Posters

Atomic Force Microscopy

2031-Pos Board B1

The Importance of Studying Biomolecular Processes at the Single Molecular Level

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Current biomolecular studies are unevenly tilted toward using statistical methods for analyzing molecular structures. However, such a procedure may be misleading when a macromolecular assmbly is involved. Two examples will be presented in which drastically different conclusions were derived when biological processes were analyzed at single molecular level using AFM and TEM. Based on statistical methods, lipoprotein(a), a molecule highly enriched in atherosclerotic plaques, was reported to consist of one or two apolipoprotein(a) bound to one low density lipoprotein (LDL). These plaques are the leading cause of cardiovascular diseases. AFM images revealed unambiguously that one apolipoprotein(a) is bound to either one or two LDLs at one or two distant sites. This discovery led to a new theory on how lipoprotein(a) might promote LDL deposition onto the plaque. Protein self assembly to amyloid fibers is an early event in numerous human diseases, including Alzheimer's and Parkinson's diseases. Accompanying this process is a conformational change to β-sheet regardless of the protein's sequence. It was generally believed that monomers, dimers or oligomers assembled directly to the tip of a growing fiber and, for prion proteins, misfolding of the molecule led to their aggregation. AFM, and later TEM, images, however, unambiguously revealed that large spheres consisting of dozens of monomers were first formed and then assembled linearly into amyloid fibers. This discovery led to the introduction of a controversial theory, which is currently challenged by some biomedical investigators while supported by many biophysical scientists, that amyloid fiber formation is a special colloidal phenomenon, starting from colloidal particle formation and followed by their linear aggregation. Formation of the spheres, an energy minimization process, drives the protein's conformational change to β-sheet (Amyloid, 2007, 14(2):119).

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AFM Studies of the Effect of Temperature and Electric Field on the Structure of DMPC-Cholesterol Bilayer Supported on a Au(111) Electrode Surface

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Atomic Force Microscopy (AFM) has been used to characterize the formation of a phospholipid bilayer composed of 70mol% 1, 2-dimyristoyl-sn-glycerophosphocholine (DMPC) and 30mol% cholesterol at a Au(111) electrode surface. Results indicate that addition of cholesterol into the membrane relaxes the elastic stress, increases membrane thickness and reduces defects density. The thickness and thermotropic properties of the mixed DMPC-Cholesterol bilayer supported at the gold electrode surface are quite similar to the properties of the mixed membrane in unilamellar vesicles. The stability of the supported membrane at potentials negative to the potential of zero charge was investigated. This study demonstrates that the bilayer supported at the gold electrode surface is stable provided the applied potential is less than −0.3V. At larger polarizations swelling is observed. Polarizations larger than −1 V cause electro-dewetting of the bilayer from the gold surface. At these negative potentials the bilayer remains in close proximity to the metal surface, separated from it by a ~2 nm thick layer of electrolyte.

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How Phosphatidylinositol 4,5-bisphosphate Regulates Membrane-Cytoskeleton Interaction in Endothelial Cells?

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Phosphatidylinositol 4,5-bisphosphate (PIP2) is a constitutional component of the cell membrane. It can directly or indirectly regulate vesicle trafficking, focal adhesion, cell migration and membrane-cytoskeleton interaction. It has been reported that sequestration of PIP2 in fibroblasts resulted in the decrease of the force needed to extract membrane tethers from the cells, a finding that was attributed to weakened membrane-cytoskeleton interaction, The commonly accepted mechanism of this phenomenon is that PIP2 inhibits the activity of actin-severing proteins such as cofilin, gelsolin, or profilin, and also activates vinculin, talin, α, β-catenin, α-actinin, and thus enhances the interaction between the cell membrane and cytoskeleton.
The aim of our research is to clarify how PIP2 regulates endothelial cell function. Bovine aortic endothelial cells (BAECs) were treated with Pleckstrin homology domain of phospholipase C (PH-PLC) and Neomycin sulfate, which can sequester PIP2, and subsequently exposed to tether extraction with AFM. The results showed that after treatment with Neomycin sulfate, the tether force of BAEC slightly increased in comparison to control, while PH-PLC did not alter the tether force at all. These findings suggest that PIP2 functions differently in BAECs than in fibroblasts. These findings also suggest the following preliminary conclusions. (1) Sequestration of PIP2 with Neomycin might release actin binding proteins that may act as actin crosslinkers, thus strengthening the cytoskeleton in BAECs. (2) PH-PLC, may not be able to sequester PIP2, due to the latter's higher affinity to cytoskeleton binding proteins, thus not affecting the membrane-cytoskeleton interaction in BAEC.
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Controlling Neuronal Growth on Au Surfaces by Directed Assembly of Extracellular Matrix Proteins

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Studying how individual neuronal cells grow and interact with each other is of fundamental importance for understanding the functions of the nervous system. However, the mechanism of axonal navigation to their target region and their specific interactions with guidance factors such as membrane-bound proteins, chemical and temperature gradients, mechanical guidance cues, etc. are not well understood. Here we describe a new approach for controlling the adhesion, growth and interconnectivity of cortical neurons on Au surfaces. Specifically, we use Atomic Force Microscopy (AFM) nanolithography to immobilize extracellular matrix proteins at well-defined locations on Au surfaces. These surface-immobilized proteins act as a) adhesion proteins for neuronal cells (i.e. well-defined locations where the cells ''stick'' to the surface), and b) promoters/inhibitors for the growth of neurites. Our results show that protein patterns can be used to confine neuronal cells and to control their growth and interconnectivity on Au surfaces. We also show that AFM nanolithography presents unique advantages for this type of work: 1) high degree of control over location and shape of the protein patterns, 2) the procedure is carried out in aqueous solutions (protein buffers), such that the proteins are very likely to retain their folding conformation/bioactivity, and 3) minimum protein feature size can be reduced down to several tens of nm (typically ~50nm).

2035-Pos Board B5

Lung Parenchymal Tissue Stiffness in Fibrosis and Cellular Responses to Substrate Stiffness

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Stiffening of the lung parenchyma is one of the cardinal features of progressive pulmonary fibrosis, giving rise to a heterogeneous microenvironment with continuous gradients in stiffness. To define the local mechanical environment of the lung fibroblast, we directly measured the local elastic modulus and elasticity gradients of tissue strips from bleomycin treated mouse lung at the cellular scale using AFM force mapping technique. Elastographs revealed a heterogeneous spatial distribution of tissue stiffness with shear moduli ranging from 0.01 to 20 kPa in both normal and fibrotic lung. Fibrotic lung presented incremental stiffness and spatial heterogeneity. The median shear modulus shifted from 0.1 kPa in normal lung to 3 kPa in fibrotic lung. We then prepared a model system using 2D collagen-coated polyacrylamide substrates with stiffness gradients ranging from 0.1 to 50 kPa to study the role that local stiffness play in focusing fibroblast growth and activation. Human lung fibroblasts growing on stiffness gradients exhibited increased spatial density as substrate stiffness increased, suggesting that proliferation and/or migration responses on stiffness bias fibroblast accumulation to the stiffest region. Immunostaining indicated that stiffness increases focal adhesion size, alpha-smooth muscle actin expression, procollagen I expression, as well as the fibrogenic effect of TGF-beta1 on lung fibroblast. Together these results demonstrate that fibroblast accumulation and activation are biased to areas of increased substrate stiffness, and more importantly, that regional mechanical factors could underpin fibrotic progression in the lung through positive feedback loops of fibroblast recruitment and activation.