Lehigh University
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Detecting Mechanosensing with Biophotonics

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Abstract

Endothelial cells (ECs) form the inner lining of the blood vasculature and are exposed to shear stress (τ), the tangential component of hemodynamic forces. ECs transduce τ into biochemical processes possibly via EC-membrane perturbations. We present evidence for membrane-phase specificity of lipoid dyes (DiIs) in confluent endothelial cells using confocal molecular spectroscopy. Our measurements of fluorescence lifetime (FL) and polarization (FP) of DiI's in BAEC cell membranes are consistent with the partitioning of DiI C_{12} (a carbocyanine dye with dual 12-carbon alkyl chains) in a liquid phase of the plasma membrane (FL~0.6 ns, FP~ 0.06), and of DiI C_{18} (a carbocyanine dye with dual 18-carbon alkyl chains) in the liquid-ordered or gel phase (FL ~ 1.0 ns, FP ~ 0.3). We exposed DiI C_{18}-labled endothelial cells to shear stress (10 dynes/cm2) and measured fluorescence lifetime and polarization. Results from time-dependent changes in the fluorescence lifetime of DiI suggest that gel-phase domains are perturbed by a fluid shear stress of 10 dynes/cm^2 and that there exists a spatial distribution of this perturbation. Such results have important implications for understanding the mechanical-biological interface of endothelial cells.

Materials and Methods

Bovine aortic endothelial cells (BAECs) were grown to confluence on glass, stained with DiI C_{12} (a liquid-phase-preferring dye) or DiI C_{18} (a liquid-ordered-phase-preferring dye) and imaged in 0.5 :m x,y increments using time-domain lifetime and anisotropy.

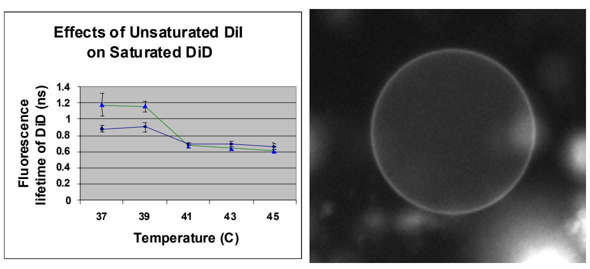
A picosecond pulsed laser was scanned across the surface of the cell, and time-correlated single photon counting (TCSPC) was used at each 0.5 :m increment to collect photon numbers and arrival times relative to the laser pulse time. These arrival times were assembled in a histogram for each 0.5 :m x 0.5 :m pixel, and curvefit to a single exponential decay with a Marquart-Levenburg least squares regression algorithm. Images were assembled with pixel values corresponding to position and intensity, lifetime, or polarization degree.

DiI C_{18}-Fluorescence lifetime as an indicator of membrane phase

50-picosecond length laser pulses excite the DiI in labeled cells grown in a temperature controlled flow chamber. Red-shifted photons emitted from the sample are transduced into electrical pulses by single photon counting photomultiplier tubes (PMTs). The time between laser pulse and emitted photons is determined by the time-correlated single photon counting PCI card in the computer. Many pulses and many emitted photons contribute to the formation of a histogram which is fitted with a negative exponential whose time constant is the fluorescence lifetime. Fluorescence polarization is calculated as the relative fluorescence intensity in PMTs sensing light which is polarized parallel and perpendicular to the excitation light polarization.

DiI C_{18} or DiD (red shifted version of DiI) is used to label giant unilamellar DPPC vesicles (fig 1) or cells (fig 3). Figure 1 demonstrates that fluorescence lifetime of DiD can be used to detect the transition from gel phase to liquid phase which occurs at 40°C. Saturated DiI disrupts the gel phase (lower curve). Figure 3 contains histograms of FP and FL. A 2-D histogram reveals increased mobility of DiI C_{12} versus DiI C_{18}.

Fig. 1



Experimental setup: Confocal Laser-Scanning Spectroscopy

Laser light is scanned across the sample using a 2-axis piezo-driven mirror. Emitted fluorescence is de-scanned and directed through a dichroic mirror and a confocal aperture to eliminate out-of focus light. A polarizing beam splitter selects light oriented parallel and perpendicular to the excitation light polarization. A multi-channel router is used to direct electrical pulses from each PMT to the correct memory bank on the time-correlated single photon counting card in a PC. (Fig. 2)

