MECHANICAL PROPERTIES OF VASCULAR ENDOTHELIAL CELLS EXPOSED TO STRETCH.

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Abstract

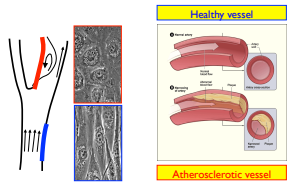
The pressure pulse present in arteries exposes vascular endothelial cells (VECs) to stretch. It is known that, under cyclic, uniaxial stretch, VECs align their actin filaments perpendicular to the direction of stretch. We study how this cytoskeletal remodeling is correlated to changes in subcellular microrheology.

We analyze the changes in the magnitude and directionality of the shear and elastic moduli of bovine aortic endothelial cells (BAECs) exposed to cyclical, uniaxial stretch using Directional Particle Tracking Microrheology (DPTM) that employs novel microrheology formulae that better account for the anisotropy of the cytoplasm.

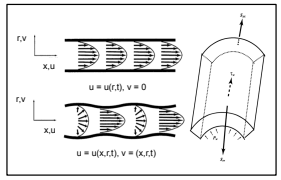
We find that, under cyclic, uniaxial stretch, BAECs stiffen and align their softest direction of mechanical polarization perpendicular to stretch.

Motivation

- **Atherosclerosis has a propensity for regions that experience biaxial stretch, low shear stress magnitude, high shear stress gradient and little net direction of flow.** In contrast, the straight regions of blood vessels, which are exposed to uniaxial stretch, high shear stress and directional flow, are spared from atherogenesis.



- **Pressure waves caused by pulsatile blood flow travels through the artery and dilates it – stretching the wall and exposing VECs to uniaxial stretch.**



Zamir, Mair. **The Physics of Pulsatile Flow**. Pgs. 114-120.

Aims

Aim 1: To improve non-invasive techniques to measure directional microrheological properties of the cytoplasm in living cells.

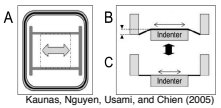
Aim 2: Monitor the changes of magnitude and directionality of cytoplasmic microrheology in VECs subjected to stretch.

Cell Culture / Microscopy

- Bovine aortic endothelial cells (BAECs, Cell Applications Inc.) are cultured in growth media composed of DMEM (Invitrogen) supplemented with fetal bovine serum (Omega Scientific), Pen/Strep (Invitrogen), L-glutamine (Invitrogen), and sodium pyruvate (Invitrogen, Carlsbad, CA).
- BAECs are maintained at 37 C with 5% CO2 / 95% air incubator until confluent.
- Videos were acquired at a rate of 5-7 frames/s for 3 minutes at 1-hour intervals.
- Images were captured using Simple PCI (Hamamatsu Corp.) using an Olympus IX70 microscope (Olympus America Inc. SEG) and a UPlanSApo 60X/1.20 water immersion lens (Olympus America Inc. SEG).

Live Cell Stretch Chamber

- BAECs seeded on a 127 μm thick silicone membrane, coated with 0.002 mg/ml fibronectin (Sigma) in DPBS (Invirtrogen), for 18-24 hours.
- The silicone membrane is affixed to a 10×10 cm plastic stretch chamber (UC San Diego Machine Shop) using a rubber gasket. 25 ml of growth media containing 1mM Hepes (Invitrogen) is added to the chamber.

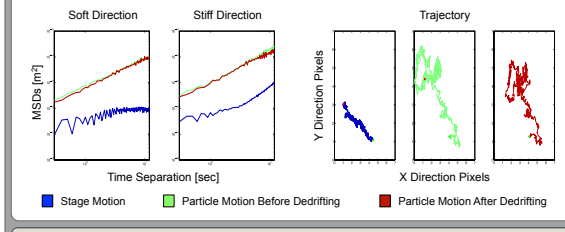


Kaunas, Nguyen, Usami, and Chien (2005)

- The stretch chamber complex is assembled onto a movable plate above a stationary I-shaped plastic indenter.
- Uniaxial stretch is created by moving the plate up and down at a frequency of 1 Hz to mimic a human pulse.
- The system is maintained at 37 C using a heater and humidified with 5%-CO_2 / 95%-air.

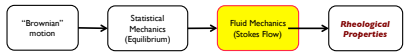
Removing Stage Drift

- Stage-motion detection algorithm optimizes correlation between two subsequent images.
- As an initial guess for the translation coordinates, we apply phase correlation.



