3800-Pos

Tumor and Micro-Environment: The Role of Pressure in Cancer Proliferation

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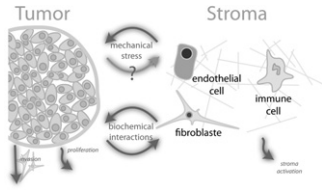
Cancer progress is a multistep process. In the case of carconimas the primary tumor growth locally before some cells evade the stroma by degrading the basal membrane. Two main elements drive the primary tumor proliferation: accumulation of genetic aberrations and the tumoral micro-environment. In contrast with the genetic aberrations, the precise role of the micro-environment and its interplay with the tumor is still poorly understood. Recent works suggest that the mechanical pressure felt by the tumor and the stroma can play a role in the tumor progression. In this project we study the physical and biological interplays of the tumor and its micro-environment focusing on the role of the mechanical stress. We present two different approaches to measure the effect of pressure on tissue growth and death. The first setup use osmotic pressure to deform a dialysis bag and exert a known pressure on a multicellular tumor spheroid. First results indicate the ability to modulate tumor growth depending on the applied pressure. The second setup is a microfluidic based integrated system which enables to feed and visualize spheroids in the same time that we apply a known pressure.



3801-Pos

Reaction of Primary Fibroblasts to Well Controlled External Strain

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Most cells of the connective tissue have to cope with sizeable mechanical strains which also serve as cues to initiate cell responses like cell shape changes, cell reorientation and rearrangement of the cellular cytoskeleton. In our experiments we cultivated human umbilical cord fibroblasts in elastic chambers and exposed them to cyclic external strain of various amplitudes. We characterized both cell morphology and cytoskeletal structure simultaneously after various durations of straining by immunofluorescence microscopy. Digital image processing was employed to achieve high accuracy and high statistical significance of the results due to large numbers of evaluated cells. Moreover, regular microstructures micromolded into the elastomeric cell culture chamber were exploited to reliably quantify the amount of strain experienced by individual cells. Measured angular distributions of cell cytoskeleton orientations are in agreement with theoretical predictions in which a steady state is determined by a set point condition. The observed dependence of the results on the effective Poisson's ratio of the stretching chamber indicates that these cells regulate their mechanical homeostasis to control strain and not stress. Intriguingly, we observed a temporal delay between cytoskeleton and cell morphology reorientation with morphology lagging behind cytoskeleton orientation. First results on simultaneous molecular processes in focal adhesion complexes and cytoskeleton will be discussed.

3802-Pos

Cell Response to a Locally Applied Force: Mechanical Stiffening Correlated to Actin Recruitment

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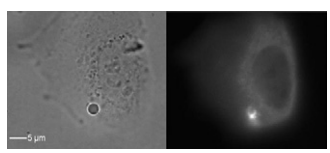
Using micrometric beads specifically bound to integrins of the cortex and trapped in optical tweezers, we applied a local constant force to myoblasts in culture. The application of a constant force allows to apply a controlled local stress to the cell and to measure its mechanical properties (creep experiment). We followed actin distribution during force application using cells that express GFP-actin. We observed within a few minutes an increase in the viscoelastic modulus of the cell, correlated to both a reinforcement of the cell anchoring to the bead (actin recruitment at the bead-cell contact) and a reinforcement of the entire actin cytoskeleton (actin recruitment up to several micrometers from the force application zone). We show that cell adaptation to mechanical environment can take place at short time scale.



3803-Pos

Does the External Environment of Cells, in Addition to their Genetic Programs, Play a Role in the Localization of their Division Sites ?

