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In this study, we investigated the effects of 2.1T static magnetic filed on the mixed culture of neurons and astrocytes obtained from rat's spinal cord, focusing on their viability and morphology. The study of the effect of these fields at cellular level are of high importance due to high incidence of exposure of human tissues to high static magnetic fields applied in magnetic resonance scanning. Our results showed that the exposure of a 2.1T magnetic field for 24 and 48 hours had no effects on the viability of either dorsal root ganglion neurons or astrocytes, checked by Ethidium Bromide/Acridine Orange staining. However, the exposure of the mentioned field for 72 hours caused neural cell death ($p<0.001$), while, astrocytes remained alive. There were neither changes in neural/astroglial cell surface area nor cellular migration detected following a two-hour exposure to the field. Analysis of the neurite growth pattern showed that when neurons were exposed to the field for four hours, the normalized parallel components of the neurites with respect to the field direction were statistically greater than the corresponding perpendicular ones ($p<0.01$). This fact indicates that the neuritis could be aligned in parallel to the applied magnetic field direction. However, this effect was not observed when cells were exposed for only two hours. There was no statistically significant change in neurite number identified after two hours exposure to 2.1T static magnetic field. The results can be further elaborated at molecular level when considered with respect to the polymerization and arrangement of microtubules in neural cells exposed to magnetic field.

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Keywords

Biophysics, neural culture, magnetic field, astrocyte

3213-Pos Switching microtubule molecular motors during melanosome transport in Xenopus Laevis melanophores

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Board B516

Xenopus laevis melanophores are one of the cellular systems commonly used to study the function of molecular motors in vivo. An unexpected but common observation in trajectories of single organelles moving along microtubules is that the organelle frequently revert its direction. This observation was explained by the switching of the motor responsible for the transport but the mechanism by which motors with a given polarity turns on and motors with opposed polarity turns off is not known.

In this work, we explore the mechanism of in vivo coordination of microtubule motors by using a fast and precise tracking method. We analyze the interval in the trajectories where reversals of directions are observed and postulate a model to explain the reversals in living cells.

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Cell Mechanics & Motility - III

3214-Pos Analysis of Ciliary Coordination: The Making of Waves

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Board B517

Coordinated ciliary beating is essential for mucociliary transport. Discrete coordination patterns vary both with organism and cell type. In all coordination patterns, there exist orthogonal directions of synchrony and metachrony that are uniquely defined with respect to power stroke direction. Coordination parameters quantified previously include metachronal wave direction, wavelength and velocity. These parameters assume cilia are tightly coupled. But how does one quantify coordination parameters of cilia whose beat cycles are only partially coupled in space or time? This is often the case in primary ciliary dyskinesis (PCD). Here, we describe a new approach to analyze ciliary coordination using high speed DIC images of primary explant cultures from respiratory epithelium.

Digital movies (500 fps) of beating cilia were acquired using a Redlake Motion Meter camera in conjunction with a Zeiss Axioskop microscope equipped with high extinction DIC optics, including a 63X (NA 1.4) oil immersion objective. Movies were assembled into 3D stacks with Oncore Image software, the z direction representing time. Image stacks then were preprocessed and ciliary centroids were determined. For each individual cilium within a group, beat direction was precisely determined and the phase of the beat cycle was determined as a function of time. These data then were analyzed with respect to coordination for specific ciliary groups along specific directions. Coordination, in this sense, represents constant phase relationships within a ciliary group.

Using this approach, we defined the variance of beat frequency (phase) with time for single cilia. In addition, we determined how distance and positional angle between cilia, as well as the angle between their power strokes, affect coordinated beating. Finally, we defined directions of synchrony and metachrony, as well as meta-

chronal wave properties. This approach should prove useful for future analyses of PCD and other coordination phenomena.

3215-Pos Comparing Laser Trap-based Approaches to Investigate and Model Flagella Force Generation in *Chlamydomonas reinhardtii*

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Board B518

Chlamydomonas reinhardtii flagella are an ideal model of the cilia found in the human body because the *Chlamydomonas* are accessible single celled alga whose flagella mimic human cilia in structure and waveform. By characterizing and modeling force generation as the flagella regenerate, we will increase our knowledge of the numerous disorders associated with human cilia. Currently, many models exist that describe movement of flagella at only a maximum or set length. This research investigates flagella movement using the extensible system created by *Chlamydomonas* flagella regeneration. We report success in optimizing and automating laser trap-based measurement of *average* swimming force (PSD calibration and assessment of displacement from trap center) and comparing these force vs length relationships to those previously described using a system that measured *maximum* swimming force (Stoke's drag calibration and trap escape). Our research will lead to a mathematical model of how force varies with time in an extensible system, revealing certain relationships between structure and movement that are otherwise ignored in an inextensible system.