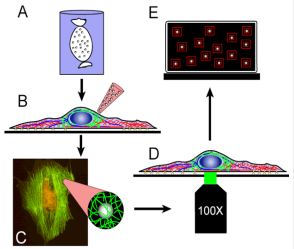
Particle Tracking Microrheology of Anisotropic Media

Generalized Stokes-Einstein Relation (GSER)



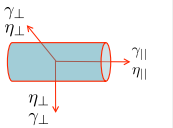
- Mechanical properties of the cell cytoplasm are calculated from the Mean Squared Displacements (MSD) of mitochondria undergoing Brownian motion, using the Generalized Stokes-Einstein Relation (GSER).
- GSER - relates Einstein's equation for diffusion of a particle in thermal equilibrium, and Stoke's drag.

$$\vec{F} = \xi \vec{V}$$



(Mason and Weitz, 1996, Wirtz *et al* 2002)

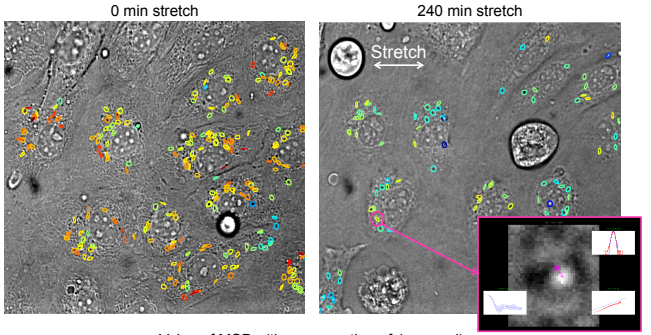
- Stokes equation assumes the liquid is isotropic – which does not apply for cell cytoplasm.
- **To improve Stokes contribution to the GSER, we model the cytoplasm as an orthotropic fluid.**
- The response functions for the orthotropic fluid are:

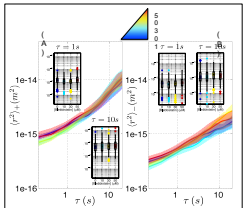


$$\xi_\perp = \frac{4\pi a \eta_\perp}{1 + \frac{1}{2}\frac{\eta_{||}}{\eta_\perp - \eta_{||}}\left[1 - \frac{\eta_\perp}{\eta_{||}}\frac{\tan^{-1}\sqrt{(\eta_\perp - \eta_{||})/\eta_{||}}}{\sqrt{(\eta_\perp - \eta_{||})/\eta_{||}}}\right]}$$

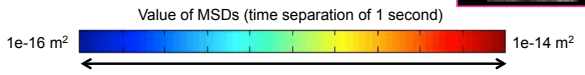
$$\xi_{||} = \frac{4\pi a \eta_{||}}{1 - \frac{\eta_\perp}{\eta_\perp - \eta_{||}}\left[1 - \frac{\tan^{-1}\sqrt{(\eta_\perp - \eta_{||})/\eta_{||}}}{\sqrt{(\eta_\perp - \eta_{||})/\eta_{||}}}\right]}$$

Results





We compared results to cells treated with Blebbistatin, a drug known to inhibit Myo II activity and saw no difference – supporting our assumption that the measured MSDs are a result of thermal fluctuations and not a result of Myo II activity.



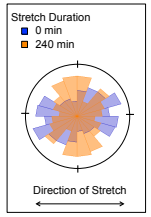


Figure 3

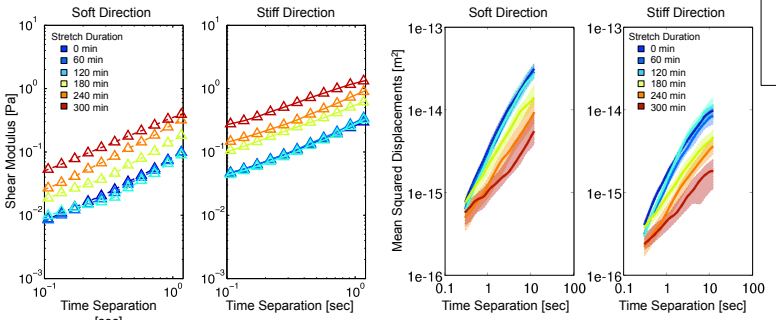


Figure 1

Figure 2

- **At time separations less than 10 seconds, the value of the MSDs were on average two to three times larger along the soft direction than along the stiff one (Figure 2). The ratios of the MSDs along each direction give an indication of the degree of anisotropy. The level of anisotropy of the MSDs did not change significantly after stretch.**
- **The values of the MSDs decreased significantly after stretch (Figure 2). The value of the shear modulus, calculated using the slope of the MSDs, increased accordingly (Figure 1). The logarithmic slopes of the MSDs with respect to time separation also varied over the course of the experiment. These slopes reveal how close the viscoelastic behavior of the cytoplasm is to being liquid-like (unity slope) or elastic-like (zero slope). The slopes remained fairly constant for the first two hours of stretch with values close to 1, and decreased approximately 10% after each subsequent hour of stretch. This decrease in slope indicates that VECs become less liquid and more elastic under stretch.**
- **Figure 3 shows the distribution of the orientation of maximum compliance for a time separation of 10 seconds. The cells aligned their softest direction perpendicular to stretch after 240 min.**

Conclusion

- ***Under stretch, we have shown that VECs become more elastic-like, stiffen and align their softest direction of mechanical polarization perpendicular to the direction of stretch. This response likely acts to minimize intracellular strain.***
- ***Both shear and stretch align the softest direction of mechanical polarization parallel to the direction of blood flow in vivo. The VEC's response to stretch likely acts to minimize intracellular strain in response to stress. The orientation of the anisotropic mechanical properties perpendicular to stretch provides the cell with a physical mechanism to relate stress and strain differently along the two directions. This reorganization may play an essential role in mechanotransduction.***