and their relative positions determined both laterally and axially, yielding insight into the competitive adsorption of LS and serum protein. Finally, the continuous steel-ribbon barrier of the new Langmuir trough maintained very low surface tensions, permitting *in situ* imaging of LS monolayer collapse.

Atomic Force Microscopy

841-Pos Combined Atomic Force/ Fluorescence Microscopy Technique to Select Aptamers in a Single Cycle from a Small Pool of Random Oligonucleotides

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Board B686

We are developing a method, which utilizes a combined atomic force microscope (AFM)/fluorescence microscope and small copy number PCR, to affinity-select individual aptamer species in a single cycle from a small pool of random-sequence oligonucleotides (oligos). In this method, a library of small beads, each of which is functionalized with fluorescent oligos of different sequences, is created. This library of oligo-functionalized beads is flowed over immobilized target molecules on a glass cover slip. High-affinity, target-specific aptamers bind tightly to the target for prolonged periods and resist subsequent washes, resulting in a strong fluorescence signal on the substrate surface. This signal is observed from underneath the sample via fluorescence microscopy. The AFM tip, situated above the sample, is then directed to the coordinates of the fluorescence signal and is used to capture a three-dimensional, high-resolution image of the surface-bound bead and to extract the bead (plus attached oligo). The extracted oligo is PCR-amplified, sequenced and may then be subjected to further biochemical analysis.

Here, we describe the underlying principles of this method, the required microscopy instrumentation and the results of proof-of-principle experiments. In these experiments, we selected aptamers in eight trials from a binary pool containing a 1:1 mixture of thrombin aptamer oligo and a nonsense oligo. In each of the eight trials, the positive control aptamer was successfully detected, imaged, extracted and characterized by PCR amplification and sequencing. In no case was the nonsense oligo selected, indicating good selectivity at this early stage of technology development.

842-Pos AFM Study Of DNA Complexes With The V(D)J Recombination Proteins RAG1/RAG2

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Board B687

RAG1 and RAG2 proteins are critically involved in the DNA rearrangement process responsible for the assembly of functional antigen receptor genes from component gene segments. These proteins bind to specific sites called recombination signal sequences (12-RSS or 23-RSS) and mediate recombination of antibody and T-cell receptor genes in developing lymphocytes via formation of synaptic complexes with a 12-RSS and a 23-RSS (the 12/23 rule). This 12/23 rule ensures that the correct regions are joined when recombination takes place. To elucidate the mechanism leading to synaptic complex formation and elucidate the architecture of RAG-RSS complexes, we have used atomic force microscopy (AFM) to directly image RAG complexes bound to RSS substrates. Various fragment designs have been used to compare protein-DNA complexes formed with the RAG proteins and intact 12-RSS and 23-RSS motifs, signal end (SE) motifs which resemble post-cleavage fragments, and nonspecific DNA. We have characterized complexes of the RAG proteins bound to a single DNA fragment (presynaptic complex), as well as complexes of the RAG proteins bound to two DNA fragments (synaptic complexes). The site specificity of RAG binding was examined by measuring the position of the protein complexes on each fragment, and the stoichiometry of the RAG proteins in these complexes was estimated by measuring the volume of the complexes. Our results show that the volume of the RAG proteins in synaptic DNA complexes is larger than in presynaptic complexes, suggesting an association model for the synaptic complex assembly. Also, when the RAG proteins are bound at the end of the stand, fragments are considerably shorter, raising the possibility that the DNA wraps around, coils within, or undergoes strand separation in such complexes. These findings highlight the structural differences between RAG pre-cleavage and post-cleavage signal end complexes.

843-Pos Probing Protein Conformations at the Oil-water Interface Using Single-Molecule Force Spectroscopy

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Board B688

Beta-lactoglobulin (BLG), a globular protein that is abundant in the milk of several mammals, adsorbing to the interface of oil-in-water emulsions and forming a protective coating that stabilizes the oil droplets against flocculation and/or coalescence. The present work aims at a deeper understanding of the conformational changes in BLG adsorbed onto the emulsion interfaces due to variations in pH. Mechanical unfolding of BLG using AFM-single-molecule force spectroscopy (AFM-SMFS) was performed on single oil droplets that were mechanically trapped in a polycarbonate filter. The changes in the contour length upon each unfolding event were determined by fitting the WLC model of polymer elasticity to each of the BLG peaks. Our results show clearly that at pH 2.5 BLG exists as a dimer in which each monomer is similar to two Immunoglobulin domains with contour lengths of 32 nm. At neutral pH (6.8) BLG on the oil droplets adopts a conformation that is different from that in its native state consisting of domains with a contour length of 11 nm. Furthermore, at pH 9 the interactions between the AFM tip and the BLG layer on the oil droplet surface are dominated by a huge repulsion due to the highly negatively charged BLG layer. This study demonstrates a novel application of AFM-SMFS to investi-

gate the underlying mechanisms by which proteins can be used to stabilize food products.