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Oriented cell division and establishment of cell polarity are central to development of many organisms [Drubin & Nelson 96]. Yeast cells exhibit defined patterns of oriented cell divisions by choosing a specific bud site on their cell cortex. The current understanding is that the site for bud formation is determined genetically, depending on cell type [Ni & Snyder 01, Aronov et al 07]. In haploid cells, bud sites are adjacent to the previous bud neck site: This is the axial pattern. In diploid cells, mother cells select bud sites either adjacent to their previous daughter cells or on their opposite end, whereas daughter cells always choose a bud site directed away from their mother: This is the bipolar pattern. It is believed that cells respond to cortical cues that mark positions on the cell cortex to establish these cell type-specific budding patterns. Recruitment of proteins to the presumptive bud site is thought to direct the cytoskeleton and secretory apparatus toward the bud site, thereby restricting new growth to the bud.
Might the external environment play an additional role in the localization of bud sites, such as confinement via some mechanical and/or chemical sensing? To address this question, we perform image analysis of budding yeast cells in a two-dimensional micro-fluidic chamber, allowing for a controlled growth environment over multiple cell cycles [Charvin et al 08].

3804-Pos

Crosslinked Collagen Films Affect Cell State

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Cells respond to the stiffness of their microenvironment by altering their morphology and even their gene expression profile [Engler et al, Cell 126, 677]. We study the behavior of human mensechymal stem cells (hMSC) cultured on thin highly ordered collagen films, and we show that hMSC are sensitive to nano-mechanical properties of collagen coated substrates. Cell morphology, cytoskeleton organization, and differentiation are evaluated in response to different collagen crosslinking agents. Cells pull on the collagen films, and their ability to deform the collagen fibrils is greatly influenced by the films'mechanical properties. Mechanically anisotropic native collagen films promote strong polarization and orientation along the highly aligned fibrils. Transglutaminase cross-linked films lose their distinct anisotropic mechanical properties and give a very different cell response. In comparison to crosslinked polyacrylamide gels coated with collagen-ligand, the morphology of the cells on the native and pure collagen films resembles that of cells on soft gels (myogenic phenotype) while cells on cross-linked collagen films appear more like cells cultured on stiff gels which can promote osteogenic differentiation. Cells cultured for two weeks on transglutaminase crosslinked collagen fibrils indeed express the osteogenic marker CBFa1 in contrast to cells cultured on non-crosslinked collagen films. Atomic Force Microscopy techniques are used to evaluate local topography as well as the mechanical properties of the cells and their surroundings at high spatial resolution. The AFM stylus is also used to deform the fibrils, mimicking cellular processes of collagen remodeling. Crosslinked collagen films require forces which are at least twice as high for similar plastic deformations of native collagen films. We also measure the elasticity or effective tension of live cells in response to the different collagen films and conclude that cells stiffen considerably after two weeks on the stiff crosslinked collagen films.

3805-Pos

Breast Cancer Cells Reduce the Stiffness of Endothelial Cells

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Metastasis formation is a key component of malignant tumor progression. The capability to metastasize depends on the cancer cells potential to invade connective tissue, adhere, and potentially transmigrate through the endothelium. How invasive cancer cells diminished the endothelial barrier function is still elusive. We hypothesize that some invasive cancer cells can decrease the endothelial barrier function through reduction of the endothelial cell stiffness. Using cell invasion assay in dense 3D collagen matrices, we observed that MDA-MB-231 breast cancer cells invade collagen matrices, and that their invasiveness is significantly increased in the presence of an endothelial cell monolayer. Using microrheology magnetic tweezer measurements, we investigated whether invasive breast cancer cells alter endothelial cells mechanical properties. Indeed, we found that these invasive cancer cells reduce the stiffness of co-cultured microvascular endothelial cells compared to mono-cultured endothelial cells. The reduction of cellular stiffness in endothelial cells may explain the break down of the barrier function of endothelial cells that was induced by breast cancer cells. In summary, the mechanical measurements of cells help to identify molecules and signal transduction pathways that control biological processes such as cell invasiveness and metastasis. These measurements

may emerge as a powerful tool to analyze whether cancer cells may be able to break down the endothelial cell barrier by altering endothelial cells mechanical properties.

