

# 2041-Pos Board B11 Viscosity Effect On The AFM Force Measurement

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Atomic force microscopy based techniques have been used in the investigation of protein folding/unfolding in order to study the protein properties at the single molecule level. These experiments are performed in buffer solution, where the AFM cantilever is used to exert and measure the mechanical forces on the protein molecules under study. Due to the motion of the cantilever relative to the liquid, a viscous drag force, which can be significant when the liquid is viscous or the cantilever's speed is high, acts on the cantilever. The irregular shape of the liquid chamber and cantilever, and the special boundary conditions of the fluid make this problem complicated and difficult to solve analytically. We measured the viscous drag forces on different cantilevers for several different cantilever speeds in solutions of different viscosities. The results show that the viscous drag on a cantilever is determined by its geometry, its relative speed, the viscosity of the solution, and the separation between the cantilever and the sample surface. This method will be useful to make corrections to the unfolding force data of proteins as well as that of AFM based force measurements.

# 2042-Pos Board B12

## AFM Visualization and Force Spectroscopy of Clathrin Triskelia

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We have applied atomic force microscopy (AFM) and single molecule force spectroscopy (SMFS) to characterize the structure and molecular mechanics of clathrin triskelia. The latter are the basic building blocks of the protein coat surrounding plasma-membrane-derived vesicles involved in receptor-mediated endocytosis. Here we resolve variable profiles of individual triskelia on mica surfaces for the first time by AFM, at a resolution comparable to that of electron microscopy. Classical three-leg, filamentous pin-wheel shapes, as well as dimers and non-planar triskelion conformations, are readily observed. Time sequences of AFM images clearly demonstrate conformation fluctuations in single triskelia when the latter are in physiological solutions. Additionally, AFM SMFS reveals, also for the first time, a series of internal energetic barriers that characterize triskelion heavy chain domain folding, corresponding to numerous alpha-helix hairpins of ca. 30 amino acid residues, and variable unfolding of larger, cooperative hairpin domains up to the size of the known repeating motif of ca. 145 amino acid residues. The dynamic domain rupture force ranges from a few 10s pN to over 500 pN, increasing continuously as the stretching loading rate changes over the range from 100 to 10<sup>5</sup> pN/s. The results once again show AFM to be a powerful tool for biomedical imaging and nanometric single molecule characterizations.

# 2043-Pos Board B13

## Force Spectroscopy Of The Interaction Of Fibrinogen With Erythrocytes And Platelets

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Increased values of erythrocyte aggregation are associated with an augmented risk of cardiovascular and cerebrovascular conditions. The prevailing hypothesis for the mechanism of erythrocyte hyperaggregation is due to an increase in plasma adhesion proteins, particularly fibrinogen. Fibrinogen is a blood-borne glycoprotein comprised of three pairs of distinguishable polypeptide chains with three potential integrin-binding sites, but can also interact with cells through non-integrin receptors. Currently, fibrinogen-induced erythrocyte aggregation is thought to be caused by non-specific protein binding to erythrocyte membranes. In contrast, platelets are known to have integrin receptors expressed on the membrane surface (the  $\alpha_{IIb}\beta_3$  glycoprotein complex). In the present work we evaluated the possible existence of a binding between human fibrinogen molecules and an unknown receptor on human erythrocytes. For the sake of comparison, an equivalent study was conducted with human platelets. The interactions were studied under physiological conditions by force spectroscopy, using an atomic force microscope (AFM) with fibrinogen-functionalized tips. Blood cells were deposited on poly-L-lysine treated glass slides. Single molecules were stretched by tip pulling after pressing the cantilevers on the samples. AFM images were carried out in tapping mode and force spectroscopy measurements in contact mode. The interaction forces between a fibrinogen-modified AFM probe and platelet/erythrocyte surface were determined from

pN to nN levels. The main conclusion from our results is that there is a single molecule interaction between fibrinogen and an unknown receptor on erythrocyte membrane, with a lower affinity when compared with platelet binding. The existence of a membrane receptor involved on the fibrinogen-induced erythrocyte aggregation suggests the possibility of a drug therapy that could result in a significant decrease of erythrocyte aggregation in patients with different pathologies (e.g., hypertension and diabetes).