844-Pos The Stress-Strain Behavior of Single Fibrin Fibers

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Board B689

We used a combined Atomic Force Microscopy (AFM)/Fluorescence Microscopy technique to determine the stress-strain behavior of single fibrin fibers. The AFM tip was used to stretch the fibers, which were suspended over ridges, and to take force measurements, while the fluorescence microscope was used to observe the stretching process. This technique may also be applicable to other fibers.

Crosslinked and uncrosslinked fibrin fibers showed similar, viscoelastic behavior. When held at constant strain, stress relaxes exponentially with a fast and slow relaxation time of 3 s and 58 s (crosslinked), and 2 s and 55 s (uncrosslinked). The average elastic modulus was 4.7 MPa (crosslinked) and 4.3 MPa (uncrosslinked); the average total stretch modulus at pulling speed 2 μm/s was 7.6 MPa (crosslinked) and 7.0 MPa (uncrosslinked). Fibrin fibers show significant strain hardening as the total stretch modulus increases sigmoidally from 4 MPa at low strains to 12 MPa at high strains. The amount of dissipated energy per stretch cycle increases dramatically and also sigmoidally with increasing strain from less than 10% at low strains to 70% at high strains.

To explain these data and the extraordinarily large extensibility of fibrin fibers, we propose a model in which individual fibrinogen monomers within a fiber can extend via two major molecular mechanisms: an α-helix to β-strand conversion of the coiled coils and a partial unfolding of the globular γ-C-domain.

845-Pos AFM Study Of KcsA Potassium Channels Reconstituted In Model Supported Lipid Bilayers

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Board B690

KcsA, first identified in the gram-positive bacterium *Streptomyces lividans*, is a tetrameric potassium channel with 160 amino acid units. The determination of its three-dimensional structure has provided fundamental understanding of the physical basis of ion permeation and selectivity. We have used atomic force microscopy in physiological environments to study the morphology of KcsA channels in a supported lipid bilayer. The KcsA channels can be clearly observed with the atomic force microscope. These were reconstituted in POPE / POPG (3:1) lipid vesicles that were spread onto a mica substrate to form the supported lipid bilayer. A comparison was performed between two different constructs: the full-length KcsA wild-type channel and a truncated wild-type version lacking the M2 C-terminus portion (amino acids 126–160 removed). In our preliminary results many of the images show instances where the full-length channel protrudes approximately 4–5 nm above the lipid bilayer and it is possible to distinguish the four subunits. On the other hand, the truncated channel protrudes only about 1–3 nm. These results indicate that these channels have their intracellular face exposed to the AFM probe. In the truncated channel measured at pH 3, a central cavity is observed, which is consistent with the known observation that at this pH the bundle crossing is in the open configuration.

846-Pos Correlating Cellular Response To Nanoscale Morphology And Mechanical Properties Utilizing Atomic Force Microscopy And Optical Microscopy

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Board B691

Cell behavior is known to be affected by the mechanical properties of the extracellular matrix. Cellular response to mechanical stimuli has been achieved previously using the tailored bulk properties of a variety of biological and non-biological materials, but less has been reported about response to nanoscale mechanical features. We have used Atomic Force Microscopy (AFM) and optical microscopy to correlate cell response to the nanoscale mechanical properties of Type 1 collagen fibrils. Dehydrating films of collagen fibrils causes fibrils to become stiffer, and results in greater spreading and proliferation of vascular smooth muscle cells (Biophys, J., 2006). Here we provide additional insight into how matrix properties on the nanometer scale influence cell behavior using quantitative AFM techniques. Collagen films that have been subjected to dehydration display reduced surface roughness, suggesting a decrease in fibril-fibril interspacing, likely due to the removal of water. In addition, force-distance curves obtained at varying loading rates before and after dehydration show that the viscoelastic behavior of the collagen fibril network is significantly diminished after dehydration. We observe that in addition to a higher rate of proliferation and greater spreading, the quantified changes in the nanoscale properties due to dehydration of the thin collagen fibril films lead, to a more highly organized actin cytoskeleton in cells and greater focal adhesion maturation. We suggest that these effects result as a direct response to the mechanical characteristics of the fibrils. Comparisons of dehydrated collagen with enzymatically cross-linked collagen suggest similar effects.