3216-Pos Micromanipulation Techniques Provide A New Insight Into Selforganization Mechanism Of The Mitotic Spindle

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Board B519

The mitotic spindle segregates replicated genomes into two daughter cells equally during cell division. In this process, miss-segregation causes birth defects and canceration. Spindles are mainly composed of microtubules and molecular motors. At metaphase, size and shape of the spindle are maintained constant. Various studies have addressed the mechanisms that regulate the spindle morphology, and the roles of key regulators (kinesins, cytoplasmic dyneins, microtubule-associated proteins, etc.) have been characterized using chemical and protein-based inhibition of spindle components *in vitro* and *in vivo*. In spite of the morphological stability of the metaphase spindle, spindle microtubules spontaneously change the state between growth and shortening (dynamic instability). Additionally, they are transported toward the spindle pole by molecular motors (poleward flux). So the stability of the spindle indicates that microtubule dynamics and the interplay between microtubules and motors are well coordinated during metaphase.

In this study, we developed micromanipulation techniques of cutting and connecting mitotic spindle. Spindles were spontaneously assembled in *Xenopus* egg extract, and we changed their shape by using these two micromanipulation techniques with glass microneedles. After cutting the spindle along its pole-to-pole axis, the elongated pieces were re-organized into two distinct spindles. In the case of connecting the two spindles, they fused together and formed one bipolar spindle. These results demonstrate that the mitotic spindle has an ability of self-organization. Our findings provide a new insight into how metaphase spindle maintains its stable structure.

3217-Pos Sorting Between Theories of Tether Formation by Experiments

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Board B520

Membrane tethers, nano-tubular structures from cell membrane have drawn much attention due to their biological importance, such as providing inter- /intra- cellular pathway, enrolling in leukocytes rolling process and cancer metastasis. Different techniques, such as optical and magnetic tweezers, atomic force microscopy (AFM) and micropipette aspiration, have been used to extract tethers from variety of cell types (e.g. endothelial cells, fibroblast and neutrophils). Tether formation is a complex, dynamic process, which has been described by different theories. According to the dependence of the tether force, F, needed to elongate a tether, on the velocity of extraction, v, these theories fall into two main classes. One depicts F as a linear function of v, while the other presents a non-linear relationship either due to shear thinning or hydrodynamic narrowing of tethers. By using AFM we have systematically investigated the functional relationship F(v) using two different cell types, Chinese hamster ovary cells and bovine aortic endothelial cells. Taking advantage of AFM, we were able to perform either a dynamic or "a static study" of tether formation. In the dynamic study, we extracted tethers with constant v and measured the constant force needed to continuously elongate tethers. In this mode, we were able to vary the pulling speed from 0.2 μm/s up to 40 μm/s (v_{max}). In the static mode, which corresponds to v=0, we hold the extracted tether at a fixed length, and monitor the relaxation of the tether force to its equilibrium value, the static tether force. When considered in the

entire velocity regime, [0, v_{max}], our results for both cell lines show a non-linear F(v) relationship. Latrunculin treatment affected the details of the F(v) function, but not its non-linear character.

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3218-Pos Force-Deformation Characteristics of the Red Blood Cell Dimple Measured with an Optical Trap