Fig. 2

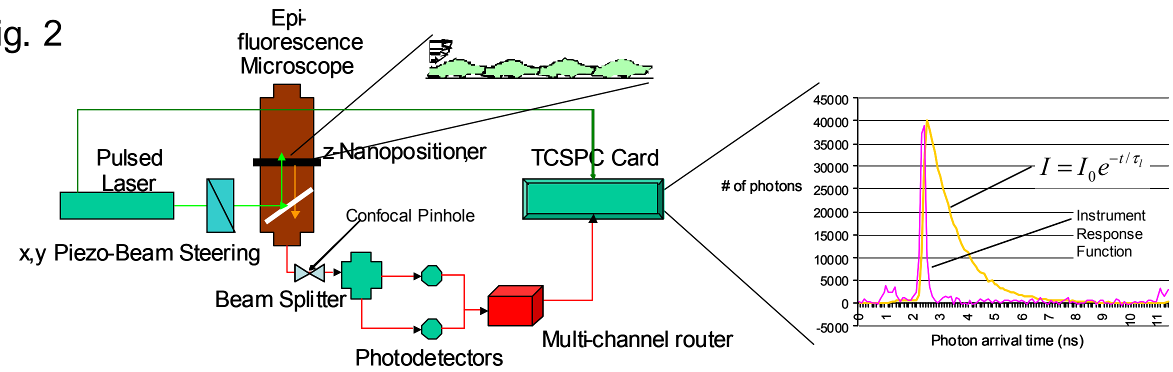
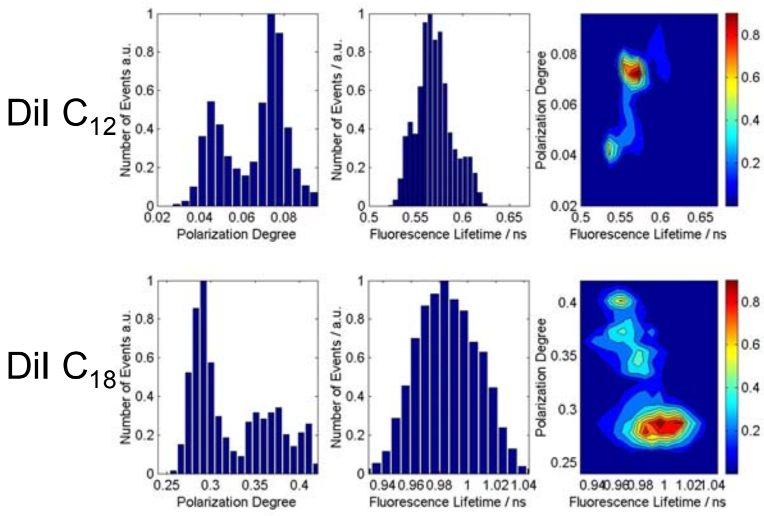


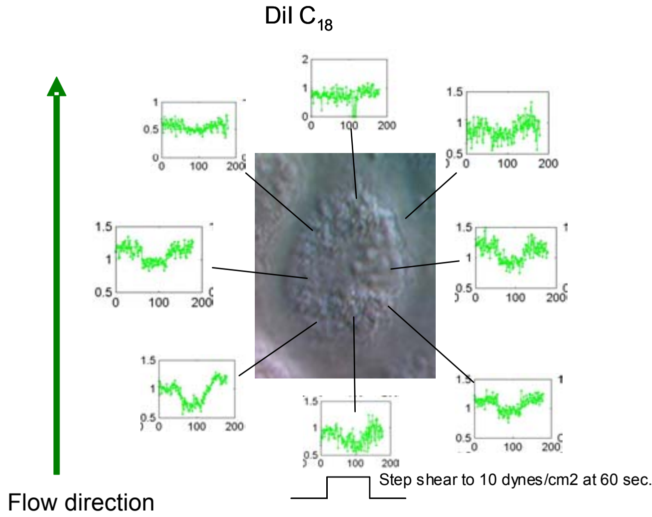
Fig. 3



Shear stress induces reduction of FL of DiI C_{18}: Evidence for shear-induced membrane gel-phase perturbation

Shear stress was stepped from 0 to 10 dynes/cm^2 at t=60 seconds and maintained at 10 dynes/cm^2 for one minute at which time it was stepped back to 0 dynes/cm^2. Fluorescence lifetime was monitored at various positions on the cell membrane. Figure 4 is an illustration of 8 points on the cell measured at 1.25-second intervals.

Fig. 4



Summary

Fluorescence spectroscopic tools along with membrane-phase specific lipoid dyes allow the rapid detection of membrane phases on the EC membrane and the quantitative measurements of changes in shear-induced lipid dynamics which have been correlated to changes in biochemical processes in ECs. Biophotonics allows high spatial and temporal resolution investigations of molecular processes fundamental to cellular health.

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