3806-Pos
Feeling for Cells with Light: Illuminating the Role of Biomechanics for Cancer Metastasis
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Light has been used to observe cells since Leeuwenhoek's times; however, we use the forces caused by light described by Maxwell's surface tensor to feel for the cellular cytoskeleton. The cytoskeleton a compound of highly dynamic polymers and active nano-elements inside biological cells is responsible for a cell's stability and organization. It mechanically senses a cell's environment and generates cellular forces sufficiently strong to push rigid AFM-cantilevers out of the way. The active cytoskeleton is described by a new type of polymer physics since nano-sized molecular motors and active polymerization overcome the inherently slow, often glass-like brownian polymer dynamics. The optical stretcher exploits the nonlinear, thus amplified response of a cell's mechanical strength to small changes between different cytoskeletal proteomic compositions as a high precision cell marker that uniquely characterizes different cell types. Consequentially, the optical stretcher detects tumors and their stages with accuracy unparalleled by molecular biology. As implied by developmental biology the compartmentalization of cells and the epithelial-mesenchymal transition that allows cells to overcome compartmental boundaries strongly depend on cell stiffness and adhesiveness. Consequentially, biomechanical changes are key when metastatic cells become able to leave the boundaries of the primary tumor.

3807-Pos
Refrigerated Versus Fresh Human Red Blood Cells Response to Sheer Stress
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By fully calibrating 1064 nm infrared laser traps we have studied the mechanical deformations caused by shear stress on healthy human RBCs. In this study we have investigated how the mechanical deformation of RBCs to such a stress varies when we use fresh and refrigerated RBC (at 4°C for a period of about 2 weeks). Fresh and refrigerated red blood cells from a healthy donor are suspended in the donor's blood plasma. Then the cells are subjected to viscose drag force by translating the microscope stage holding the blood sample while the cells are kept trapped by the laser to cause sheer deformation on the cells. Under these conditions the areal, longitudinal, and transverse deformations of the cells as a function of the sheer stress have been investigated. The results for these deformations have revealed significant difference with a nonlinear behavior as a function of the net force acting on the cells. Moreover, the results indicate the elasticity of the cells drastically decreases due to refrigeration.

3808-Pos
How Deep Cells Feel
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Cellular organization within a multicellular organism requires a cell to assess its relative location, taking in multiple cues from its microenvironment. A cell engages ECM and actively probes the matrix, sensing in deformation the elastic resistance that seems to characterize different tissues, and so to assess how far the feedback extends - by analogy to the 'princess and the pea'fairy tale - we have generated substrates of different elasticity and different thickness on top of rigid supports. The elastic properties of our gels are characterized by AFM-based micro-rheology - a tool that probes at the cellular scale, and mesenchymal stem cells (MSCs) are studied because these cells have proven particularly sensitive to matrix elasticity and microenvironment in terms of their adhesion, their morphology, and even - after days - their differentiation. Cell morphology changes generally take hours, and we find that spread area, focal adhesions and cytoskeleton organization of MSCs on thin and soft gels resemble structures in cells on thick and stiff gels. Thickness sensitivity decreases with stiffness, and initial computational modeling of cell and matrix mechanics lends insight into the sub-cellular sensitivity. Furthermore, continuity of deformation from matrix into the cell and around the cytoskeleton-caged and linked nucleus also suggests mechanisms to affect processes such as differentiation. The results ultimately show that even if one's cells are not of royal descent, they seem to feel the difference between stiff or soft and thick or thin surroundings.