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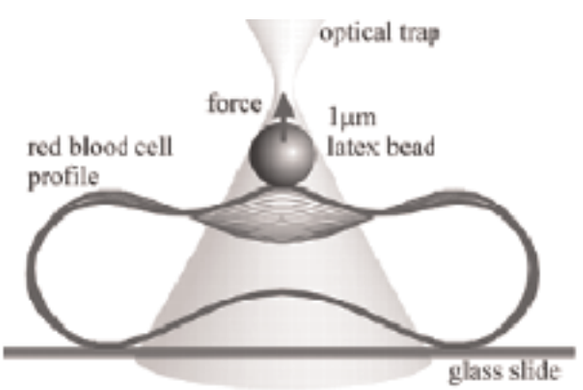
The deformation of red blood cells fixed to a glass surface was measured by binding a 1 μm-diameter latex bead at the dimple center and applying a vertical force to it with an optical trap (stiffness κ_z ~ 13 pN/μm). The force was varied by moving the glass surface relative to the stationary trap while monitoring the bead position. This axisymmetric experiment lends itself to an exact numerical simulation in terms of intrinsic elastic properties of the cell membrane. We used an iterative relaxation algorithm based on the theory of Parker and Winlove (Biophys. J. *77*:3096 (1999)) to solve for the geometrical configuration of the membrane and the distributions of tension and bending moments at mechanical equilibrium for a given applied force. The measured linear stress-strain relationship does not provide sufficient information to determine *H*, the in-plane shear modulus, and *B*, the out-of-plane bending modulus, independently. However, the spring constant of the dimple (~5 pN/μm over an extension range of ~1 μm), together with the simulation, does provide a relationship between *H* and *B*: 5 pN/nm $\approx 0.7\ H + 13\ \mu m^{-2} \times B$.



3219-Pos Multimodal Perturbation of Intracellular Ca^{2+} using Dorsal Cell Adhesion

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Board B522

The cell's chemical and mechanical milieu provides a range of stimuli that must be effectively integrated into intracellular signaling events for the regulation of cell function and physiology. Ca^{2+} is a ubiquitous messenger responsible for governing cell processes ranging from muscle contraction to gene transcription. To examine Ca^{2+} response to both purinergic (P2Y) stimulus and mechanical perturbation through mature, dorsal focal adhesions, we cultured NIH 3T3 cells overnight within our pseudo-3D (P3D) culture environment, consisting of ordered polypropylene microscaffolds constructed over coverglass and mounted into an open-bath perfusion chamber. The P3D system represents a new technology capable of inducing more physiological cell morphology, similar to 3D culture environment, but confining the cell to a single focal plane for imaging. The scaffold encompasses a 3–6 μm gap size, previously shown (Beningo et al., PNAS 2004) to allow dorsal cell attachment. We found cells formed more physiological, stellate/dentritic morphologies when attached between the glass and fiber compared with the typical laminar morphology observed with fibroblasts cultured on glass. For Ca^{2+} imaging, cells were loaded with Fluo-4-AM, and imaged with 150 ms exposures every 5 s. Both 100 μM ATP (using continuous flow perfusion) and 10 μm dorsal-bound fiber shift (using a micromanipulator) were applied as stimuli to measure purinergic and mechanical responses, respectively. Our initial results indicate that cells can frequently produce robust Ca^{2+} spikes in response to G-protein coupled receptor (GPCR) initiated stimuli, reset their spatiotemporal Ca^{2+} signal, and subsequently respond to mechanical stimulation (n = 7 cells), providing evidence that the P3D system allows for the rapid manipulation of chemical milieu as well as the mechanical environment through stretching of mature focal adhesions of stellate fibroblasts cultured *in vitro*.

3220-Pos Experimental Analysis Of Erythrocyte Flicker: Its Relationship To Membrane Tension, Bending And Shear Moduli And The Lack Of Evidence For A Metabolic Driving Force

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We have developed and employed two methods for measuring erythrocyte flicker based on video analyses of a bright field image with a 415nm interference filter. The first measures the projected periphery around the cell and the second measures thickness fluctuations of the cell based on the optical density of the cell's haemoglobin. Using the first method we have applied a range of osmolarities of the medium to change the internal volume of the cell. By analysing the flicker characteristics in the space frequency

domain q(m-1) we were able to show that within a range of volumes around the isotonic state there is an approximate q-3 dependence with mean square amplitude. According to theories (Pécréaux, J., et al 2004. Eur Phys J. E,*13*:277–290) developed for fluctuating vesicles this suggests this behaviour is bending modulus dominated. Once the cell had been swollen to nearly its maximum volume before lysis a q-1 dependence with mean square amplitude became apparent and the same theory indicates a tension dominated behaviour. Using the same experimental method we examined cells depleted of ATP, but before shape change, and found the fluctuation amplitude to remain unchanged, giving no evidence for a metabolic driving force, which contradicts findings by some workers (Tuvia, S., et.al. 1997. PNAS *94*:5045–9). Using the second method we measured the fluctuation amplitude of the cell as a function of radius from the cell's projected centre. A peak in the fluctuations occurs at the region of the cell with the lowest Gaussian curvature. Thus we were able to calculate the shear modulus using the theoretical relationship based on the ratio of bending to shear modulus (Peterson, M.A., et.al. 1992. J. Physique *2.2*:1273–1285).

