experimentally reported. We attempt to establish correlation between the mechanistic models presented here and an earlier heuristic model that we have developed [6].

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Water Potential of Cell Microenvironments Modulates their Proliferation

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Microenvironmental conditions in interstitial spaces can change rapidly after inflammatory insults. Hydration potential shifts of *50-150 mmHg* occur during burn and ischemia-reperfusion injury in dermal and myocardial extracellular matrices, respectively, presumably due to increased mechanical tension, fluid flux, and water activity as edema accumulates (McGee et al., *Biophys J 2012;Circ Res 2012a;Wound Rep Reg 2013*).While cell responses to mechanical and flow-related components of the hydration potential are increasingly studied and understood, responses to concomitant changes in water's chemical potential are not. Here, we explore its effects on *HL60*, an anchorage-independent, human leukemia cell line that readily differentiates towards various cell lineages. Cells *(10^5 /ml)* were grown in static suspension cultures at *37 °C*, in liquid media supplemented with *2.5%* fetal calf serum and at colloidosmotic pressures adjusted to between *1-100 mmHg* with inert polymers. After 24 hours, the cells' growth rate changed with the water chemical potential in direct proportion to the colloidosmotic pressure of the growth solution. Linear regression analyses showed that the slope of the growth rate versus pressure was *(2.4%)/day/mmHg(R^2 = 0.875)*. The observed rate changes were independent of the physicochemical characteristics of the inert polymer; *polyethyleneglycol 8000* or *dextran 10* enhanced cell proliferation. Cell differentiation pathways also appeared to change as determined by the cells morphology and size in Giemsa stained cytocentrifuge preparations and further suggested by a shift to the right in the frequency-distribution of their nucleus/cytoplasm ratios. These results show that changes in water's chemical potential modulate proliferation regardless of media-stiffness or flow sensing by the cell. Hydration potential components other than the mechanical play significant roles in cells' adaptation to changes in their microenvironment.

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Rapid Disorganization of Mammary Acini Driven by Long-Range Mechanical Interaction

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Cells and multicellular structures can mechanically align and concentrate fibers in their extracellular matrix (ECM) environment but can also sense and respond to mechanical cues by differentiating, branching, or disorganizing. Mechanically induced collagen concentration and alignment into arrangements variously referred to as fibers, tracts, cables, straps or lines has been seen in experimental systems ranging from single cells and tumor explants to human clinical samples. Here we show that pairs or groups of Ras-transformed mammary acini with thinned basement membranes and weakened cell-cell junctions can generate collagen lines that then coordinate and accelerate transition to an invasive phenotype. When two or more acini mechanically interact by collagen lines, the pairs or groups of acini begin to disorganize within 12.5 ± 4.7 h in a spatially coordinated manner, whereas acini that do not interact mechanically with other acini disorganize more slowly (21.8 ± 4.1 h) and to a lesser extent ($p<0.0001$). Overall, disorganization of mechanically interacting pairs of acini is more probable, rapid, and extensive than of single acini. When the directed lateral mechanical interconnections between paired acini were laser-severed, the acini reverted to the slow disorganization phenotype. When acini were mechanically isolated from other acini and also from the bulk gel by box-cuts with a side length below 900 μm, transition to an invasive phenotype was blocked in 20 of 20 experiments. Thus, pairs or groups of mammary acini can interact mechanically over long distances through the collagen matrix and these directed mechanical interactions are necessary for rapid transition to an invasive phenotype.

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Dorsal Adhesion Slows Glioblastoma Migration in Perivascular Mimics

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Glioblastoma multiforme (GBM), the most prevalent primary brain cancer, is characterized by diffuse infiltration of tumor cells into brain tissue, which severely complicates surgical resection and likely gives rise to the almost universal tumor recurrence. This diffuse infiltration is frequently guided by anatomical "tracks" in the brain in the form of blood vessels or white matter tracts, which give rise to the highest migration speeds observed in vivo. Despite this observation, little is known about the biophysical and biochemical mechanisms through which these tissue interfaces promote invasive motility, which in turn may derive from a lack of appropriate culture paradigms. To address this need, we developed a culture system in which tumor cells are sandwiched between a ventral fibronectin-coated dorsal surface representing vascular basement membrane and a dorsal hyaluronic acid (HA) surface representing brain parenchyma. We find that inclusion of the dorsal HA surface induces formation of adhesive complexes and significantly slows cell migration relative to a free fibronectin-coated surface. This retardation is amplified by inclusion of integrin binding peptides in the dorsal layer and expression of CD44, suggesting that it acts through biochemically specific mechanisms rather than simple physical confinement. Moreover, both the reduction in migration speed and assembly of dorsal adhesions depend on myosin activation and the stiffness of the ventral layer, implying that mechanochemical feedback directed by the ventral layer can influence adhesive signaling at the dorsal surface.

