Micro-Mechanical Properties of Endothelial Cells Measured With Optical Tweezers

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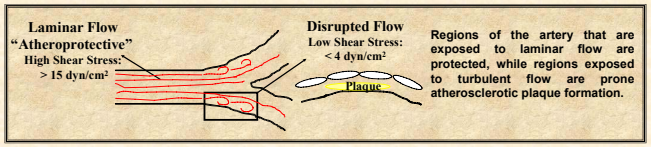
Introduction

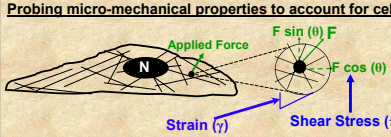
Characterizing the mechanical properties of cells is important for understanding many cellular processes, such as cell movement, shape, and growth, adaptation to changing environments, and even the development of disease. In this study, we explore the mechanical properties of endothelial cells that form the endothelial lining of blood vessels, whose dysfunction leads to the development of atherosclerosis, a cardiovascular disorder characterized by plaque formation in arteries and hardening of the arterial wall. Understanding the mechanical properties of endothelial cells will provide a better insight into the molecular basis of atherogenesis.



Regions of the artery that are exposed to laminar flow are protected, while regions exposed to turbulent flow are prone atherosclerotic plaque formation.

Like all cells, endothelial cells are viscoelastic, exhibiting both liquid-like (viscous) and solid-like (elastic) behaviors. Their intracellular space is spanned by a system of semi-flexible protein polymers known as the cytoskeleton. The cytoskeleton, which is a cell's mechanical framework is composed of actin filaments, microtubules, and intermediate filaments, and is responsible for most of the cell's mechanical functions that allow it to adapt to its local environment. The cytoskeletal network gives rise to an intracellular space that is inhomogeneous and dynamic, making the study of mechanical properties difficult in cells. We applied the optical tweezers technique, a microrheology approach, to measure mechanical properties of endothelial cells. This methodology allowed us to account for endothelial cell inhomogeneity by probing micro-mechanical properties, and their dynamics by collecting as many as 100 data points per second.

Probing micro-mechanical properties to account for cell inhomogeneity



Stress-Strain Relationship:

$\tau = G^*\gamma$, $G^*(\omega) = G'(\omega) + i\,G''(\omega)$

G' – measure of elasticity

G'' – measure of viscosity

In this interdisciplinary effort, our main research objectives were to use optical tweezers to (i) measure frequency-dependent micro-mechanical properties of endothelial cells to account for their inhomogeneous intracellular space (*figure 1*), (ii) detect endothelial cell dynamics by measuring viscoelastic changes over time (*figure 4*), and (iii) alter cytoskeletal composition by depolymerizing actin filaments with cytochalasin B, to study its effect on endothelial cell mechanics (*figure 3*).

Methods

1. Bovine Aortic Endothelial Cells (BAECs) were isolated and cultured

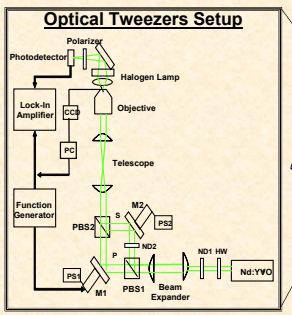
2. Treatments
   a. 0.9-um polystyrene beads introduced for use as probes for optical trapping
   b. Cytochalasin B introduced to alter cytoskeletal composition by depolymerizing actin filaments

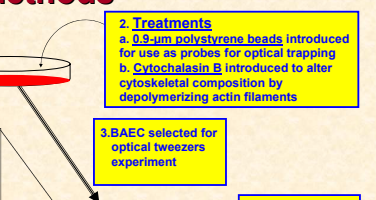
3. BAEC selected for optical tweezers experiment

4. Beads or intrinsic structures used as probes for optical trapping

5. OT Output:
   Displacement – D
   Phase Shift - δ

Optical Tweezers Setup





$$G'(\omega) = \frac{k_{ot}}{6\pi a}\left(\frac{A\cos\delta(\omega)}{D(\omega)} - 1\right)$$

$$G''(\omega) = \frac{k_{ot}}{6\pi a}\left(\frac{A\sin\delta(\omega)}{D(\omega)}\right)$$