3809-Pos
Fibroblasts Sense Substrate Viscosity
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Mechanosensitivity of fibroblasts, their ability to sense and respond to viscoelastic changes in their surrounding environment, is believed to critically affect cell adhesion and morphology and to play an important role in multiple cellular processes, such as cell differentiation and migration. Previous studies in cellular mechanoresponse using μm-thick polymeric films suggest that fibroblasts mainly sense changes in substrate elasticity. Here we present comparable experiments using a biomembrane-mimicking substrate which show that plated fibroblasts may respond similarly to changes in substrate viscosity. These 8-40nm thick substrates consist of stacks of multiple, polymer-interconnected lipid bilayers where cell-linker fluidity and substrate viscosity are tuned through the degree of stacking. In this experimental system, the amount of frictional coupling affecting substrate viscosity is reduced with increasing distance between the outermost (cell-exposed) lipid bilayer and underlying glass. The integrity of the multi-bilayer system, containing mobile laminin linkers, in the presence of plated cells is confirmed through combined differential interference contrast (DIC) microscopy and fluorescence recovery after photobleaching. Optical microscopy (DIC, phase contrast, EPI-fluorescence) data of GFP-actin transfected cells illustrate profound changes in adsorption, phenotype, and cytoskeletal organization, in response to substrate viscosity. Furthermore the impact of substrate viscosity on projected cell area and cellular migration is discussed.

3810-Pos
Control of Extracellular Matrix Organization through Coupled Mechanical and Chemical Inputs
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The extracellular matrix (ECM) is a critical cellular component that provide structural support and organization as well being linked to a variety of cell responses including motility, proliferation, and apoptosis. Fibronectin (FN) is an ECM protein that is secreted by many mammalian cells as a soluble dimer and assembles into insoluble multimeric fibrils at the cell surface. This specific extracellular component has been linked to wound healing, cell adhesion, blood coagulation, cell differentiation and migration, maintenance of the cellular cytoskeleton, and tumor metastasis. In addition, FN is constantly subjected to mechanical and chemical stimulations, resulting in a highly dynamic microenvironment that is constantly being remodeled by the cell. While many studies have examined FN organization through various modes of chemical stimulation, there is limited work on examining the effects of mechanical stimulation or in examining the coupled affects of mechanical and chemical stimulation. In our present study we used a custom fabricated device to probe the effects of mechanical and chemical stimulation on FN organization. We exposed single cells to equibiaxial stretching and observed an increase in localized FN fibrils relative to unstimulated cells. The response patterns of the FN were markedly distinct when examining intracellular versus extracellular organization. We also perturbed this system by coupling the mechanical stimulation with chemical stimulation by exposing cells to equibiaxial stretching while inhibiting Rho activity. These dual mode stimulated cells revealed similar responses to cells exposed to mechanical stimulation in that increased FN fibrils was observed, indicating mechanics may play more of a dominate role in ECM organization with respect to Rho activity. These results have implications in a variety of fields including biophysics, cell mechanics, and mechanotransduction.

3811-Pos
Cell-Matrix De-Adhesion Dynamics Reflect Contractile Mechanics
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Measurement of the mechanical properties of single cells is of increasing interest both from a fundamental cell biological perspective and in the context of disease diagnostics. In this study, we show that tracking cell shape dynamics during trypsin-induced de-adhesion can serve as a simple but extremely useful tool for probing the contractility of adherent cells. When treated with trypsin, both $SW13^{-/-}$ epithelial cells and U373 MG glioma cells exhibit a brief lag period followed by a concerted retraction to a rounded shape. The time-response of the normalized cell area can be fit to a sigmoidal curve with two characteristic time constants that rise and fall when cells are treated with blebbistatin and nocodazole, respectively. These differences can be attributed to actomyosin-based cytoskeletal remodeling, as evidenced by the prominent buildup of stress fibers in nocodazole-treated $SW13^{-/-}$ cells, which are also two-fold stiffer than untreated cells. Similar results observed in U373 MG cells highlights the direct association between cell stiffness and the de-adhesion response. Faster de-adhesion is obtained with higher trypsin concentration, with nocodazole treatment further expediting the process and blebbistatin treatment blunting the response. A simple finite element model confirms that faster contraction is achieved with increased stiffness.