3800-Pos
Tumor and Micro-Environment: The Role of Pressure in Cancer Proliferation
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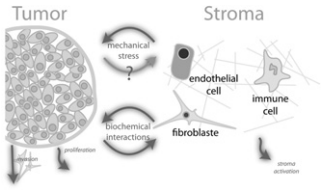
Cancer progress is a multistep process. In the case of carconimas the primary tumor growth locally before some cells evade the stroma by degrading the basal membrane. Two main elements drive the primary tumor proliferation: accumulation of genetic aberrations and the tumoral micro-environment. In contrast 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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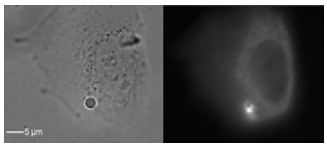
Using micrometric beads specifically bound to integrins of the cortex and trapped in optical tweezers, we applied a local constant force to myoblasts in culture. The application of a constant force allows to apply a controlled local stress to the cell and to measure its mechanical properties (creep experiment). 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