Results

I. Frequency-dependent Viscoelastic Properties of Endothelial Cells

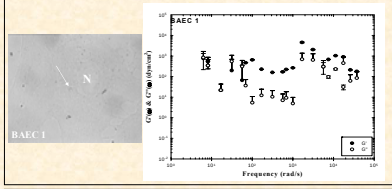


Figure 1: G' and G" measurements were made in BAEC 1 using an endocytosed polystyrene bead (arrow) as a probe for optical trapping. The frequency-dependent behaviors of the elastic (G': closed symbols) and viscous (G": open symbols) moduli were obtained over a frequency range of 0.1 Hz to 6,000 Hz.

Note: BAEC 1 exhibits no frequency dependence, with G' dominating G" throughout. Cell also exhibits fluctuating viscoelasticity.

II. Effect of nutrient-depletion on BAEC mechanics

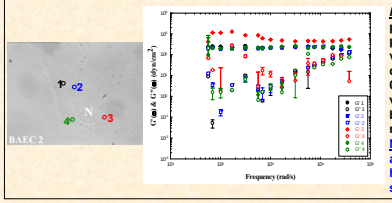


Figure 2: BAEC 2 had endocytosed 4 polystyrene beads (1-4), which were used to probe the elastic (G': closed symbols) and viscous (G": open symbols) moduli of the different regions within the same cell, over ω: 0.1-6,000 Hz. Viscoelastic behaviors in the vicinity of beads 1-4 are represented by black, blue, red, and green symbols, respectively.

Note: Fluctuating behavior seen in fig.1 goes away. G' in the vicinity of bead 3 (red) is higher than in the other 3, representing a stiffer nuclear region.

III. Disrupting cytoskeletal composition of BAECs

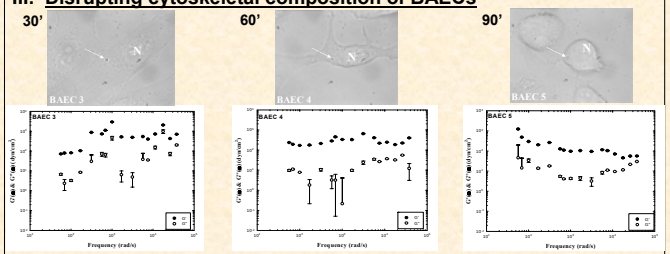


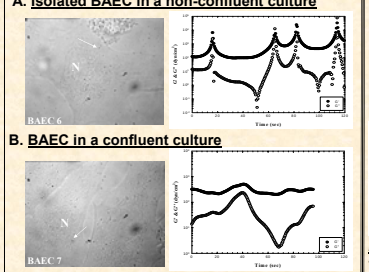
Figure 3: BAECs 3, 4, and 5 were treated with cytochalasin B for 30, 60, and 90 minutes, respectively, to depolymerize F-actin. Endocytosed beads (arrows) were used to obtain the frequency-dependent viscoelastic properties of the disrupted intracellular space of the BAECs.

Note: With increased exposure to drug, i.e. F-actin depolymerization, BAECs undergo more drastic morphological changes, and decrease in dynamics & elasticity (G'~700, 300, 200 dyn/cm², respectively).

IV. Time-dependent Viscoelastic Properties of Endothelial Cells

A. Isolated BAEC in a non-confluent culture

B. BAEC in a confluent culture



C. Autocorrelation Function $\langle G'G'(\tau)\rangle$

$$\langle G'G'(\tau)\rangle = \frac{\sum_{i=1}^{N-\frac{\tau}{\Delta t}} \frac{1}{\left(N-\frac{\tau}{\Delta t}\right)}\left[G'(i\Delta t)\,G'(i\Delta t+\tau)\right]}{\sum_{i=1}^{N}\frac{1}{N}\left[G'(i\Delta t)\right]}$$

i. $\langle G'G'(\tau)\rangle$ for BAEC 6 ii. $\langle G'G'(\tau)\rangle$ for BAEC 7

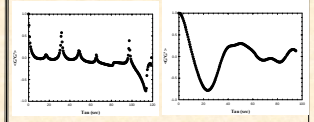


Figure 4 (A&B): Intrinsic granular structures with $n_{structure} > n_{Cytoplasm}$ (arrows) were used as probes for optical trapping. Time-dependent changes in G' and G" were tracked over 120 seconds in isolated BAEC 6 (A), and over 100 seconds in BAEC 7grown to confluence (B).