3221-Pos Human Tumor Cell Migration Through Extracellular Matrix In 3-d

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Board B524

A prerequisite for metastasis formation and malignant tumor progression is the ability of tumor cells to invade and migrate through connective tissue. In this study, we analyzed the role of matrix-degrading enzymes, adhesion receptor expression, contractile force generation, and remodeling of cytoskeletal structures for cell invasiveness. We studied 51 well-established tumor cell lines regarding their ability to migrate through a dense collagen matrix. 27 cell lines were found to be non-invasive, and 24 cell lines were invasive to different degrees that we quantified by the number density of cells that invaded into the gels over a time course of 3 days, multiplied with the average invasion depth. 2-D and 3-D traction microscopy was used to measure contractile forces. Adhesion strengths, cytoskeletal stiffness and molecular turn-over rates were measured using magnetic tweezer microrheology. The speed of cytoskeletal remodelling processes was characterized using nanoscale particle tracking. MMP-14 matrix metalloproteinase and integrin adhesion (α1, α2, α3, α4, α5, α6, α9β1, αvβ3, αvβ5, β1, β4 and αÅβ7) receptor expression was measured using FACS analysis. We found that cell invasiveness correlated with increased expression of MMP-14 matrix metalloproteinase and integrin adhesion receptors (α3 and α5 integrin subunits), increased contractile force generation, and increased speed of cytoskeletal reorganization. Each of those biomechanical parameters, however, varied considerably between cell lines of similar invasivity, suggesting that tumor cells employ multiple invasion strategies that cannot be unambiguously characterized using a single assay. Taken together, our results may help identify molecules and signal transduction pathways that control tumor invasion and metastasis formation.

3222-Pos Cilia Dynamics and Membrane Response in Human Airway Epithelial Cells

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Board B525

Cilia in the Human airway epithelial cells play the important role of transporting a biphasic liquid consisting of a viscoelastic mucus layer and a periciliary liquid layer to facilitate sterility in the airways below the larynx. In Cystic fibrosis, the mucus layer becomes solid-like which inhibits this clearance process by impeding cilia movement. To understand the phenomena of mucociliary clearance, it is important to understand cilia dynamics as well as its potential role as a force transmitter to the cell membrane. We have successfully been able to measure the force response of a single cilium through the use of our custom designed magnetic force system, the 3-dimensional force microscope. We find the force required to stall a single cilium is 180pN. The response of the cell membrane observed through strain field mapping and force curves shows a coupling between the cell membrane mechanics and cilia dynamics and discuss our results in the context of stretch activated channels.

3223-Pos Dynamical Measurement Of The Physical Properties Of Single Cells

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Board B526

The mechanical response of living cells to external forces has attracted the attention of many researchers. We have developed a new tool that takes advantage of an 'L' shaped micropipette to micromanipulate a single cell and put it in contact with an adhesive surface mounted on a translation stage. The spring constant of the micropipette is carefully measured and its deflection is used to apply a calibrated force, and probe the mechanical properties of the cell. As the cell is compressed between the pipette and substrate, dynamical measurements of the elasticity of the cell and the adhesion of the membrane to the substrate are obtained by monitoring the displacement of the micropipette. This technique gives access to real time monitoring of the cell response to a constant applied force, thus exploring the relaxation processes of the cell when subjected to deformation.

