Effect of Cholesterol on Plasma Membrane Biomechanics of HEK293 Cells

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Introduction
This study deals with the effects of cholesterol on human embryonic kidney (HEK293) cells’ plasma membrane mechanics using an optical tweezers setup.

Cholesterol regulates trans-membrane protein movement and plasma membrane-to-cytoskeleton attachment mechanics. Testing the effect of differing cholesterol concentrations on the biomechanics of HEK cells is to use understanding of the role cholesterol plays.

Using the optical tweezers setup (Fig. 1), we extract nanotubes (tethers) from the plasma membrane under cholesterol-enriched and cholesterol-depleted conditions to determine the tether force.

We are assuming that a second-order Maxwellian spring = dash-pot model (Fig. 2) of viscoelastic holds for the mechanics of these tethers.

Due to this, we also study the tether force with a degree of time resolution so as to better understand how the viscoelastic properties change. Thus, we study the force after a relaxation period of one minute and two minutes.

Materials and Methods
Static Force Calibration
The trapping force was calibrated by passing DIMEM Complete (Dulbecco’s Modified Eagle Medium with FBS and Penicillin/Strep) Serum-enriched through a trapped bead using a piezoelectric stage at various velocities (in µm/s) and measuring the velocities when the bead is displaced from the trap. From these, we use a modified version of Stokes’ Law to calculate the Trapping Force constant (Fig. 3).

Static Tether Force Measurement:
Using the Optical Tweezers setup, we brought a single cell in contact with a trapped bead and held the two together for a short time. After this time, the cell was moved away (at 1 µm/s) to pull a tether of length 10µm, 15 µm, or 20 µm. At this time, during the time-resolution = no delay, one minute, or two minutes delay), the laser power was lowered until the bead jumps out of the trap and returns rapidly to the cell (indicating a tether had formed). The final steady current was recorded to calculate the Tether Force (pN).

Cholesterol Manipulation:
HEK 293 cells are incubated in cholesterol-enriched and depleted media with the following concentrations: 3 mM, and 5 mM. Cholesterol is depleted using M-CD (Methyl-Beta-Cyclodextrin), and cholesterol-enrichment is done using water-soluble cholesterol from Sigma-Aldrich®. The prepared media are vortexted for 3-4 minutes, followed by incubation for 30 minutes (37°C at 5% CO2) before experimentation.

Results
The static calibration results are presented first, to show the result of our Stokes’ Law calculation.

The above graph shows that the tether forces increase as the cholesterol is depleted. This is a very significant result, and can be backed up with statistical t-tests that show that the means are statistically different.

There seems to be a clear positive, and linear trend of forces for the concentrations studied – no delay. The order of tether forces is:

Cholesterol-Depleted > Untreated > Cholesterol-Enriched.

Also, the higher the concentration, the stronger the effect in either direction. This becomes more evident in Fig. 5.

Conclusion and Future Work
We have determined that depletion cholesterol through the use of M-CD-Cyclodextrin causes the tether force to increase significantly over the untreated value, while enriching the plasma membrane with cholesterol causes a decrease in the tether force. With both cholesterol-enrichment and -depletion, a greater increase or decrease, respectively, is visible as the tether length is increased from 10 µm to 20 µm.

We have also determined that the presence of a time-resolution study inherently highlights the viscous component of the second-order Maxwellian spring – dash-pot model. With no delay, the elastic regime more accurately portrays the peak tether forces. As the viscous regime takes over, the tether forces are much lower, and are much less correlated. This means that the viscous component of force is more dominant over time.

In the future, we will be doing similar pulling velocities must be tested to further examine the time-resolution effects, as measurements must be taken into account. Finally, a quantification scheme for the amount of cholesterol present in the membrane must be devised.

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References


For further information
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www.engineer.berkeley.edu/sharadgupta.html