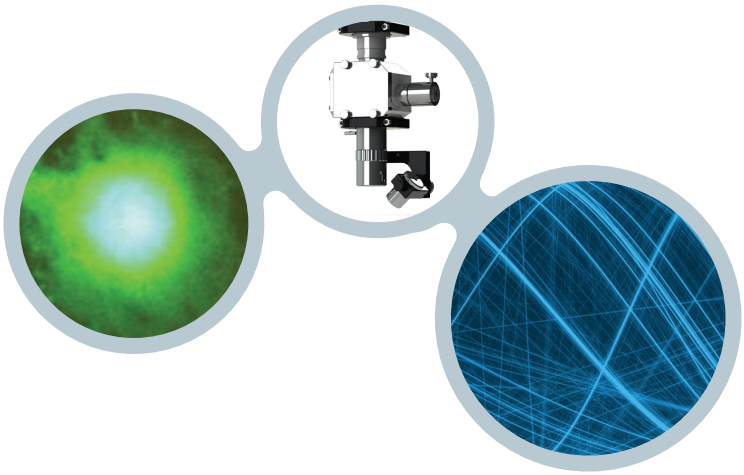

Application Note #1523

Compression Test of a Living Cell

Infection and disease frequently result in variations in the mechanical properties of living cells. Quantitative nanomechanical tests of cell membrane stiffness can help identify if and how a single cell was affected. The Fluorescence Microscope option for Bruker's Hysitron® TI Series TriboIndenter® enables clear visualization of green fluorescent protein (GFP) transfected cells kept in a phosphate buffered saline (PBS) solution. However, identification of initial contact between a flat end probe and a very soft cell membrane can be very challenging. Contact forces are very low even compared to PBS meniscus forces and membrane adhesive forces acting on the probe. Monitoring phase shift during oscillatory approach clearly identifies the contact point. The superior sensitivity and dynamic characteristics of Bruker's lightweight transducer technology enables the superposition of very small oscillatory forces at high frequency on the quasi-static force approach signal. Any discontinuity in phase shift signal indicates changes in contact conditions.

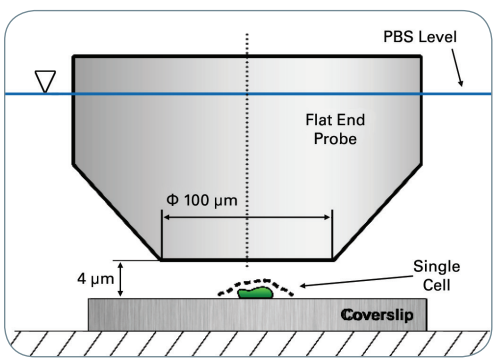

Figure 1. Schematic of a single cell compression test.

Procedure

A Hysitron TI Series TriboIndenter was used to perform compression tests on a single cell with a diamond fluid cell 100 µm flat end 90° conical probe. Cells were treated in a PBS solution to maintain natural testing conditions. The transducer's dynamic characteristics were calibrated for the whole displacement range while in contact with the PBS solution. Single cells were identified using an integrated fluorescence microscope and then positioned under the flat end probe, approximately 1-2 µm underneath the probe face (Figure 1).

Bruker's Hysitron nanoDMA® III technique enables the approach of an oscillating probe at very low frequencies. The lock-in amplifier monitors a phase shift of oscillatory force and displacement amplitude while the flat end probe is moving toward a cell membrane. Initially, the experiment is performed "out-of-contact" before contact is determined by changes in phase shift signal. Detection of initial contact is extremely important for precise data as the compressive force-displacement curve is offset from this value. The compression test was performed by force-controlled nanoDMA indentation starting out of contact.

Results

The compressive loading rate was 0.5 µN/s, while 0.3 µN of AC load amplitude was used at 200 Hz. To clearly differentiate between contact with a cell membrane versus contact with the glass coverslip, the phase shift signal was also independently monitored during an approach to the glass coverslip (Figure 3b). A phase shift of approximately -160° corresponds to the transducer's dynamic characteristic calibration in PBS. The red curve indicates contact with the coverslip by a phase shift increase from -160° up to -15°. The blue curve shows a phase shift decrease from -160° down to -250°, which refers to adhesive contact with the cell membrane. The phase shift increase of the blue curve at the end from -250° to -15° indicates contact with the coverslip after cell compression. The phase shift difference allows for exact determination of both the beginning and the end of the cell compression test and thus a calculation of the total deformation, which for this study was approximately 1500 nm (Figure 3b). Bursting of a cell can be traced by a force drop in load/displacement data (Figure 3a).