3224-Pos Using Microtome Technology to Probe the Effects of Alternating Substrate Stiffness on Cell Morphology and Cytoskeleton Distribution

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Board B527

Adhesion to extracellular matrices in most mammalian cells is a prerequisite for migration, growth, division and differentiation. An increasing body of research is demonstrating that in addition to biochemical signals, mechanical force acting at the interface between cells and their environment is a key regulator of these processes. At these interfaces with respect to extracellular factors, cells recognize substrate elasticities resulting in distinct morphological and functional responses. At the cell-substrate contact area, interactions through connections such as focal adhesion complexes are also believed to act both as force transmission and signaling centers. We are developing a novel method to control the microenvironment of the substrate through generating controlled localized stiffnesses to probe the structural behavior of cells as they interact with the substrate. We first fabricate polymeric films with alternating layers of materials that have different stiffnesses; in this work, we used poly(dimethylsiloxane) with a stiffness of kPa and an epoxy-based polymer with a stiffness of MPa. We then slice this polymer-epoxy composite perpendicular to the plane of the film using the glass knife of a microtome. This method enables us to prepare a substrate with control over the location and repeating sequences of materials with varied stiffnesses. Cells such as fibroblasts attach and spread on both poly(dimethylsiloxane) and epoxy-based polymer without the specific surface coating of extracellular matrix thus cells can be interfaced with this composite system in order to examine behaviors such as cell motility and structural response. Through this approach, we will test cellular responses of various cell types such as fibroblasts and neural cells that will be useful in understanding cell behavior such as cell morphogenesis and cell-surface interactions.

3225-Pos Neural Cell Culture Response To The Applied DC Weak Electric Fields

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Board B528

External constant electric field has shown marked effects on the growth of non mammalian neurons in culture. Nerve branching (1), orientation (2), growth rate(3) the number of neurites (4) were different on the cathodal, cathodotrophic effects, and anodal side of the neurons in culture. However, there are very few reports on the effects of electric field on mammalian CNS neurons in culture. In the present study we attempted to apply and monitor electric field effects on different properties of neurons, including the size of soma, number of neurites per cell, orientation, migration distance and direction and so on in mixed spinal cord cell culture. DC electric fields of 150 mV/mm and 300 mV/mm were applied to the 2–3 weeks old cells in culture by means of agar bridges for a duration of 15, 30 and 45 minutes. According to our preliminary results, neural cells are arranged towards the cathode. Furthermore, cells are elongated perpendicular to the field's plain. However, similar observation and morphological changes didn't presented by astrocytes in culture. There are some changes observed in soma sizes, though, the final approval requires further statistical analysis on the vast number of photographs taken from different cultures.

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3226-Pos Physical Properties Of The Cytoskeleton Of The Adherent Living Cell

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Board B529

Mechanical properties of the adherent living cell are largely determined by its cytoskeleton (CSK), a complex biopolymer network consisting of filamentous actin, microtubules, and intermediate filaments, all of which are associated with crosslinkers, motor proteins, and regulatory proteins. Although the CSK is structurally complex, its dynamics can be described by a small number of 'simple universal laws'. These laws appear to capture the essence of cytoskeletal behaviors with very few parameters, and, although the mechanistic bases are not presently understood, they are virtually universal among many adherent cell types. In particular, the CSK of the living cell 1. is soft and prestressed; 2. exhibits scale-free dynamics; 3. fluidizes promptly and then recovers slowly in response to stretch; and 4. remodels in a fashion that is intermittent and superdiffusive. Here we report data further corroborating laws 3 and 4. We used forced nanoscale motions of RGD-coated microbeads tightly bound to the CSK to study the mechanical properties of the human airway smooth muscle cell and its alteration by a large shear. We used spontaneous nanoscale displacements of such a microbead to show evidence of molecular trapping and caged dynamics. The existence of such generic laws suggest that some integrative cell functions such as cell contraction, adhesion, or spreading, might be controlled not so much by specific protein interactions but rather by generic physical principles.

3227-Pos Microinjection into Schizosaccharomyces pombe cells for studying intracellular diffusion

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Board B530

Microinjection is a common technique used to transfer nano-particles, DNA or other sub micron substances into the intracellular

space. However, microinjection of yeast cells such as Schizosaccharomyces pombe (S. pombe) is difficult because their rigid cell wall makes direct microinjection impossible. Therefore, we have developed a three-step procedure to successfully microinject these cells. Firstly, the cells are transformed into protoplasts by enzyme degradation of their cell walls, using lysing enzymes. After which, the protoplast is microinjected using a micro-needle and a small forward pressure applied by a pressure applicator. Lastly, the cell is regenerated back into a healthy cell (capable of further division) by re-establishing the cell wall in a rich growth medium.