# 2044-Pos Board B14

## Force Transduction In Smooth Muscle Cells

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Mechanical forces directly affect the form and function of tissues. Transmission of force from outside the cell through focal and junctional adhesions controls the maturation or disassembly of these adhesion sites and initiates intracellular signaling cascades that alter cellular behavior. To understand the mechanism by which living cells sense mechanical forces, and how they respond and adapt to their environment, a critical first step is to develop a new technology able to investigate cellular behavior at sub-cellular level that integrates an atomic force microscope (AFM) with total internal reflection fluorescence (TIRF) microscopy and fast-spinning disk (FSD) confocal microscopy, providing high spatial and temporal resolution. The integrated system is broadly applicable across a wide range of molecular dynamic studies in any adherent live cells, allowing direct optical imaging of cell responses to mechanical stimulation in real-time.

Thus, we are able to: (i) image with high spatial resolution or stimulate the apical cell surface using AFM, and (ii) quantitatively time-lapse image the cell-overslip interface using TIRF, or FSD confocal image to study molecular dynamics and protein translocation between different sub-cellular structures. Significant rearrangement of the actin filaments and focal adhesions was shown due to local mechanical smooth muscle cell stimulation at the apical cell membrane that induced changes into the cellular structure throughout the cell body. By exploring innovative approaches like those used in these investigations, new information for understanding live cell restructuring and dynamics in response to mechanical force can be provided. Understanding how live cells adapt to mechanical force and how they are able to recognize and respond to mechanical stimuli represents an important biophysical problem.

# 2045-Pos Board B15

## Using Atomic Force Microscopy to Measure Mechanical Strength of Nanometre Scale Protein Fibrils

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Mechanical properties are routinely measured on both biological and man-made fibres on the macro- and micro-scale to give valuable information on properties such as elasticity (Young's modulus), shear and rupture force. Here, methods will be described to progress such mechanical measurements to samples on the nanometer size scale, using atomic force microscopy (AFM). This technique is used to first localise and visualise a single fibril on a patterned surface with regularly repeating plateaus and troughs; controlled motion of the tip then is used to push down on a fibril hanging over a trough or pull it sideways while recording the force exerted on the fibril as it is being stretched. Performing such measurements over a range of forces allows the determination of the elastic Young's modulus as well as observation of non-elastic (irreversible) extension and ultimately the rupture force of protein fibrils with diameters down to a few nanometers.

Results will be presented for a range of protein fibrils. In particular, the properties of collagen fibrils with diameters on the order of 5 nm will be compared to those obtained for thicker collagen fibres.

# 2046-Pos Board B16

## Mechanics of Spontaneously Beating Cardiac Myocytes Investigated by AFM

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Cardiac myocytes *in vitro* contract spontaneously as they are cultured under appropriate conditions. In order to clarify the contractile mechanism of cardiac myocytes, it is basically important to investigate the mechanical properties of cardiac myocytes. The atomic force microscope (AFM) is now a powerful tool for measuring the mechanics and the dynamics of cells at a single cell level. Previous studies revealed that the AFM allowed us to measure the amplitude and frequency of cells at their several positions, however the dynamic behavior has not been investigated as a function of cell position. In this study, we measured the spatial-dependent contractility of spontaneously beating

cardiac myocytes with AFM. The contractility of cardiac myocytes was measured by using a contact-mode operation, in which the deflection of cantilever with a colloidal probe that contacted the cell surface was kept a constant value with an electronic feedback loop. A force mapping technique was employed to measure the spatial-dependent of the contractility of spontaneously beating cardiac myocytes. We succeeded to measure the amplitude and the frequency of spontaneously beating cardiac myocytes with the AFM technique, and it was observed that the dynamics of the cells was kept in a steady state under appropriate conditions. Interestingly, the cells distended in a region around the center of cells while they exhibited a contraction in a peripheral region of cells. Such a spatial-dependent contractility was observed as the beating was externally perturbed with chemicals. This work is partially supported by the GCOE Program from MEXT of Japan.

