P3.41. Role of Labyrinthine Fluid on Sound Energy Attenuation and Endolymphatic Hydrops : a Physical Model

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Sound energy attenuation by the middle ear muscles and stapes footplate is well-recognized concept. We have designed a novel mathematical model explaining the basis of the new concept for the sound energy attenuation due the ionic nature of the labyrinthine fluid. This model was developed using the Coulomb's law. According to this model, excess of given sound energy either in form of increase in the amplitude or increase in the frequency is attenuated due to, increase in the energy consumption for the oscillation of the charged molecules in the labyrinthine fluid. This model also explains the basis of low frequency hearing loss in the early stages of the endolymphatic hydrops. Endolymphatic hydrops manifests as increase in the intralabyrinthine pressure. Because of that, there will be decrease in the distance between two charged particles in the labyrinthine fluid. This phenomenon increases the consumption of the given sound energy for the oscillation of the charged molecules of the labyrinthine fluid and attenuating the energy being transmitted to the neuroepithelia. In cases of low frequency sounds the energy input to the ear is low. Since being attenuated by the above phenomena, the energy reaching the neuroepithelia is less then the threshold for hearing leading to hearing loss. Pharmacologically decreasing the intra-labyrinthine pressure brings back the normal distance between the molecules and reduces the energy being attenuated.

P3.42. The Importance Of Microtubule Lattice Defects In Katanin-Mediated Microtubule Severing *In Vitro*

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The microtubule-severing enzyme katanin uses ATP hydrolysis to disrupt the noncovalent bonds between tubulin dimers that stabilize the microtubule lattice. The resulting microtubule severing may be important in fundamental cellular processes including mitosis and axonal outgrowth. ***In vitro*** severing of paclitaxel-stabilized microtubules nucleated by surface-bound axonemes had been observed previously in real time by VE-DIC: microtubules were found to undergo a multiple-step severing process in which they became kinked for 1.1 ± 1.7 min (N = 44) before breaking. Because severing appeared to be localized to well-separated points on the microtubule (average separation = 4.0 ± 2.6 μm, N = 44), it was hypothesized that katanin exploits infrequent defects in the microtubule lattice. In support of this hypothesis, Monte Carlo simulations of several alternative severing models showed that assuming a defect-free microtubule lattice does not allow prediction of the experimentally observed breaking rate, the number of severing events, the final level of severing, and the sensitivity to katanin concentration over the range 6–300 nM. On the other hand, a defect-containing model, in which lattice defects increase severing activity at infrequent, localized points along the microtubule, was consistent with the experimentally observed severing characteristics. (Supported by NSF BES 9984955)

P3.43. Micropatterned Surfaces To Control Cell Spreading And Quantify Adhesion Strength

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Integrin-mediated cell adhesion to extracellular matrices provides anchorage and triggers signals that direct proliferation and differentiation. This process involves initial receptor-ligand binding followed by strengthening arising from cell spreading, receptor clustering, and cytoskeletal recruitment. Using a hydrodynamic spinning disk adhesion assay, we showed that initial adhesion strength is proportional to the number of receptor-ligand bonds. The current work focuses on quantifying adhesion strength for cells adhering to micropatterned surfaces to analyze the contributions of cell spreading and focal adhesion (FA) assembly. Microcontact printing of self-assembled monolayers of alkanethiols on gold was used to engineer patterns of fibronectin (FN) adhesive "islands" (2-20 μm diameter) on a non-adhesive background. Cells adhered and were constrained to the FN islands, and cells on 2 and 5 μm patterns remained nearly spherical. Immunofluorescence staining demonstrated localization of $\alpha_5\beta_1$ integrin and FA proteins, including vinculin, talin, and paxillin, to micropatterned islands. The relative contributions of receptor clustering and FA assembly to adhesion strength were analyzed by comparing adhesion experiments with similar available contact areas (5 μm dia islands) at different time points (15 min and 16 hr). Integrin clustering, FA formation, and cytoskeletal recruitment resulted in 5-fold increases in adhesion strength. Current work focuses on pharmacological and genetic experiments to analyze the role of specific FA components.