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Length Scale Dependent Micro-Rheology of Cellularized Type I Collagen Gels

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Collagen gels are commonly used as the substrate for experiments on cell mechanics because collagen is the most abundant protein in the extracellular matrix of most animals. The gels are commonly approximated as homogeneous elastic materials; however, on smaller length scales, the inhomogeneity of the collagen fiber network becomes very apparent. During gelation, collagen fibers can group together to form larger fiber bundles, with the size, shape, and distribution of these bundles depending on the collagen concentration and the temperature during gelation. In addition, when cells are embedded in the collagen substrate, the cell adhesion forces deform the collagen and alter its elastic properties. We study local variation in the elastic modulus of type I collagen gels and characterize inhomogeneity caused by cell adhesions and fiber bundles in the collagen network. We expect the cell adhesions and the collagen fiber bundles to each have distinct length scales over which the elastic properties will vary. These length scales will be calculated by separating the gel into domains in which the elastic properties of the collagen change in a characteristic way. We map the local elastic modulus of type I collagen gel using active two-point micro-rheology. Optical tweezers are used to perturb microscopic particles embedded in the gel and in-line holographic particle tracking is used to calculate the particle displacements. The local elastic properties are calculated by cross-correlating the trajectory of the perturbed particle with the trajectories of the surrounding particles. Then confocal reflection microscopy is used to image the collagen fiber network, showing the locations of cells and fiber bundles. These images are used to compare the distribution of cells and fiber bundles to the results of the local micro-rheology calculations.

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Intermediate Filament Structure, Assembly and Nanomechanics

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Intermediate filaments (IFs) consist of two-stranded coiled coils that form anti-parallel, half-staggered tetramers. By time-lapse electron microscopy, complemented with total internal reflection fluorescence (TIRF) microscopy, we have investigated the in vitro assembly of vimentin to define the assembly pathway for vertebrate cytoplasmic IFs. First, we have characterized the physical and structural state of the soluble vimentin subunits by analytical ultracentrifugation (AUC) and X-ray crystallography. Assembly is induced by a change in the ionic strength and starts with the lateral association of tetramers to full-width unit-length filaments (ULFs) driven by the interaction of the

basic, non-structured head domains with the acidic coiled-coil rods. In a next step, ULFs longitudinally anneal by an end-on-addition mechanism to yield filaments. This mechanism is also exhibited by muscle desmin and the epithelial keratins, whereas the nuclear IF proteins, i.e. the lamins, do not assemble into ULFs. In a next step, the subunit composition of ULFs and IFs of different IF proteins was analyzed by scanning transmission electron microscopy (STEM) and cryo-electron tomography of native specimens. Depending on the ionic conditions used for assembly, on average keratin IFs harbor 8, vimentin IFs 16 and desmin IFs 24 coiled-coil dimers per filament cross-section. The formation of ULFs was investigated further by small-angle X-ray scattering (SAXS) and AUC, employing a mutant vimentin variant that is arrested in the ULF state. With these data at hand, we investigated the impact of human disease mutations found in desmin that cause myofibrillar myopathy. Last but not least, we explored the network formation of lamin A and some of its disease variants, which strongly deviates from that of cytoplasmic IFs. These data give a first mechanistic clue how the lamin network provides mechanical stability to the nuclear envelope and nuclear architecture.

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The Role of Central Microtubules in the Beating of Eukaryotic Flagella, Revealed by High-Speed Holographic Microscopy

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Understanding the mechanics of the eukaryotic flagellum is a key challenge in biophysics. As well as being of scientific interest, there are clear therapeutic applications, not least in reproductive medicine. The axoneme lies at the heart of the flagellum, and its structure is known: nine microtubule doublets surround a central pair of microtubule singlets. The role of the central pair in this canonical '9+2' arrangement has been the subject of speculation for some time, though they are known to assist in regulating the flagellar beat. Our group has developed high-speed holographic microscopy that allows us to numerically refocus a digital image off-line. By generating a stack of refocused images from each frame in a video, we obtain scans of the sample volume at the frame rate of our video camera. Effectively, this allows us to image 500-1000 volumes per second. By analyzing this volumetric data, we can measure the waveform of a eukaryotic flagellum to within 200 nm in three dimensions, and with millisecond time resolution. We have previously used this method to measure the waveform of a naturally occurring, isolated flagellum: the microgamete of the rodent malaria parasite Plasmodium berghei. In order to assess the role of the central pair, we take advantage of a newly-available mutant strain that is lacking one or both central microtubules. By comparing the three-dimensional shape and movement observed in mutant flagella to that in the wild-type, we can get some insight into how the central pair helps to regulate the beating action.