847-Pos Identification and Activity of Adsorbed Fibrinogen Measured by Novel AFM Techniques

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Board B692

Biomaterial induced thrombosis remains a problem in development of blood-contacting medical devices. Under physiologic conditions, multiple proteins adsorb onto rough polymeric biomaterials. Adsorbed fibrinogen serves as a ligand for platelets via the platelet binding epitope in the γ- chain dodecapeptide (γ400–411). The complex nature of adsorbed proteins makes conventional atomic force microscopy (AFM) challenging. In this study, we have developed novel AFM techniques to characterize the distribution and activity of adsorbed fibrinogen.

All these AFM techniques are based on immunolabeling. Gold nanobeads (1.4 nm) were conjugated to a polyclonal anti-fibrinogen antibody to produce a marker for labeling of fibrinogen in dual protein layers prepared on mica and biomedically relevant polymer substrates. Bovine serum albumin (BSA) was patterned on substrates by micro-contact printing and subsequently backfilled with human fibrinogen to yield a featureless protein layer. Gold labeled antibodies were infused and fibrinogen was detected by AFM mechanical property imaging. This AFM immunodetection approach is applicable to complex multi-component protein films adsorbed on clinically-relevant polymers used in medical devices.

For functional studies, AFM probes were modified with monoclonal antibodies recognizing fibrinogen γ392–411, which includes the platelet binding dodecapeptide sequence. Adhesive interactions between modified probes and adsorbed fibrinogen were used to observe time-dependent changes in the exposure of this important dodecapeptide region. The probability of antigen recognition was determined as a function of fibrinogen adsorption time. Statistical analysis shows that the probability of antibody-antigen recognition peaks at ~45 minutes post-adsorption and decreases with increasing adsorption time. Macroscale platelet adhesion measurements on the same substrates were carried out using washed human platelets and a standard lactate dehydrogenase (LDH) assay. Platelet adhesion was determined to be highest for fibrinogen adsorption times of ~45 minutes, correlating well with molecular scale AFM results.

848-Pos Conductive AFM Study on Electrical Properties of Microbial Surface Appendages

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Board B693

Recent discoveries indicate that charge transfer processes in nanoscale microbial surface appendages like pili might play a fundamental role for anaerobic microbiological processes, including the degradation of pollutants. Moreover, they represent a compelling target for future nanotechnological applications as biological nanowires.

In order to investigate pili-like structures of metal respiring bacteria like *Shewanella oneidensis*, *Geobacter* species and *Anaeromyxobacter dehalogenans* we combine proteomics and conductive atomic force microscopy studies. Due to the fact that the measurements of the conductive property of biological molecules are influenced by contact chemistry, geometrical arrangement and deformation of the molecule, we made a comparative study of materials and samples and measured the current-voltage behaviour of these appendages by the point contact method.

849-Pos Photo-Oxidative Stress In The Presence Of NanoTiO2: Single-Cell-Level AFM Assay

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Currently, nanoTiO_2 is the most popular semiconductor system used in heterogeneous photocatalysis and UV filtering materials. In particular, anatase nanoTiO_2 has been reported to efficiently generate reactive oxygen species (ROS) under UV illumination. Here, we report on *single-cell-level* atomic force microscopy (AFM) study of the ability of nanoTiO_2 to generate oxidative stress on individual living human skin fibroblasts (CCL-110). Fibroblasts were exposed to the deleterious action of ROS in the presence of the commercially available anatase nanoTiO_2, with the primary particle size of ~6 nm. Force-spectroscopy AFM measurements were performed for cells exposed to UV-A (8 and 20 mW/cm^2) or UV-C light (0.1 mW/cm^2) in physiological buffer (PBS) containing low concentration of nanoTiO_2 (4 μg/mL). The AFM results obtained for cells illuminated with UV-A revealed a rapid drop (time scale $<$ 1 min) of the cellular stiffness (~30–60%), which scaled with the exposure to the oxidative stress. In contrast, illumination with UV-C light showed a marked drop in the cellular stiffness, which was independent of the presence of the photocatalyst. These findings point to ROS-mediated reorganization of the cytoskeleton and/or to damages occurring at focal adhesions. They also highlight the sensitivity of AFM to detect early changes in mechanical properties of cells exposed to the photo-oxidative stress.

