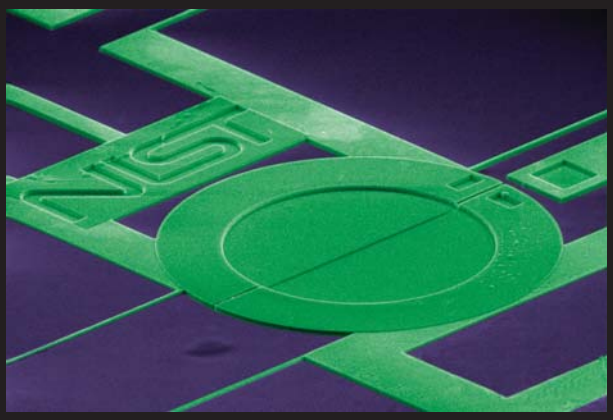
Single Cell Mechanics

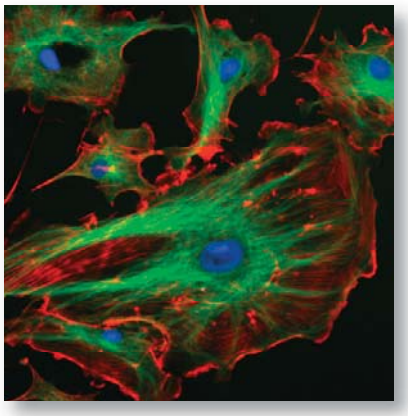
Objective

Our goal is to develop fundamental tools to measure the response of live cells to mechanical stimulation. The mechanisms by which cells convert mechanical forces into biochemical responses are increasingly recognized as playing a key role in tissue formation, disease progression, and disease treatment. We are applying physical measurement principles from conventional materials testing to provide biologists with a new micro-fabricated platform with which they can further evaluate the underlying mechanisms of cell mechanics.



Impact and Customers

- Cancer, heart disease, and cerebrovascular disease account for 57 % of all deaths in the U.S. Annual healthcare costs resulting from cancer and cardiovascular disease exceed $700 billion. Early diagnosis and advanced therapeutics are critical steps in reducing these costs.

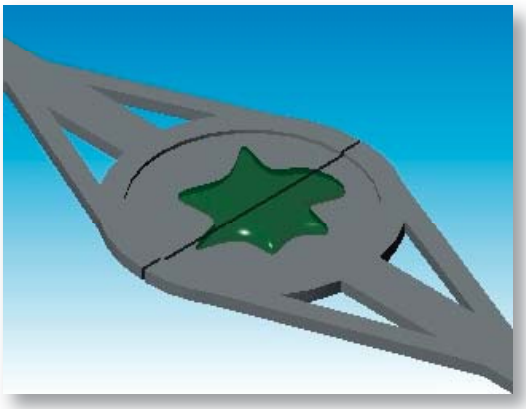


- Although the concept that cells are inherently adaptive is widely accepted, the molecular basis for this behavior is not well understood. Unlocking the mysteries of cell mechanics will accelerate the research cycle for new therapeutics by providing a more complete picture of human response at the individual cell level.

- Sectors benefiting from advanced understanding of cell mechanics include pharmaceuticals, regenerative medicine, cosmetics, chemicals, and homeland security.

Approach

We have developed a unique bio-MEMS device that stretches a single live adherent cell and measures its corresponding mechanical response. The resulting data provide an accurate, quantitative assessment of single cell elasticity. By coupling our platform with conventional bioimaging techniques and biomarkers, force-displacement data can be correlated with cell physiology to provide insight into the relationship between mechanical stress and disease.

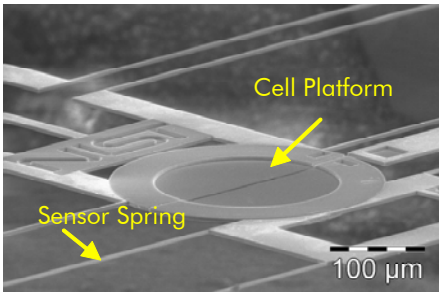


Our device mimics a conventional tensile test apparatus, consisting of a split circular platen with a pull rod attached to one end. The platen is coated with a protein, such as fibronectin, to promote cell adhesion, and the entire device is housed in a miniature bioreactor to provide a nutritive and temperature-controlled environment. A probe station externally controls displacement by hooking the pull rod, while force is measured via calibrated serpentine springs. Taken together, the data provide a complete representation of the force-displacement curve for a single cell.

