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Behavioral Thresholds for Force-Sensation Determined by an Integrated Video-Tracking and Force-Clamp System

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Neurosensory mechanotransduction, the conversion of a force stimulus to an electrical signal, is the fundamental process governing touch sensation. Understanding how the touch receptor neurons (TRNs) of Caenorhabditis elegans mediate this conversion can unravel how touch works. The classical method is to score its response to mechanical stimuli applied with an eyebrow hair or a micro-von Frey hair. However, both methods lack repeatability, precision and resolution. In particular, the sensitivity and resolution possible with micro-von Frey hairs is at least an order of magnitude larger than the threshold for activation of force-gated currents in TRNs in vivo. To overcome these issues, we developed a force clamp system capable of applying fixed loads with high force resolution (sub-nN), a fast response (1 kHz), and a large dynamic range (50 dB) to enable the quantitative evaluation of C. elegans touch. We developed a piezoresistive micro-cantilever force probe and integrated it with a piezoelectric actuator and a programmable real-time controller. We implemented a computer vision-based x-y tracking system in parallel with the force-clamp system, which allows the desired force to be applied precisely at a selected location on a moving C. elegans. We first measured the threshold for behavioral responses to touch is between 0.1 and 1 μN. We have confirmed that mutations affecting MEC-4, a subunit of the force-gated ion channel expressed in TRNs disrupt responses to stimuli applied to the animal's body, but not its nose. The effect of additional mutations that selectively disrupt distinct classes of mechanosensory neurons and body mechanics will be presented. This system is a powerful tool for determining force sensitivity in wild-type and mutant nematodes and provides a new method to understand how factors like body mechanics affect touch sensitivity in this tiny nematode.

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Analysis of Gold Nanoparticles Effect on RSV Using AFM

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Respiratory Syncytial Virus (RSV) is one of the most common viral causes of upper and lower respiratory tract infections in infants and result in pneumonia. In the present study, we used AFM to observe Gold nanoparticles effect on the RSV virus particles. The results indicated that RSV particles were almost round in shape with a polymorphous distribution via off-line particle analysis of AFM, most of the RSV particles had a diameter of approximate 135nm. When we mixed RSV with gold nanoparticles, we observed that gold nanoparticles bound around RSV and RSV start shrinking in size. Furthermore, *in vitro* infection of RSV alone and RSV with Gold nanoparticles were performed in Hep-2 cells. Our studies showed that when we incubated RSV with the gold nanoparticles for 30min, RSV size reduced only 5% but when we increased the incubation time to 1h, RSV size reduced 25 %, and increased the incubation time to 2 h, RSV size reduced 71%.Moreover, we observed that the nuclear envelope has deteriorated in RSV infected cells but some nuclear structure is still visible when these cells were incubated with the virus for 24 hours. On the other hand, uninfected cells have very well defined nuclear structures and the nuclear membrane is easily distinguishable from the interior organelles. In addition, the membranes appear fuller and more uniform. The nuclear envelope has also deteriorated in RSV+gold nanoparticle that prepared by incubated only 30min, infected cells for 24h. Since RSV size reduction is only 5% when virus incubated with the gold nanoparticles for 30min, virus still can damage the nuclear envelope. However, when we infected the cells with 1h and/or 2 h incubated RSV+Gold nanoparticle mixture, the damage on the nuclear envelope is reduced and cells have well defined nuclear structure.

