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 um diameter) coated with fibronectin (FN), collagen type-I (CNI), collagen type-IV (CNIV), laminin (LN) or vitronectin (VN). ECM-coated beads induced clustering of a5 and b3 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 a5 and b3 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 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

no modification of cell surfaces is required, so that artifacts between the probe and the cell surface may be minimized compared with magnetic twisting cytometry and optical tweezers, in which microbeads are attached to the cell surface before measurements. However, the statistical properties of cell rheology, which are important to know the universality of cell mechanics, have not been so far investigated in the AFM method. In the present study, we measure a large number of cells in the force modulation measurements with a microarray technique. The amplitude and phase of fibroblast NIH3T3 cells ($n > 100$) were measured in the frequency range from sub-Hz up to 200 Hz, and $G^*(\omega)$ was estimated. The results indicated that the storage and loss moduli exhibited clearly a log-normal distribution Moreover, these moduli were well fitted to a power-law model, the so-called structural damping model, and the exponent was around 0.2 with a normal Gaussian. The relationship between the rheological properties and the internal structures of cells will be discussed.

2052-Pos Board B22

Single DNA Molecule Reaction Based On Dip-pen Nanolithography

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Controlling a reaction at the single molecule level has attracted substantial attention from chemical, physical and biological society for its scientific importance and potential applications. Some examples have been given using small molecules by scanning tunneling microscopy (STM). Challenges still remain in control of single-molecule reactions on the soft bio-molecules, which may reveal meaningful information on biological processes. Conventional techniques including magnetic tweezers and fluorescence microscopy have been used to study the enzymatic digests of single DNA molecules. Herein we report a new technique for localized digestion of single DNA molecules on solid substrate with nonspecific endonuclease, by employing an atomic force microscope (AFM) and the concept of Dip-pen nanolithography (DPN). We choose the solution containing DNase I with Mg2+ ions as the ink. As we known the DNase I can catalyze the hydrolysis of DNA strands without the requirement of specific sequence when Mg2+ ions or Mn2+ ions is presented. However, in our system, when DNase I ink solution was deposited on a local position of a single DNA molecule by an AFM tip, the followed digestion location of the DNA strands could be restricted with nanometer precision, so that the site cutting reaction with nonspecific endonucleases was achieved. This AFM site-specific reaction approach, although there is certain degree of shifting and broadening due to lateral diffusion of enzymes on surface, promises a deeper understanding of molecular surgery at the molecular scale and may become very important in the emerging field of nanobiotechnology.

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2053-Pos Board B23

High-speed, Thermo-chemical Nanolithography for Biological Applications

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Scanning probe-based chemical nanolithography has been recognized as an essential part of future nanofabrication processes. However, most of the present strategies still have significant limitations in terms of throughput, resolution and substrate variety. Recently, we have developed a new chemical nanolithography technique called thermo-chemical nanolithography (TCNL). TCNL utilizes a resistively-heated atomic force microscope tip to thermally activate a chemical reaction on an arbitrary organic or inorganic substrate surface. TCNL can write well-defined chemical features at a rate of mm/s, with sub-15 nm resolution [1]. In particular, we have demonstrated that carboxylic ester groups on a polymer surface can be deprotected by TCNL to give carboxylic acids and further modified to form anhydrides [2]. Therefore, TCNL can tune surface wettability with in situ write-read-overwrite capability. In addition, nanoarrays of TCNL-activated amine groups have been used as anchor sites to immobilize a variety of biological ligands [3]. This proves TCNL to be a powerful tool to control the physical placement of biomolecules and cells. It can be applied to a range of molecular cell biology studies such as ligand-receptor recognition and cell signaling.

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2054-Pos Board B24

Direct Mapping Of Surface-bound Liquid With Sub-nanometer Resolution

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At the solid-liquid interface molecules of the liquid adopt a particular arrangement which depends primarily on the interfacial energy and geometry. At the nanoscale this molecular arrangement is of central importance in a wide range of fields from molecular biology to surface physics, heterogeneous catalysis and electronics. In biology the role of interfacial liquid is further emphasized by the soft nature of most biomolecules whose conformation and dynamics depends on the surrounding medium. This is the case for protein function and folding (1), self-assembly processes and bio-electronics where a complex interplay between surface-bound liquid molecules and ions strongly affects any motion. Using amplitude modulation atomic force microscopy (AM-AFM) operated in liquid and in a particular regime, it is possible to simultaneously image the topography of the surface-bound liquid while measuring its adhesion energy to the solid investigated. We have used this method to map the binding energy of water to gold nanoparticles coated with mixed ligands, self-assembled in controlled patterns (2, 3). Such functionalized nanoparticles can mimic the typical surface of proteins (hydrophobicity, charge, surface domains) while allowing careful control of the domains' size and properties (2). Our results show that the average binding energy of water to the surface of the nanoparticles strongly depends on the spatial arrangement of the ligands molecules. The geometry as well as the size of the ligand domains both affect the local adhesion energy of the solvent in a non-linear fashion. Our findings provide experimental and quantitative insight into the interplay between solvent and surfaces in nanoscale biophysical processes.

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2055-Pos Board B25

Visualizing Lipid/Water and Lipid/Ion Interactions at the Biological Interfaces with Angstrom-Resolution by Frequency Modulation Atomic Force Microscopy

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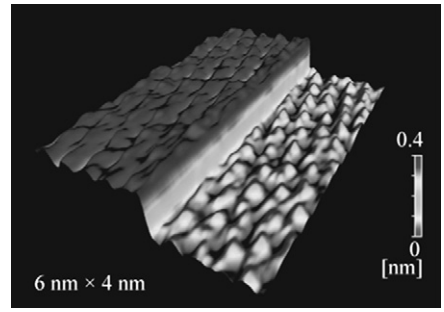
At the interface between biological membranes and their surrounding physiological solution, various interfacial phenomena take place through the interactions between lipid headgroups and water molecules (or ions) and hence have a great impact on the functions and structure of the biological membranes. However, it has been a great challenge to experimentally access such interfacial phenomena with Angstrom resolution.

Recently, we have developed an atomic force microscopy (AFM) technique that is capable of visualizing Angstrom-scale structures and phenomena at the solid/liquid interface with piconewton order loading forces. Here we apply this technique to the investigation of the lipid/water and lipid/ion interactions at the biological interfaces.

The figure shows an AFM image taken at the interface between the DPPC bilayer on mica and PBS solution. The image shows the molecular-scale corrugations of the lipid headgroups (lower half) and their primary hydration shells (upper half), revealing the existence of a stable hydration layer on the DPPC bilayer with nanometer-scale lateral extent.



Fluorescence Spectroscopy II

2056-Pos Board B26

Improved Global Spectral Analysis Method for Simulating Excitation-Emission Maps of Semiconducting Single-Walled Carbon Nanotubes

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Recent research breakthroughs in the field of semiconducting single-walled carbon nanotubes (SWCNTs) have centered strongly around the acquisition and analysis of photoluminescence excitation-emission maps (EEMs). EEMs have been shown to provide both qualitative and quantitative information of