BIOMATERIALS

Accomplishments

Our Bio-MEMS devices are fabricated using a custom in-house microfabrication process that consists of a single structural single-crystal silicon (SCS) layer combined with a silicon nitride structural layer over a silicon oxide sacrificial layer. The cell platform is an 800 nanometer silicon nitride membrane suspended by a SCS annulus. This provides a transparent substrate onto which the cell can adhere. A backside etch through the wafer allows an optical path through the entire cell platform. When released, the adherent cell on the silicon nitride platform can be directly imaged.

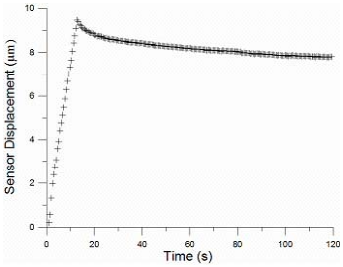


Cell stretcher apparatus

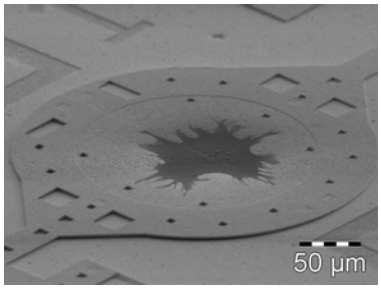
Rather than using an electrostatic comb-type actuator or a thermal actuator, our device employs a simple design with a ring that can be hooked by a probe-station tip for external displacement control. Displacements large enough to de-adhere the cell can be applied (> 25 % strain) or the cell can be cycled at lower strains to examine stress-strain response.

To demonstrate the functionality of the platform, we placed individual hamster fibroblast cells onto our bio-MEMS platforms. These cells are a type of connective cell typically associated with muscle and cartilage. Displacements were applied to stretch the cell at a rate of 40 nanometers/second to a maximum displacement of 90 micrometers. Through a series of such experiments, we were able to determine the de-adhesion force for these cells and also to quantify their viscoelastic response.

In addition to direct mechanical measurements, force-displacement data

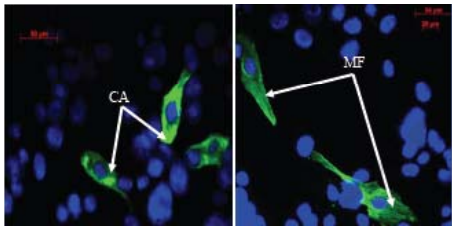


Viscoelastic behavior of fibroblast cell



Platen with adhered cell in center

can also be used to predict cell phenotype. Determining phenotypic differences between cell populations can be difficult, but is especially important when those differences are the result of disease. To demonstrate that our bio-MEMS device can distinguish phenotypes, we conducted experiments using fibroblast cells from a 10 to 20 % confluent culture and an 80 to 90 % confluent culture.



Actin staining of the different cultures

The 80 to 90 % confluent culture exhibited a linear force-displacement response, while the 10 to 20 % culture exhibited a knee in the response curve, indicating significant mechanical differences between the two cell populations. When stained for actin, the 10 to 20 % confluent cells showed loosely organized actin networks (CA), whereas the 80 to 90 % confluent cells showed a more organized network of stress fibers (MF), confirming the expected differences. These results show that by testing just a few cells, phenotype differences can be determined. Additional demonstration experiments are underway involving desmin (for cardiomyopathy) and hypoxic cells (for pulmonary hypertension).

Learn More

Andy Slifka
Nikki Goldstein
Tammy Oreskovic

David Serrell
(Materials Reliability Division)
303-497-4659
serrell@boulder.nist.gov
http://www.boulder.nist.gov/div853

Publications

Serrell DB, Oreskovic TL, Slifka AJ, Mahajan RL, Finch DS, *A uniaxial bioMEMS device for quantitative force-displacement measurements*, Biomed Microdev 9 (2): 267 (2007)

Panchawagh HV, Faheem FF, Herrmann CF, Serrell DB, Finch DS, Mahajan RL, *A flip–chip encapsulation method for packaging of MEMS actuators using surface micromachined polysilicon caps for bioMEMS applications*, Sens Act A: Phys 134 (1): 11 (2007)

Slifka AJ, Panchawagh H, Mahajan RL, Finch DS, Rorrer RA, Static and quasi-static calibration of a bio-MEMS device, Biomed Sci Instrum. 40:429 (2004)