The successful microinjection of S. pombe cells has been proven by microinjecting the membrane-impermeable fluorescent dye Alexa Fluor 488 into the intracellular space. Our microinjection protocol is a fundamental step towards future biophysical studies on the intracellular space of yeast cells. Recently, intracellular diffusion has been studied by optically trapping intracellular, micron-sized lipid granules [1]. In future studies, we will extend this study by optically trapping microinjected nanoparticles. The final goal of our work is to characterize intracellular diffusion processes in the different stages of cell division.

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3228-Pos A New Mechanism for Epithelial Morphogenesis in *Drosophila melanogaster* Oogenesis

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Board B531

The follicle cell epithelium (FCE) that surrounds the developing oocyte and nurse cells undergoes significant morphogenetic changes during oogenesis. The most dramatic example of this is the transition of the initially cuboidal monolayer of follicle cells into distinct populations of columnar and squamous cells by stage 10A. This process provides an excellent system to study epithelial morphogenesis; a crucial element in the development of all multicellular organisms. We have performed a detailed quantitative analysis of cell morphologies at different time points during this process and developed a physically realistic computational mechanics model of the process. Extracted wild type follicles have been stained for cell boundaries after fixation and mounted in a manner to preserve the native shape. Through confocal imaging and subsequent image processing we have reconstructed individual cell shapes throughout the FCE at several time points between stages 8 and 10A. Cell morphology data and theories on the cellular forces were then incorporated into a finite element model that included cell growth, interior force generation, and variation in FCE material properties. Our results have revealed a new mechanism in which the growth of the interior cells stretches the squamous cell population while the columnar cells resist deformation. These results refute previous explanations that cited apical constriction or active migration of columnar cells as the dominant driving forces of this process. This new understanding has resulted from a completely novel approach to the subject and can now guide biological studies that aim to determine the molecular mechanisms controlling this process.

3229-Pos Z-ring Force Generation and Cell Wall Dynamics During Division in Rod-Like Bacteria

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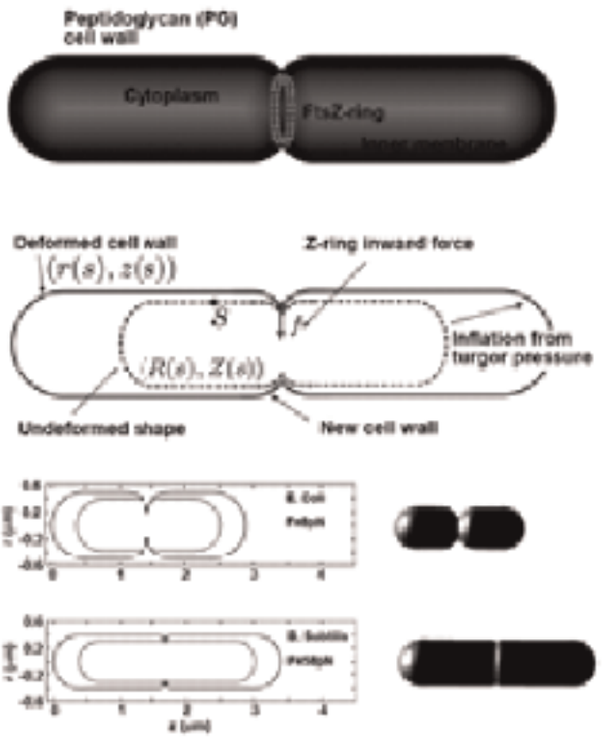
Board B532

Cell division is a critical step during the life cycle of bacteria. During division, a filamentous ring structure made of a tubulin analog, FtsZ, is seen at the mid cell. Together with several other proteins, FtsZ is essential for cell division. Visualization of strains with GFP labeled FtsZ shows that the Z-ring contracts before septum formation and pinches the cell into two equal halves. Thus, the Z-ring has been postulated to act as a force generator. Based on the in vitro measurements of FtsZ polymerization kinetics, we propose a mechanism for Z-ring contractile force generation. We also present a mathematical model to describe the cell wall dynamics during the division step. We explicitly study the division process in rod-like bacteria, make predictions about the contractile force from Z-ring, and describe the cell wall response to the contractile force. We show that only a small Z-ring force is needed to achieve contraction, and the shape of the dividing cell is computed from our model.



