Public Abstract
First Name:Mingzhai
Middle Name:
Last Name:Sun
Adviser's First Name:Gabor
Adviser's Last Name:Forgacs
Co-Adviser's First Name:
Co-Adviser's Last Name:
Graduation Term:SP 2008
Department:Physics
Degree:PhD
Title:Cell mechanics studied using Atomic Force Microscopy

Cholesterol plays an indispensable role in regulating the properties of the cell membrane. In particular, lipid rafts, specific membrane domains, which are thought to be required for a number of cell functions, such as receptor-mediated signaling and membrane trafficking, are dispersed when cell cholesterol is extracted. There is also evidence showing that cholesterol affects the cell's deformability, an important factor in the development of atherosclerosis. In this study, we investigated the effect of cellular cholesterol on the mechanical properties of bovine aortic endothelial cells (BAECs) and their correlation with the development of atherosclerosis.

To compare the mechanical properties of cells with different cholesterol content, we have developed a method to measure the forces needed to extract nanotubes (tethers) from their membranes, using atomic force microscopy (AFM). Our observations show that cholesterol depletion of BAECs resulted in significant increase of membrane-cytoskeleton adhesion. An increase in cellular cholesterol to a level higher than that in normal cells caused decrease of the membrane-cytoskeleton adhesion and dramatic decrease of the effective surface viscosity of their membranes. While cholesterol depletion and enrichment had no apparent effect on the intensity of F-actin specific fluorescence, disrupting F-actin with latrunculin A abrogated the observed effects. Fluorescence recovery after photo-bleaching experiments were also performed to measure the lateral mobility of a lipid probe (DiIC12 ) at different cholesterol contents. The results are consistent with the AFM measurement.

To investigate the molecular bases of the phenomena, we focussed on the regulatory phospholipid, phosphatidylinositol 4,5-biophosphate (PIP2), which is involved in a variety of cell functions, especially the regulation of cytoskeleon, and membrane-cytoskeleton adhesion. In the plasma membrane, PIP2 accumulates in cholesterol-rich domains, and its concentration decreases upon cholesterol depletion. By culturing BAECs with neomycin or by transfecting them to express the GFP-tagged PH domain from phospholipase C-delta, we sequester PIP2 to mimic the effect induced by cholesterol depletion. Interestingly, PIP2 sequestering by either approach decreases cell membrane deformability as cholesterol depletion does. This result suggests that cholesterol depletion affects cell mechanical properties by altering the concentration/distribution of PIP2, which may further change the cortical F-actin network. Furthermore, our studies demonstrate that AFM can be used to relate and correlate biomolecular and biophysical properties.