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AFM tip. Here, we present a combined approach to answer the question of how much of the high force tail can be attributed to either cause. We found that the presence of multiple attachments, while significant, accounts for only a fraction of the events in the high force tail of the distribution.

846-Pos Board B626

Nanomechanical Mapping of Eye Tissue

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Mechanical properties are key to bioactivity, whether in artificial or natural tissue. Variations in mechanical properties can also be an indicator of chemical content and tissue structure, with implications for material function. Here we present results from nanoscale mechanical mapping of several tissues in the eye, with possible correlations to preliminary tip-enhanced Raman (TERS) data. Our results demonstrate the importance and utility of mechanical measurements, particularly mechanical mapping, in understanding natural tissue, with an eye toward early mechanical indicators of disease.

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Probing of PNA-DNA Hybrid Duplex Stability with AFM Force Spectroscopy

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Peptide nucleic acids (PNA) are synthetic polymers the neutral peptide backbone of which provides elevated stability to PNA-PNA and hybrid PNA-DNA duplex. It was demonstrated recently (J.Org.Chem.2011, 76, 5614-5627) that additional modification of the backbone such as incorporation of diethylene glycol (miniPEG) further increases the duplex thermal stability, so the hybrid duplex with 10 miniPEG units (γ-PNA) has the melting temperature 23°C higher compared to the regular PNA-DNA duplex. Here we applied AFM force spectroscopy to probe the strength of the γ-PNA-DNA duplex (5′GAGTAG GTAG-3') containing ten miniPEG-modified units. Single-stranded PNA and DNA oligonucleotides containing terminal thiol groups were immobilized on amino-functionalized AFM tip and mica substrate, respectively via bifunctional PEG tethers and the interaction between the DNA and γ-PNA polymers was analyzed by multiple approach retraction cycles over various locations on the mica substrate. Such single molecule probing experiments produced force curves with well-defined rupture events corresponding to the dissociation of the duplex formed during the approach step. The experiments were performed at various pulling rates (300-3000 nm/sec) enabled us to characterize the hybrid duplex stability using dynamic force spectroscopy (DFS) approach. The DFS measurements yielded rupture forces varying in the range 60-70 pN. The data analyzed in the framework of the Bell-Evans approach yielded a dissociation constant, $k_{off} \sim 10^{-9}$ sec^{-1} and rupture distance $x_{-1} \sim 1.7$ nm. Similar published DFS measurements of DNA duplex are characterized by k_{off} with $\sim 10^8$ times less values, which is in line with elevated stabilities of the γ-PNA-DNA duplexes compared with the DNA duplexes.

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Investigation of Fibrin Fiber Internal Structure

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The major structural component of a blood clot is a meshwork of about 150 nm thick fibrin fibers. It is well understood how fibrin monomers assemble into the double-stranded, half-staggered protofibrils. However, how these protofibrils laterally assemble to form mature fibers is poorly understood. There is evidence that fibrin fibers are very porous with a protein content of only 20-30%. We performed two types of experiments to investigate the internal structure of fibrin fibers. We formed fibrin fiber from fluorescently labeled fibrinogen and determined the light intensity of a fiber, which is proportional to the number of monomers, as a function of fiber diameter. We found that the intensity, I, scaled as $I \sim D^{1.43 \pm 0.2}$ (wet fibers) and as $I \sim D^{1.2 \pm 0.14}$ (dry fibers). This implies that cross-sectional monomer density also scales as $D^{1.4}$, and not as D^2, as would be expected for fiber with a solid, homogeneous cross-section. We also determined the Young's modulus, E, as a function of fiber diameter, and found that E scales as $E \sim D^{-1.4}$. Thus, E decreases dramatically with diameter. These modulus data suggest that the number of bonds per cross-section scales as $D^{0.6}$, consistent with a fiber model that has a dense core and a very loosely connected periphery.

In summary, our data suggest that fibrin fibers have a very inhomogeneous cross-section with a dense core and a very loose periphery.

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Using Force Mapping to Obtain Data on Live Bacteria in Fluid

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Atomic force microscopy has been previously used to image various biofilm-forming bacteria live in fluid, followed by performing force curves on selected areas to learn about the biophysical properties of the cells. However, some bacteria do not adhere sufficiently strongly to a surface to be analyzed in this fashion. Here we have used force mapping to obtain force curves on live cells that would otherwise be difficult or impossible to collect. Predatory B. bacteriovorus cells, which are highly motile and form no biofilms, can be loosely trapped on polylysine-coated slides. Force mapping is sufficiently gentle to obtain data on these cells without dislodging them from the slide. Comparison of these predatory B. bacteriovorus cells with those of their ''host-independent,'' non-predatory counterparts indicates that predatory cells are much less stiff, presumably enabling them to squeeze into prey cells' periplasm. Moreover, host-independent B. bacteriovorus cells demonstrate considerable adhesion to AFM tips, whereas predatory B. bacteriovorus cells show little or no interaction with AFM tips. The molecular basis for these interactions is being further explored.

850-Pos Board B630

Afm Monitoring of Elasticity Changes Accompanying Differentiation Towards Neural Cells

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Mesenchymal stem cells (Msc) differentiate typically to chondrocytes. In our lab we have created neuronal-like cells from Msc. In this presentation results of atomic force microscopy (AFM) measurements of mechanical properties such as Young modulus or friction during this differentiation will be reported. Differentiations leads to noticeable changes in mechanical properties, in particular elastic maps are modified. Effects of pyrethroids, widely used neuroactive substances, on the AFM images will be also discussed. Biophysical characterization of cell differentiation process should help to optimize control and manipulation on stem cells. This knowledge is important for tissue engineering and regenerative medicine.

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Titin is a Spatially Homogenous Linear Expander

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Titin, a giant filamentous intrasarcomeric protein, is a serial chain of more than 300 globular (Ig or FN) domains and numerous unique sequences. Although force-dependent unfolding has been extensively investigated in recombinant homopolymeric constructs of titin domains, neither the global kinetics, nor the spatial pattern of mechanically-driven domain unfolding is known within the complexity of the full-length molecule. To follow the global kinetics of domain unfolding, we stretched individual titin molecules isolated from rabbit m. longissimus dorsi using high time- and force-resolution optical tweezers in force- and velocity-clamp modes. When clamped at high forces, the molecule extended in discrete steps via unfolding of its constituent globular domains. In an apparent violation of mechanically-driven activation kinetics, however, neither the global domain unfolding rate, nor the folded-state lifetime distributions of titin were sensitive to force. The contradiction can be reconciled by assuming a gradient of mechanical stability so that domains are gradually selected for unfolding as the magnitude of force increases. To explore whether there is a spatial pattern in this gradient of domain unfolding, we carried out a topographical screening of individual titin molecules stretched to varying degrees with receding meniscus. We found that unfolded domains were distributed homogenously along the entire length of the overstretched titin molecule. The spatially randomized domain stability ensures that titin is a quasi Hookean expander across a wide range of stretch and loading rates, thereby