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Tip-Free Method Used to Locate Single Purple Membrane Patches for Atomic Force Microscopy

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In atomic force microscopy (AFM), it can be difficult and time-consuming to locate sparsely distributed regions of interest. Typically, the tip is scanned until the desired object is located. This process can damage a fragile biological sample, particularly at high scan rates. Scanning over large areas can also degrade the tip from mechanical wear. Moreover, proteins from the surface may adhere to the tip, degrading its imaging capabilities and/or its chemical specificity (e.g., gold-coated tips binding to cysteine-containing proteins). We have previously developed an ultrastable AFM in which the tip and the sample positions are independently measured by, and stabilized with respect to, a pair of laser foci. This 3D stabilization is based upon back-scattered light from lasers passing through a glass substrate. Motivated by previous work locating single viruses using back-scattered light, we speculated that our tip-stabilization laser could detect patches of single lipid bilayers. Purple membrane - bacteriorhodopsin embedded in a lipid bilayer - was adsorbed onto a glass cover slip. We scanned the sample through the laser focus with the AFM tip retracted a few microns above the surface and the electronic gain turned up to detect the sub-10-nm height of a single bilayer. The high signal-to-noise ratio of the resulting images, enabled in part by intensity stabilization of the detection laser, allowed us to locate single patches within a 30 x 30 μm^2 region. After optical detection, the AFM tip was lowered back down and used to validate patch detection. Thus, this rapid technique minimizes unnecessary tip-sample interaction during AFM study of biological samples, which could be especially useful in force-spectroscopy experiments that require a specific attachment between the tip and the sample.

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The Mechanical Properties of Individual Crosslinked and Uncrosslinked Fibrin Fibers

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Blood clots have the mechanical task of stemming blood flow; yet despite decades of work our understanding of the mechanical properties of blood clots is still poor. In fact, currently there is no good mechanical model of a blood clot available. One of the key missing elements in constructing a model of a blood clot is knowledge of the mechanical properties of the clots constituents, fibrin fibers.

We have used a combined atomic force/fluorescence microscopy technique to determine numerous mechanical properties of single fibrin fibers both crosslinked by clotting factor XIIIa and uncrosslinked. In this technique, fibers are suspended across grooves and the atomic force microscope is used to stretch the fibers and record force data. Meanwhile, the fluorescence microscope records images of the stretching process.

We have determined uncrosslinked fibers are very soft, with a modulus of 3.9 MPa, but increase in modulus (stiffness) at ~100% strain by a factor of 3. They also have a sigmoid shape to their energy loss, at low strain fibers display little energy loss while at high strains 70% of the energy used to stretch the fiber is lost. In comparison, crosslinked fibers were stiffer, with a modulus of 8.0 MPa, less extensible, deformed at strains as low as 10%, strain hardened by a factor of 1.9 and showed large energy loss beginning at low strains. The viscoelastic properties of fibrin fibers were determined and it was shown crosslinking has the effect of decreasing extensibility and increasing modulus and dissipated energy.

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The Contribution of Body Wall Muscles to C. Elegans Body Mechanics Determined Using Piezoresistive Microcantilevers

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Body mechanics in the nematode C. elegans are central to both mechanosensation and locomotion. Previous work revealed that the mechanics of the outer shell, which is composed of both the animal's skin (cuticle) and attached muscles, rather than internal hydrostatic pressure, dominates stiffness [S.-J. Park et al., PNAS (2007), 104:17376]. This finding suggested that the striated body wall muscles could contribute significantly to the mechanics of this tiny invertebrate. To test this idea, we utilized pharmacological and genetic tools to modulate muscle contraction and assayed stiffness using a piezoresistive microcantilever displacement clamp system. Relaxing the muscles with 2-3-butanedione monoxime (BDM) decreased stiffness. Levamisole, a nicotinic acetylcholine receptor agonist known to paralyze C. elegans, induced hypercontraction, but did not significantly alter measured stiffness. We infer from these results that resting muscle tone contributes to body stiffness. A uniform increase in muscle tone, by contrast, has no detectable effect on stiffness. Since levamisole-induced hypercontraction is associated with significant changes in body geometry, it is possible that the internal hydrostatic pressure is decreasing and offsetting contraction-induced stiffness increases. Preliminary studies of mutants defective in muscle contraction follow an analogous trend. These results provide new insight into the role of the body wall muscles in C. elegans body mechanics and are a step towards developing better models of the mechanics of both locomotion and mechanotransduction.