#### 2047-Pos Board B17

##### Mechanical Activity At Focal Adhesion Sites

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We investigated whether mechanical force applied to extracellular matrix proteins (ECM)-integrin focal adhesion sites would induce mechanical activity characteristic of specific ECM type. We used atomic force microscopy (AFM) to apply forces to ECM adhesion sites on vascular smooth muscle cells (VSMC) isolated from resistance arterioles. The tip of the AFM probes were fused with a borosilicate bead (5  $\mu\text{m}$  diameter) coated with fibronectin (FN), collagen type-I (CNI), collagen type-IV (CNIV), laminin (LN) or vitronectin (VN). ECM-coated beads induced clustering of  $\alpha 5$  and  $\beta 3$  integrins and actin filaments at sites of bead-cell contact indicative of focal adhesion formation. Step increases of an upward (z-axis) pulling force (800–1600 pN) applied to the bead-cell contact site for FN specific focal adhesions induced a force-generating response from the cells resulting in a downward pull by the cell. Depolymerization of the actin cytoskeleton with cytochalasin D blocked whilst stabilization of the actin cytoskeleton with jasplakinolide enhanced this micromechanical event. Myosin light chain kinase inhibition (ML7) and an inhibitor of cSrc tyrosine kinase (PP2) also blocked the response. Furthermore, inhibitory antibodies to  $\alpha 5$  and  $\beta 3$  integrins blocked the micromechanical cell event in a concentration-dependent manner. Similar experiments with CNI, CNIV, VN, or LN failed to induce micromechanical events. Our results demonstrate that mechanical force applied through FN at single focal adhesion sites induces a micromechanical event that is actin, myosin light chain kinase and tyrosine kinase dependent. Importantly, the data illustrate that there are different mechanical characteristics for focal adhesions formed by different ECM proteins. FN appears of particular relevance in its ability to induce a force-generating reaction from sites of focal adhesion in VSMC in response to applied forces.

#### 2048-Pos Board B18

##### Cells and Gels: A Comparison of Indentation Behavior

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Understanding and modeling the mechanical behavior of biological systems requires high quality experimental data to be acquired and analyzed. Conventional indentation tests assume homogeneous linear elastic material properties, and therefore are of limited use in characterizing inhomogeneous biological tissues that generally exhibit complex nonlinear elastic response. Despite their shortcomings, the Hertzian models commonly applied in the analysis of the indentation of soft materials are accurate for determining the quasi-static elastic properties of many synthetic gels when applied within their restrictions, including small strain deformation. The lack of practicable, nonlinear elastic contact mechanics models has compelled the application of the Hertz theory to the indentation of biological soft matter, including cells, often with erratic results. We evaluated the accuracy and limitations of the Hertz equation of spherical contact as it pertains to the AFM indentation of chemically crosslinked poly(vinyl alcohol) gels and murine chondrocytes. We then derived and validated via numerical and experimental methods, nonlinear elastic contact equations based on different hyperelastic strain energy functions. The data were reanalyzed using the new models. The results revealed that the linear elastic limit of the cells is dramatically smaller than that of the synthetic gels. All hyperelastic models considered capably described the mechanical response of the gels while only the Fung and Ogden phenomenological models proved suitable for the chondrocytes, which exhibited pronounced strain stiffening even at small deformations. We propose the use of these mathematically simple alternatives to the Hertz theory for modeling the indentation of intrinsically nonlinear soft materials, including live cells and soft tissues, at strains exceeding the Hertzian regime.