P3.44. Measuring Ligand Binding And Diffusion Within Cell-Membrane Contact Area

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Formation of cell-cell contact area is an integral step in the development of immunological synapses. This process is regulated by the diffusion of adhesion and signaling receptors into, and their binding of ligands in the contact area. A method was developed to evaluate the ligand binding kinetic rates and diffusivity of the receptors within an established contact area. It consists of an experiment that measures the fluorescence recovery after photobleaching and a model that analyzes the coupled diffusion-reaction process. Jurkat cells expressing CD2 or CHO cells expressing $CD16a^{GPI}$ or $CD16b^{NA2}$ were respectively placed on glass-supported lipid bilayers reconstituted with fluorescently-labeled lipid-anchored CD58 or rabbit IgG to allow formation of stable contact areas wherein the molecules accumulated. The reverse-rates k_r of $CD16a^{GPI}$/IgG and $CD16b^{NA2}$/IgG interactions were 0.0025 and 0.012 s^{-1}, respectively. The k_r of CD2/CD58 interaction was too fast (> 0.2 s^{-1}) to be evaluated. The diffusivities of free CD58 and IgG inside their respective contact areas were, respectively, a few folds and 1-2 orders of magnitudes smaller than those outside (~1 $\mu m^2 s^{-1}$). The unrecoverable fractions were ~50% for the two CD16/IgG systems but <10% for the CD2/CD58 system. These data provide insights to the physical chemistry of contact area formation.

P3.45. The Temporal Effects Of Cell Adhesion On The Mechanical Characteristics Of Single Chondrocytes

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Cell adhesion to material surfaces is a fundamental phenomenon in tissue response to implanted devices, and an important consideration in biomaterial design and development. The objective of this project was to measure the mechanical adhesiveness characteristics of single rabbit articular chondrocytes as a function of seeding time to provide further understanding of the cell adhesion process. The hypothesis was that cell mechanical adhesiveness increases with culture time. After culturing chondrocytes on glass coverslips for 1, 2, 4, 6 hours cytodetachment tests were performed on individual chondrocytes to measure mechanical adhesiveness. The results of this study indicated that cell adhesiveness increased from 230 ± 140 Pa at 1 hour to 1080 ± 210 Pa at 6 hours. The cell-substratum contact area with the substrata increased from 161 ± 52 um2 at 1 hour to 369 ± 105 um2 at 6 hours while the cell height decreased from 10.25 ± 1.14 um at 0.5 hour to 6.20 ± 0.95 um at 6 hours. Moreover, fluorescence staining by rhodamine-phalloidin demonstrated the process of spread in the cytoskeleton from 0.5 hour to 6 hours.

P3.46. Effect Of Vibration On IL-8 Production By Dermal Microvascular Endothelial Cells

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Hand-arm vibration syndrome (HAVS) is an occupational illness that occurs in workers who use vibrating hand tools. Symptoms of HAVS include severe vasoconstriction in the fingers in response to cold (also called Raynaud's phenomenon of occupational origin or vibration white finger), paresthesia and numbness in the fingers, and a loss of grip strength. Some evidence suggests that repeated vibration exposure results in a chronic inflammatory response, which may be responsible for neurological and vascular tissue damage. The purpose of this study is to use a cell culture model to investigate the effects of vibration on vascular cells. We grew human dermal microvascular endothelial cells on gelatin-coated 35 mm culture dishes and exposed them to mechanical vibration using an electromagnetic shaker. After 24 hours, supernatants were collected from vibrated cells and from stationary controls. Interleukin-8 (IL-8) protein levels in the supernatant were measured using an enzyme immunoassay. Preliminary results indicate that IL-8 release from vibrated cells was greater than from non-vibrated controls. Our results suggest that vibration stimulates the release of inflammatory mediators from microvascular endothelial cells and that this may be involved in the etiology of HAVS.

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