Frictional Property Measurement of Individual Vascular Smooth Muscle Cells

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Statement of Purpose: The main goal of the current study was the development of an AFM-based technique for micro-scale measurement of individual cell surface frictional properties, as well as elucidation of the cellular physical constituents responsible for governing frictional behavior. Vascular smooth muscle cells were chosen for this research due to the clinical relevance of their frictional properties with respect to endovascular surgical procedures. While significant steps have been made towards understanding the bulk mechanical properties of living cells, comparatively little is known about their surface frictional properties. The study of cellular frictional properties is of interest for a variety of reasons. In regard to endovascular surgical procedures, the deployment of endovascular devices results in the exertion of mechanical shear forces on underlying vascular endothelial and vascular smooth muscle cells (VSMCs). The AFM tip has been shown to be a model for nanoasperities of metallic implants [1], such as endovascular stents, in contact with SMCs once deployed in the vascular lumen.

Methods: Rat aortic smooth muscle cells (P4-P6) were seeded onto collagen-coated coverslips at a density of 150,000 cells/coverslip. Cells were maintained in DMEM with 10% FBS prior to and during AFM experiments. AFM lateral force microscopy experiments were performed on individual cells using a 5 μm borosilicate spherical probe on a 0.12 N/m cantilever. The lateral deflection sensitivity of the cantilever was calibrated using the modified wedge method [1, 2].The AFM (Dimension 3000 Veeco Metrology, Santa Barbara, CA) was operated in scan mode at a 90° scan angle, with a reciprocating cutoff length of 10 μm, and a speed of 20 μm/s. Normal force was incrementally increased up to 100 nN for each cell that was tested. Some cells were treated with 5 μM cytochalasin D (Sigma, St. Louis, MO) for 30 minutes prior to experimentation, while others were fixed with 3% glutaraldehyde (Sigma) overnight. Controls were untreated. Following AFM experiments, data were processed using custom MATLAB scripts, and frictional coefficients (μ) were calculated using a linear fit of the equation, $F_L = \mu \cdot F_N$, where F_L is lateral force and F_N is normal force.

Results: For the AFM cantilever used in these experiments, the lateral sensitivity, α, was found to be approximately 68 nN/V. In total, 16 data points were collected at each given normal force, for each individual cell. One-way ANOVA comparison of friction coefficients indicated a statistically significant difference between groups, $p<0.001$. Subsequent pairwise comparisons indicated significantly greater friction coefficients for glutaraldehyde treated VSMCs ($\mu=0.21 \pm 0.04$) vs. controls ($\mu=0.06 \pm 0.02$), $p = 7.8 \times 10^{-6}$, and significantly lower coefficients for cytochalasin D treated VSMCs ($\mu = 0.01 \pm 0.01$) vs. controls, $p = 3.4 \times 10^{-6}$.

Conclusions: The principal goal of the current study was to investigate the development of an AFM-based method for measurement of cell surface frictional properties on the micro-scale, with a specific interest in vascular smooth muscle cells. To the best of the authors' knowledge, the current data represent the first reporting of cellular surface frictional coefficients obtained via AFM. The mean coefficient of friction for untreated VSMCs found in the current study (0.06 ± 0.02) is similar to macroscale values previously reported for endothelial cells ($\mu = 0.03–0.06$) [3] and corneal epithelial cells ($\mu = 0.05\pm 0.02$) [4]. Each of these studies was carried out in media with 10% serum, meaning there was undoubtedly significant protein adhesion to the probe. Such protein adhesion however, is precisely what one would expect from almost any implant material once it comes in contact with blood. No detachment or noticeable displacement of cells was observed in any of the VSMC sample groups during friction testing, indicating that the cells were firmly adhered to the substrate. The exception to this was the observation that cytochalasin D treated VSMCs underwent a noticeable bulk reciprocating motion in phase with probe motion. Through microscopic observation, cells did not appear to be physically damaged as a result of AFM probe contact. The observation that glutaraldehdye (a crosslinking agent) and cytochalasin D (an actin depolymerizing agent) caused significant increases and decreases in frictional coefficients, respectively, serves as evidence that bulk cellular modulus does correlate positively with frictional coefficient, as has been predicted for whole tissue [5]**.** Measurement of cellular surface frictional properties via AFM appears to be a viable, albeit complex technique. In the current study, frictional coefficients of untreated VSMCs were found to be approximately 0.06. Frictional coefficients were increased by cellular crosslinking, and decreased by cytoskeletal depolymerization. Further study using this technique is needed to determine the precise mechanisms underlying cellular lubrication. Additionally, the AFM-based cellular friction technique provides the ability to modify the AFM probes with any number of different surface chemistries, either based on charge or hydrophilicity to evaluate the effect of implant surface treatment on cell frictional properties.

References:

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