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Probing the Nanomechanical Behavior of Cells and Cell Nuclei

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Abstract

Atomic force microscopy (AFM) is a nanoscale characterization technique that at its most basic level employs a nanometer-scale probe tip to physically trace a surface, generating a topographical map of the sample. However AFM has many applications beyond topography, including nanomechanical property analysis via cantilevered nanoindentation. In this project, tipless AFM probes functionalized with a 10 μm diameter glass bead have been used to measure the elastic modulus of live multipotent stromal stem cell nuclei before and after vibration treatments and/or structural component knockouts. The goal of these nanoindentation measurements of nuclear stiffness is to gain a better understanding of how mesenchymal stem cells respond to mechanical (in addition to chemical) signals in their environment to guide differentiation into osteoblasts, chondrocytes, or other cell types.

Probing the Nanomechanical Behavior of Cells and Cell Nuclei

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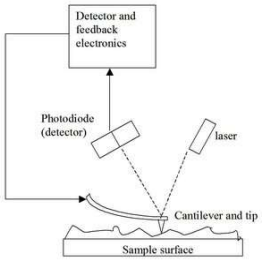
Atomic Force Microscopy (AFM)

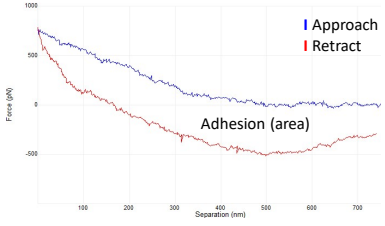
AFM is a scanning probe microscopy (SPM) technique used to generate high resolution topographical maps of a surface. Images are obtained by mechanically oscillating a cantilever with a sharp tip while scanning over a small (< 100 µm wide) area.



Bruker Dimension Icon FastScan AFM[1]

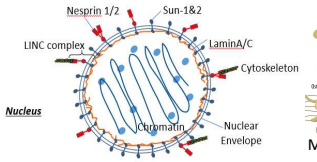
Nanomechanical Measurements

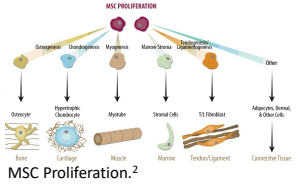
The cantilever acts as a spring. If its spring constant (applied force required per unit distance of deflection, expressed in N/m or nN/nm) and deflection sensitivity (relative photodiode signal as a function of deflection, in V/nm) are known, the cantilever's measured deflection can be converted into a force measurement at each point along the scan. Piezoelectric actuators can precisely control the displacement of the probe, which allows the acquisition of a force-displacement curve and subsequent mechanical properties analysis. The interaction between a spherical probe and the sample can be modeled with either Hertzian or JKR (Johnson, Kendell, and Roberts) dynamics. JKR is an extension of the Hertzian model that takes into account the effect of adhesive forces between the tip and sample during the retract curve.



Nanomechanical Properties of MSC Nuclei

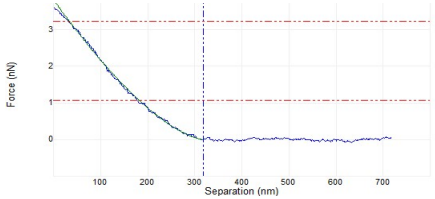
Mesenchymal stem cells (MSCs) are multipotent, capable of differentiating into bone, cartilage, muscle, or fat cells. A combination of mechanical and chemical signals guide this transformation; the goal of this study is to understand how mechanical signals affect MSCs.



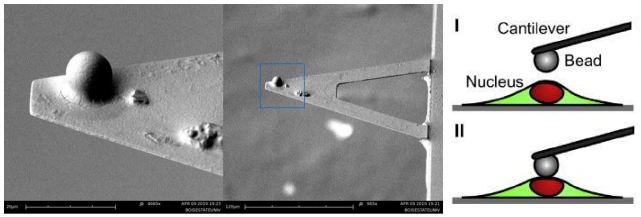


MSC Proliferation.[2]

One measurable response seen in MSCs is a change in the elastic or Young's modulus (i.e., the "stiffness") of the nucleus, which can be calculated from the slope of a force-displacement curve.



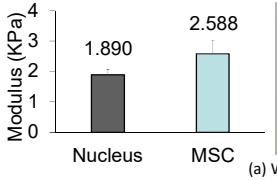
Rather than a sharp AFM tip, a 10 µm diameter glass bead is used to indent on nuclei because it has an ~5,000x larger radius of curvature. This disperses the applied force, preventing the probe from puncturing the cell membrane. It also allows a more holistic measurement of the nucleus's mechanical properties.

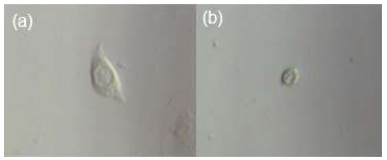


AFM cantilever with 10 µm diameter glass sphere attached.

Cells vs. Isolated Nuclei

Nanoindentation was performed on MSCs and isolated MSC nuclei. Noticeable structural differences were observed between whole cells and isolated nuclei, including a decrease in nuclear area.

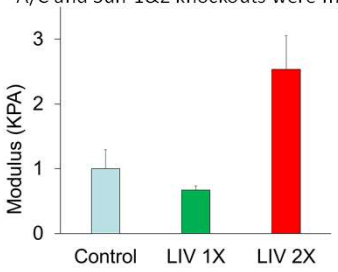


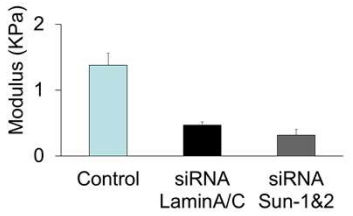


(a) Whole MSC vs (b) isolated nucleus at same magnification.

Low Intensity Vibration and Structural Knockout

- Low intensity vibration (LIV) was applied to unaltered MSCs before isolating and testing the nuclei. LIV was performed for 20 minute intervals with 1 hour rests in between. LIV 1X represents two intervals and LIV 2X represents four intervals.
- In a separate group, structural component knockouts were performed on MSCs before isolating and testing the nuclei. The effects of both Lamin A/C and Sun-1&2 knockouts were measured (separately).





Conclusions

- Removing the cytoskeleton causes the nucleus to shrink and significantly reduces nuclear stiffness.
- Extended LIV treatment introduces a stressful mechanical environment for the nucleus, triggering an increase in nuclear stiffness.
- Lamin A/C and Sun-1&2 provide structural support for the cell. When removed, nuclear stiffness decreases significantly. This suggests that these components also impart structure to the nucleus.

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1. Bruker.
2. Chan, Coucouvanis, Tousey, Andersen, and Jessie. "Improved Expansion of MSC Without Loss of Differentiation Potential" R&D Systems, Inc.

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