850-Pos Creep and Stress Relaxation of Living Cells Measured by AFM

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Board B693

Living cells respond to external mechanical stimuli and emerge their functions, so that it is crucial to understand their viscoelastic properties under the physiological conditions at a high resolution. The atomic force microscope (AFM) is a powerful tool for measuring the rheological properties of living cells at nanoscale under their

physiological conditions. Recently the authors demonstrated a stress relaxation test with the AFM to living cells [1], in which an indentation force was applied to the cell by the AFM tip, and then the deflection signal of the cantilever was measured as a function of time at the fixed position of its displacement. One of the advantages of such a time-domain technique is that it is capable of exploring the wide dynamic features of sample in a short measurement time compared with a frequency-domain technique such as the force-modulation mode. In this study, we combined a creep test [2], in which the applied force was kept at a constant value, and then the position of the cantilever displacement was monitored as a function of time, with the stress relaxation test to estimate the dynamics of living cells more quantitatively. Fibroblast cells were used in this experiment. The creep test was conducted subsequently to the stress relaxation one at local regions of the cell surfaces, and the observed relaxations were well fitted to linear models. The comparison of both the time-domain techniques will be discussed.

This work was supported by Industrial Technology Research Grant Program in 2006 from New Energy and Industrial Technology Development Organization (NEDO) of Japan.

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851-Pos Cell Adhesion And Cell Cortex Tension Determine Tissue Self Assembly

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Tissue self-assembly and germlayer organization are proposed to be governed by adhesive and mechanical properties of the constituent progenitor cells. In this study, we measured adhesion and cortex tension of the three germ layer progenitor cell types with the help of Atomic Force Microscopy. By comparing these data with results of in-vitro cell sorting experiments we find that differences in adhesion alone cannot drive tissue envelopment in cell sorting experiments but that tissue self-assembly and germlayer organization are sensitive to acto-myosin activity.

852-Pos Probing The Nanomechanical Properties of Intra-coronary Thrombus with Piezoresponse Atomic Force Microscopy

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Board B697

Fibrin clot stiffness is associated with thromboembolism. The molecular basis of thrombus elasticity has not been elucidated. Current methods to quantify clot elasticty with the torsion pendulum and hemodyne are imprecise and error-prone. Furthermore, no work has been done to quantify in-vivo clots. Piezoresponse atomic force microscopy (PAFM) is a novel method that applies an electrical stimulus to a biological sample and quantifies the mechanical response of the sample. Here we present novel PAFM data on the viscoelastic properties of the fibrin clot within thrombi harvested from patients with myocardial infarction (MI).

Intracoronary thrombus was retrieved from coronary vessels during coronary intervention of patients with acute MI (n=20) with the EXPORT catheter. The catheter was maneuvered across the thrombosed coronary vessel, the thrombus removed and analyzed within 2–4 hours. A Picoforce AFM was employed in the piezo-response mode. Specialized conductive tips were used. An AC current was applied in increments of 100mV whilst the sample simultaneously scanned in contact mode. As a control, the scanning was done with the current switched off. Data was collected in height and piezoresponse mode.

High resolution images demonstrated a regular sinusoidal pattern consistent with the nanomovement of the fibrin fibres within the thrombus. The periodicity of 25 nm observed correlated well with the FXIII crosslinking sites of fibrin clots. The amplitude of displacement of the fibrin fibres increased exponentially with the quantity of the current applied. The current was increased up to a point where the fibrin fibers eventually ruptured. When the current was switched off, no contrast could be observed in the piezoresponse data.

PAFM is a novel method that can precisely quantify the visco-elastic elastic propetires of a fibrin clot and may help elucidate the molecular basis for clot elasticity.