Note: BAEC 6 undergoes larger fluctuations and exhibits sharp increases in viscoelasticity not seen in BAEC 7.

Figure 4 (C): The autocorrelation function, $\langle G'G'(\tau)\rangle$, defined by the formula above, was computed for G' measurements obtained in BAEC 6 (i.) and BAEC 7(ii.) to determine if the fluctuating behaviors were random signals.

Correlation: G'G' >0; No correlation: G'G' ≤ 0.

Note: G' fluctuations are non-random events in both BAECs. BAEC 6 exhibits reoccurring autocorrelation but not BAEC 7.

Summary & Conclusions

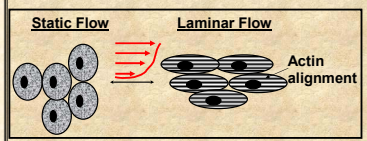
Optical Tweezers as a Tool to Study Micro-Mechanical Properties

- ✓ Intracellular **Inhomogeneity** – by measuring very local properties
- ✓ Cellular **Dynamics** – by collecting up to 100 data points per second

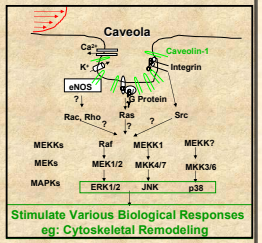
Mechanical Properties of Endothelial Cells

- ❖ **Different from non-biological complex fluid studies (eg. polymer gels, colloids)**
  - ❖ Frequency-dependent viscoelastic modulus (Maxwel Model)
  - ❖ No fluctuations in viscoelastic modulus
- ➢ **No strong frequency-dependence of either the elastic (G') or viscous (G") moduli (fig.1)**
  - ➢ **Dominantly elastic (G'>G"), with G"/G' < 0.1, over ω: 0.1 - 6,000 Hz**
  - ➢ **G' and G" undergo synchronous changes**
- ➢ **G' measurements reflect inhomogeneous intracellular space**
  - ➢ **G' measured in 10 BAECs fall within 2 values:**
    - ➢ G' ~ 300 dyn/cm² represented by BAEC 1 (fig. 1)
    - ➢ G' ~ 700 dyn/cm² (data not shown)
  - ➢ **4 regions were probed in nutrient-depleted BAEC 2**
    - ➢ G'~ 200 dyn/cm² in the vicinity of beads 1, 2, and 4
    - ➢G' ~ 600 dyn/cm² in the vicinity of bead 3
    - ❖ **Increase in elasticity corresponds to region closest to the nucleus**
- ➢ **Elastic and Viscous moduli reflect cellular dynamics**
  - ➢ **Fluctuations disappear in nutrient-depleted cells (fig. 2)**
    - ➢ Fluctuations were not instrumental artifacts
  - ➢ **Time-dependent studies showed large fluctuations in G' and G" (fig. 4)**
    - ➢ Fluctuations depend on cell density
      - ➢ **Isolated cells undergo larger fluctuations in viscoelasticity**
    - ➢ Fluctuations were non-random events
      - ❖ **Fluctuations = intracellular dynamics**
- ➢ **Actin cytoskeleton plays important role in EC mechanics (fig. 3)**
  - ➢ **↓ in cell elasticity and dynamics with ↑ in loss of actin filaments**

Future Directions



Endothelial cells also respond to changes in flow in culture. They have a mechanism of "sensing" differences in flow (mechanosensing), and adapt to changes by carrying out mechanotransduction.



Stimulate Various Biological Responses eg: Cytoskeletal Remodeling

We will look at:

1. Mechanical properties of endothelial cells under flow (10-20 dyn/cm² fluid shear stress)
2. Mechanical changes in the vicinity of caveolae, potential mechanosensors, as a response to flow
3. The role of MAPK signaling proteins in mechanotransduction and flow adaptation

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