

## **Investigating the correlation between membrane mechanics and morphology of adhered cells on polymeric surfaces**

P. Sidney Sit, Stephanie R. Tully  
Biomedical Engineering and Institute for Micromanufacturing  
Louisiana Tech University, Ruston, LA 71270

Responses of adhered cells is a primary factor that determines the performance of implanted polymeric biomaterials. Micromechanical properties of the cell membrane, mediated partly by cytoskeletal components, and cell morphology are two parameters that reflect the interactions between the cell and the underlying substratum. Membrane stiffness ( $E$ ) of cells adhered to different polymeric surfaces, as measured by Atomic Force Microscopy, appears to be dependent on the extent of surface hydrophilicity. This also affects the extent of cell spreading, indicated by the spreading area ( $A$ ). In this study, the relationship of  $E$  and  $A$  on different polymeric surfaces with known physiochemical properties is examined.

Rat lung fibroblasts (RLF) proliferated to confluence and were deposited on polymers spin-coated on glass coverslips. Polymers used are as follows: (i) poly(DTR glutarate)s ( $R$ =ethyl and dodecyl) and (ii) poly(DTE-co- $x\%$ PEG<sub>1000</sub> carbonate)s ( $x=0,2,4,8\&10$ ) (DTR: Desaminotyrosyl-tyrosine alkyl ester; PEG: polyethylene glycol). In poly(DTR glutarate)s, shorter alkyl ester pendent chain (ethyl) results in a more hydrophilic surface than long one (dodecyl). Additionally, increasing the PEG content in poly(DTE-co- $x\%$ PEG<sub>1000</sub> carbonate)s increases the surface hydrophilicity. Tissue culture polystyrene (TCPS) was used as a reference surface. A Nanoscope III Bioscope AFM (Veeco Metrology) equipped with a G-scanner and operated in fluid force-volume mode was used to image individual cells. Silicon nitride cantilevers (nominal spring constant  $\sim 0.32$  and  $\sim 0.06$  N/m) were used as probes to indent the membrane of RLF adhered to different polymeric surfaces. The membrane stiffness is obtained by best-fitting the plot of applied force against the indentation depth. Cell morphology was analyzed by Image-Probe Plus (Media Cybernetics), in which the cell boundary was outlined and the corresponding spreading area was calculated using a built-in algorithm.

The measurement of  $E$  is obtained on different locations of the cell membrane, each of which represents an area of about  $80 \times 80$  nm<sup>2</sup>. Statistical analysis (100 locations within the cell interior) is performed to evaluate the effect of the polymeric substratum on cell membrane stiffness. The results suggest that pendent chain induced changes in surface hydrophilicity does not appear to be a major factor as  $E$  is similar on the two poly(DTR glutarate)s and other relatively hydrophobic surfaces (e.g., TCPS). Note that a high  $E$  corresponds to a stiff cell membrane. Additionally, most cells display a high degree of spreading and hence have a high value of  $A$ . However, changes in surface hydrophilicity as a result of PEG addition elicit a substantial decrease in  $E$ . Attached cells also show a decreased cell spreading, indicated by a stretched morphology and a decreased  $A$ . Assuming a cell has a finite length of cytoskeletal filaments for structural integrity, the increased tension within the filament due to spreading on hydrophobic surfaces results in a higher value of  $E$ . However, the extent of increased  $E$  and  $A$  is markedly attenuated on hydrophilic surfaces.