3230-Pos Mechanisms Of Edema Formation: Pressure Components Of The Skin Interstitium Measured *Ex Vivo* By Osmotic Stress

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Board B533

Tissue interstitial pressure changes upon injury, causing edema. This pressure is regulated by intrinsic mechanisms not yet understood; both the fiber-glycosaminoglycan matrix and embedded cellular components may participate, but their interaction and relative contribution remain speculative. Here, we exploit osmotic stress techniques to quantitate the intrinsic components of interstitial pressure in human and pig skin. Full thickness sections of freshly isolated skin were equilibrated in physiologic solutions at colloidosmotic counter-pressures between 0 and 211 mm Hg, adjusted with polyethylene glycol, mw ~8,000. Water-volume changes at equilibrium and rates of change were determined from sequential weight measurements taken at time intervals between 0–21 h at 0, 20, and 37 °C to differentiate between energy-dependent (e.g., cell-mediated) and independent processes. In physiologic saline-solution, human skin volumes increased by 64, 54, and 41% of the original weight at initial rates of 1.3 ± 0.3, 2.5 ± 0.7 and 1.4 ± 0.2 µl of water/mg tissue/min at the three temperatures, respectively. The swelling rate decreased with increased colloidosmotic counter-pressure and temperature; equilibrium counter-pressures (i.e., resulting in no volume change) were 180, 175, and 70 mmHg at 0, 20, and 37 °C. Trends were similar in pig skin. Results show that the interstitial volume is regulated by both energy-dependent and -independent mechanisms. The energy-dependent mechanisms contribute 61% of the counter-pressure necessary to balance the swelling potential generated by the fiber-glycosaminoglycan matrix. Since the pressures measured are far greater than interstitial-capillary hydrostatic pressure differences reported from *in vivo* measurements, our results suggests that cellular mechanisms intrinsic to the matrix are important in local edema control.

3231-Pos Role of Extracellular Matrix Rigidity in Regulating Glioblastoma Cell Structure, Motility, and Physiology

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Glioblastoma multiforme (GBM) is a high-grade malignant astrocytoma of the central nervous system associated with a mean survival time of less than one year, even with aggressive surgical care, chemotherapy, and radiotherapy. This rapid progression is due in part to the diffuse infiltration of single tumor cells into the normal brain parenchyma, which is thought to involve aberrant interactions between tumor cells and the extracellular matrix (ECM). Here we explore whether the mechanical interplay between the ECM and the cellular cytoskeleton might contribute to GBM tumor cell structure, mechanics, migration, and chemotherapeutic sensitivity. By culturing GBM tumor cells on two-dimensional (2D) ECM substrates of defined chemistry and mechanics, we find that tumor cell area and cytoskeletal structure are strongly influenced by ECM rigidity. Reciprocally, pharmacologic dissipation of Rho GTPase-based contractility severely alters tumor cell structure and migration. Parallel studies in three-dimensional (3D) spheroidal culture reveal that GBM cell invasion into the surrounding matrix depends on ECM density and rigidity and offer insight into differential mechanisms of GBM cell migration in 2D vs. 3D. Collectively, our results provide preliminary, in vitro support for the importance of mechanobiological stimuli in regulating GBM progression.

3232-Pos Dynamic Friction Measurements On Living HeLa Cells

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The interaction of cells with various interfaces, and especially man-made surfaces, is an active field of research. In our experiment we use a micropipette to measure both the friction and normal force as a cell slides across a surface. A thin substrate coated with Poly-l-lysine is brought into contact with a HeLa cell. The adjustable substrate motion is used to study the response of the cell at various normal forces and speeds. Analysis of the micropipette provides dynamic measurements of both the friction and normal force. With our novel setup we are able to probe the attachment/detachment process of living cells.

3233-Pos Mechanical Characterization Of Reconstituted Collagen Type I Networks As A Cell Culture Environment For 3D Traction Microscopy And Invasion Assays

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Collagen is the most abundant extracellular matrix (ECM) protein and can be used as a 3D culture environment for cell biology assays. Cell behavior in 3D, such as migration speed, morphology and traction generation, sensitively depend on the mechanical properties of the ECM. Moreover, for computing cell tractions from the matrix

deformations around invaded cells, knowledge of the matrix rheology is necessary.