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Impact of Physical Boundaries on the Mechanics of Collagen Gels and Mechanosensation

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Cell adhesion responses to in-depth physical properties such as substrate roughness and topography are well described but little is known about the influence of lateral physical cues such as tissue boundaries on the function of adherent cells. Accordingly, we developed a model system to examine remote cell sensing of lateral boundaries. The model employs floating thin collagen gels supported by rigid grids of varying widths. The dynamics, lengths, and numbers of cell extensions were regulated by grid opening size, which in turn determined the distance of cells from rigid physical boundaries. In smaller grids (200 and 500 μm wide), cell-induced deformation fields extended to, and were resisted by, the grid boundaries. However, in larger grids (1700 μm wide), the deformation field did not extend to the grid boundaries, which strongly affected the mean length and number of cell extensions (~60% reduction). The generation of cell extensions in collagen gels required expression of the ß1 integrin, focal adhesion kinase and actomyosin activity. We conclude that the presence of physical boundaries interrupts the process of cell-mediated collagen compaction and fiber alignment in the collagen matrix and enhances the formation of cell extensions. This new cell culture platform provides a geometry that more closely approximates the native basement membrane and will help to elucidate the roles of cell extensions and lateral mechanosensing on extracellular matrix remodeling by invasion and degradation.

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Extracting Quantitative Data from AFM Indentations on Soft, Heterogeneous Biomaterials

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Atomic force microscopy (AFM) is a commonly used method for extracting mechanical information about samples ranging from soft biological matter to rigid semiconductors. AFM and other force spectroscopy techniques have been recently employed to examine how the elastic properties of metastatic cancer cells differ from their healthy counterparts and to study the contractile forces that cancer cells exert during the process of invasion through an extracellular matrix (ECM). As the samples being studied with AFM become more complex, novel analysis methods must be developed to produce meaningful and quantitative data, thus new strategies for fitting force-indentation data beyond the standard Hertz model are essential. We present a method of raw data fitting which determines the apparent Young's modulus as a function of indentation depth, providing sensitivity to sample heterogeneities such as subsurface elasticity effects. An improved AFM tip shape model is derived for a spherical apex with a smooth transition to a cone to provide a realistic representation of the experimental AFM tips used. A bonded two-layered elastic model is created to include the perturbations caused by a heterogeneous material, such as a cell embedded in ECM. This model allows for understanding the signal generated from subsurface components as well as the theoretical limits for determining the elastic properties of the underlying second layer. To validate this, we performed finite element analysis simulations and AFM indentations on polyacrylamide. We also show specific examples of how these analysis methods and finite element analysis can be employed to extract more information regarding the mechanical basis of cancer cell invasion into an ECM.

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From Rheology to Elasticity: How Can We Capture the Dynamical Properties of Cells?

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Atomic force microscopy has been introduced in the late 1980's in cellular biology to probe the mechanical response of living systems with a nanoscale resolution. However in traditional force-indentation curves obtained such a tool, the discrimination of elastic, viscous and dynamical aspects of a cell response under mechanical stress is very difficult, because it assumes that we have already designed a correct model to capture this response. Actually the response of a cell span different scales in space and in time. We propose here to revisit the interpretation of force-indentation curves without any a priori model to capture the temporal evolution of the shear modulus of living cells and compare this response to simple visco-elastic models. We show that using multi-scale analyzing tools such as wavelet transforms offers the possibility to survey in real time the different response modes of a cell during an indentation experiment.

Because cells are also very different from one tissues to another one, we take cells with different adherence for this discussion; namely strongly adherent cells such a fibroblasts or myoblasts, circulating cells such as blood cells and intermediate cells that have also a very rich metabolic role such as hepatocytes.

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Cytoskeletal Stiffness Controls the Threshold of T Cell Activation

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T cells utilize a dynamic cytoskeleton to effectively scan antigen-presenting cells and become fully activated. Cytoskeletal activities help the T cell both generate mechanical forces and receive these forces from extracellular matrix and from other cells. To study the function of cytoskeletal changes during T cell activation, we modified an atomic force microscope (AFM) to deliver antigen tethered to the AFM cantilever, simultaneously ligating T-cell receptors (TCRs) and measuring changes in cytoskeletal elasticity (stiffness). We found that T cells undergo rapid cytoskeletal changes upon TCR triggering. Because traditional AFM techniques preclude measuring these cytoskeletal changes in real time, we developed a new high-bandwidth cantilever that boosts scanning speeds by 1000-fold. We show that the cytomechanical stiffness actually