853-Pos AFM Study of Microfluidics: An Engineer's View of Astronauts' Bone Loss in νg

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Board B698

Astronauts lose 1–2% of bone minerals per month in microgravity (μg), a disuse-induced osteoporosis. In compact bone, the gravitational force causes nutrients, waste, as well as bio-signaling molecules to circulate between the capillary and lacunar-canalicular channeled network. Buried in hard, calcified tissue, the lacunar-canalicular network structure is difficult to study by conventional biochemical and bioimaging methods. However, the knowledge of channel dimensions and distribution is required for quantitative analysis of bone microfluidics. We applied AFM and confocal

microscopy to the analysis of the lacunar-canalicular network in demineralized bovine tibia. Results from our study showed that

1. the canalicular diameter, d = 419 ± 113 nm;
2. the canalicular porosity, e = 0.051 ± 0.018;
3. the canalicular channels have a specific morphology and location with respect to lamellae and lacunae;
4. the canalicular wall is made of globular structures; and
5. the canalicular depth appears constant over high and low lamellae.

From this study, we conclude that the integrity of the lacunar-canalicular network is preserved after demineralization and that AFM and confocal microscopy are powerful tools in high-resolution structural analysis of bones. The acquired results allow us to calculate diffusivities and tortuosity, which should facilitate microfluidics simulations under various mechanical stress stimuli.

854-Pos Protein Adsorption and Platelet Adhesion on Polyurethane Biomaterial Surfaces

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Board B699

Understanding the surface properties influencing thrombus formation is a key to the development and application of new biomaterials in the long term use of blood-contacting medical devices. In this study we utilized a series of segmented polyurethane (PU) biomaterials with different soft segments chemistries - polycarbonate (PC), polytetramethylenoxide (PTMO), and polydimethylsiloxane (PDMS), to produce a variety of surface chemistries. Atomic force microscopy (AFM) was used to characterize the polymer surface microphase separation structure of PUs and to identify adsorbed fibrinogen on the surfaces so that relationship between biomaterial surface chemistry, fibrinogen adsorption, and platelet adhesion could be addressed.

AFM phase images show that PDMS-PUs undergo strong phase separation and suggest three phases (soft, intermediate and hard domains) present in structure, while PC-PU and PTMO-PU appear to have two-phase structures. Platelet adhesion was measured optically on the PDMS-PU surfaces and found to increase with hard segment content from the range of 35% to 52% hard segment. Fewer platelets were observed on PC-PU and PTMO-PU. Protein adsorption studies were carried out by incubating polymers in mixed protein solutions of BSA and fibrinogen for 10 min. The amount of fibrinogen adsorbed on the surface was detected through antibody recognition measurements using AFM probes modified with a polyclonal anti-fibrinogen antibody. Results show that fibrinogen adsorption was roughly correlated to platelet adhesion on the PDMS-PUs, however, more fibrinogen was measured on PC-PU and PTMO-PU surfaces despite the fact that fewer platelets were observed. This work suggests that platelet adhesion is not necessarily determined by the amount of fibrinogen, but is likely related to the activity of fibrinogen, most likely correlated to the availability of the platelet binding site in the fibrinogen γαμμα- chain dodecapeptide (γαμμα400–411).

Vibrational Spectroscopy

855-Pos Kinetics of Electron Transfer to Cytochrome c Oxidase by Time-Resolved Surface Enhanced ATR-FTIR Spectroscopy

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Cytochrome c oxidase (CcO) from R. sphaeroides genetically engineered with an his-tag on SU II was immobilized in a protein-tethered bilayer lipid membrane (ptBLM) with the cytochrome c binding site directed towards the electrode. The immobilization was followed by an in-situ reconstitution into a bilayer lipid membrane. Electron transfer was enabled by direct electronic wiring to the gold film deposited on the silicon crystal of the IR setup in an ATR configuration. The kinetics of electron transfer to the CuA, the heme a and a3 center of the enzyme was investigated by time-resolved surface enhanced ATR-FTIR spectroscopy.

Rate constants of electron transfer are obtained by a periodic application of potential pulses and analyzing the difference spectra of amide I bands assigned to the respective redox centres as a function of time. These spectral changes are monitored by surface enhanced ATR-FTIR spectroscopy. Assignment of site specific vibrational modes was facilitated using phase sensitive detection. Rate constants were measured under different conditions (pH value, anaerobic, aerobic) and compared to those obtained by electrochemistry.

The wild type enzyme engineered with a his-tag is investigated compared to the N139C

Mutant. Mechanistic implications of the results are presented.

856-Pos The Investigation of the Effects of Simvastatin on Rat Skeletal Muscle by Spectroscopic and Electrophysiological Techniques

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Board B701

Statins are widely used for the treatment of hypercholesterolemia which have some adverse effects on skeletal muscles. This study