Conclusions

Identifying the initial contact of a probe and cell membrane was crucial for correct interpretation of load-displacement data. Superimposing a very small dynamic oscillation to a quasi-static force during the approach enabled precise determination of initial contact. Bruker's transducer technology with electrostatic force actuation provided superior force sensitivity and low noise floors, which allowed for precise operation in load regimes of just a few micronewtons. The directly integrated fluorescence microscope enabled precise targeting and positioning of a single cell. Load-displacement curves were used to determine the bursting force and membrane stiffness, which can be treated as a quantitative parameter for any comparative study.

References

1. Sepitka, J., Grznarova, P., Fuzik, T., and Lukeš, J., Computer Methods in Biomechanics and Biomedical Engineering (2014).

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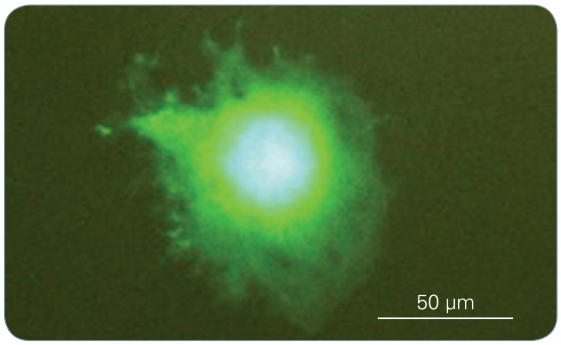

Figure 2. A cell derived from an African Green Monkey kidney transfected by GFP. Fluorescence image was taken at blue excitation and green emission.

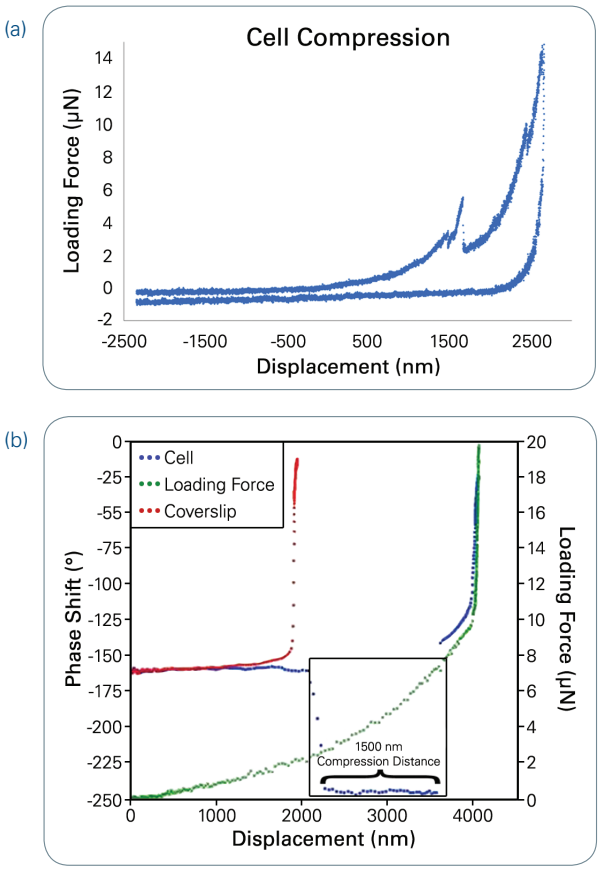

Figure 3. (a) Load-displacement curve of a single cell compression. Bursting of a cell membrane is clearly indicated by a force drop in the graph. (b) A detection of contact by nanoDMA mode. The red curve represents coverslip approach and the blue curve is the cell compression. Contact between the tip and the cell is indicated by a drop of phase shift. The last part of the blue curve (from 2250 nm) has a consentaneous trend as a last part of the red curve that confirms contact with the coverslip again. Steps in phase shift signal allow identification of the compression cycle, represented by the green curve.

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