Confocal images of collagen gels (2.4mg/ml of a 1:1 mixture of bovine and rat collagen type I) showed a narrowly distributed pore size of Ø1μm. Macrorheological measurements using a parallel-plate rheometer revealed predominantly elastic behavior that was approximately linear for strains <5%, with a shear modulus G' of 80Pa, a loss modulus G" of 11Pa, and a weak frequency dependency of both moduli according to a power-law with exponent 0.09. Microrheology was measured by applying a 21nN "point" force to a ferrimagnetic Ø4.5μm bead, and tracking the resulting displacements of Ø1μm fluorescent beads dispersed in the gel (average distance 10μm) in three dimensions. Alternatively, we applied a distributed force using a steel sphere (Ø100μm) placed onto the gel surface, or we sheared the gel with a parallel glass plate. Under all three conditions, the microscopic gel deformations closely followed that of a linear elastic, isotropic and homogeneous continuum with a Poisson ratio of 0.34. In summary, for small strains and length scales down to typical bead separation distances, marker positions in reconstituted collagen type I networks deform as expected for an affine, predominantly elastic, isotropic, homogeneous continuum.

3234-Pos Determination of Cell Elasticity through Hybrid Ray Optics and Continuum Mechanics Modeling of Cell Deformation in the Optical Stretcher

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The optical stretcher is a dual-beam trap capable of stretching individual cells. At this time there is no direct method for measuring the optical stress distribution that is responsible for the action of the optical stretcher. Previous studies have used either ray- or wave-optical models to compute the optical pressure on the surface of a spherical shell (1–2). We have extended the ray-optics (RO) model to account for focusing by the spherical interface and the effects of multiple internal reflections. Using the exact ray-optics solution for the stress distribution, cellular deformation in the stretcher is determined by a numerical solution of the Euler-Lagrange equations appropriate for thin spherical shells. Our simulation results for red-blood cells (RBCs) show that internal reflections can lead to significant perturbation of the deformation. Even in the absence of internal reflections, the RO model produces stress distributions that can deviate from the cosine-squared approximation, potentially leading to a systematic error in the determination of cellular elasticity. Calibration studies with polystyrene spheres show excellent agreement between model predictions and experimental escape force measurements, and RBC elasticity measurements are consistent with literature values. We have also used the stretcher to measure the elasticity of murine 2T3 osteoblast-like cells, and find these cells are approximately 20 times stiffer than red blood cells. Results from our current efforts to use fluorescence monitoring to assess physiological changes in stretched cells will also be discussed.

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Signal Transduction in Contractile & Motile Cells

3235-Pos The Giant Sarcomeric Protein Obscurin as a Potential Regulator of RhoA Signaling in Skeletal Muscle

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Obscurin is an 800 kDa protein of the titin superfamily that organizes myofibrils and the sarcoplasmic reticulum in striated muscle. Unlike titin, obscurin surrounds myofibrils at the level of the M-bands and, to a lesser extent, the Z-disks. The multidomain protein contains 49 immunoglobulin domains and 2 fibronectin-III-like domains at the amino-terminus, followed by an IQ domain, more immunoglobulin domains as well as some nonmodular sequence, an SH3 domain, a guanine nucleotide exchange factor (RhoGEF) domain and tandem plextrin homology (PH) domain, and several consensus phosphorylation motifs for ERK kinases at the carboxy-terminus. As aberrant signal transduction has been linked to numerous pathophysiologies, including cardiac hypertrophy, we have begun to investigate the role of the RhoGEF domain of obscurin in muscle development and physiology. Through co-localization studies and co-immunoprecipitation of exogenously over-expressed proteins in COS-7 cells, as well as immunofluorescence of endogenous proteins in developing myotubes and adult rat striated muscle, we identified the small GTPase, RhoA, as a ligand of the RhoGEF domain. The RhoGEF domain does not interact with several other related Rho family members, including Cdc42. RhoA appears to organize with obscurin at the same time developmentally, when the two proteins both concentrate primarily at the M-band. Over-expression studies indicate that obscurin is critical for RhoA organization in developing myotubes. Both proteins also concentrate at M-bands in adult rat tibialis anterior muscle, but RhoA redistributes to other sites when muscle is injured in high strain lengthening contractions. Our results suggest that RhoA activity is regulated by its interaction with the RhoGEF domain of obscurin.

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