#### 2049-Pos Board B19

##### Physical Properties of Native Biofilm Cells Explored by Atomic Force Microscopy

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Biofilms are complex microbial communities that grow at interfaces. Bacteria in biofilms are phenotypically different than their planktonic (free swimming) relatives; they adapt to the communal, sessile lifestyle by optimizing their motility, adhesion, and metabolism. We used Atomic Force Microscopy (AFM) to directly probe the physical properties of native bacterial cells in simple biofilm communities and demonstrated that widely dissimilar biofilm-colonizing cells all have a high cellular spring constant, indicating that they are quite stiff. However, the lab strain *E. coli* ML35 that does not form robust biofilms is much less stiff, hinting that stiff bacteria may preferentially colonize surfaces in the early stages of biofilm formation. Adhesive forces between the retracting AFM tip and bacterial cells vary between cell types in terms of the force components, the distance components, and the number of adhesion events, reflecting differences in associated extracellular polymeric substances (EPS), pili, and flagella. Because biofilms are dynamic, robust, and challenging to control or destroy, potential removal agents are of great interest in industrial, medical, and agricultural settings. We also explored the changes that occur in *E. coli* biofilm cells as they are devoured by *Bdellovibrio bacteriovorus*, a bacterial predator of other bacteria. Invaded prey cells, called bdelloplasts, undergo substantial chemical and physical changes that we probed directly with the AFM tip. Bdelloplasts are significantly shorter than uninvaded *E. coli* biofilm cells, and prey cells clearly lose elasticity after invasion by *B. bacteriovorus* predators. On average, the spring constant of uninvaded *E. coli* cells was three times stiffer than that of bdelloplasts. The retraction portions of the force curves indicate that bdelloplasts adhere to the AFM tip with larger pull-off forces as compared to uninvaded *E. coli*. Thus, AFM provides provocative information about native biofilms.

#### 2050-Pos Board B20

##### Viscoelastic Indentation of Extremely Soft Biological Samples

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Though tissue mechanics serve an important biological function, as in embryonic organ development, a paucity of experimental methods exists to measure these interactions. We used an atomic force microscope (AFM) to measure local mechanical properties of unfixed cryosections of mouse embryonic tooth, which served as a model for organ morphogenesis. AFM is commonly used for tissue indentation; however, most of these studies analyze adult tissues that are mechanically more robust than embryonic tissues, and thus existing experimental protocols do not address the technological limitations of AFM indentation into extremely soft materials (<10 Pa). Importantly, in the range of small applied forces (100 pN), environmental noise, reaction time and drift of the AFM cantilever can apply small, but significant, forces to the sample that affect analyses. Therefore, these artifacts should be incorporated into the indentation load history for viscoelastic analysis. To measure the viscoelastic creep function of embryonic tooth tissue, we performed load-prescribed indentations with an AFM that produced time-dependent load-indentation curves. Our analyses revealed dramatic deviations (>50%) between the software-prescribed and actual applied loads that arise from tissue deformation and the artifacts just mentioned. Since there is no closed-form solution to the integral of the noisy load history, analysis of the indentation data is more complicated. To address this, we took a numerical approach, which produced an average correction of 11% in the instantaneous shear modulus, 10% in the infinite-time shear modulus and 19% in the creep function time constant of relaxation in data from 20 indentations. In addition, this approach was able to successfully resolve subtle differences (<10 Pa) in local tissue stiffness. Thus, our method produces a more sensitive and accurate viscoelastic measurement of extremely soft embryonic tissues, which may greatly aid in uncovering the micromechanical determinants of embryonic tissue development.

#### 2051-Pos Board B21

##### Statistics of Cell Rheology Measured by AFM

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The atomic force microscopy (AFM) proved to be a useful method for measuring the rheological behaviors of living cells. The force-modulation mode of AFM allowed us to measure the complex shear modulus,  $G^*(\omega)$ , quantitatively in a wide frequency range. One of the advantages of the force modulation is that