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How do Cells Sense Physical Forces? Cellular Mechanosensing and Motility in Biomimetic 3D Environments

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The cellular microenvironment regulates processes such as cell division, cell migration and cancer progression. Cells *in vivo* constantly sense the physical properties of the environment such as adhesion and **mechanical confinement** and - accordingly to them - switch in between different migration modes. Embryonic progenitor stem cells derived from zebrafish embryos under mechanical confinement show **Myosin II** enrichment at the cell cortex. As a consequence cell contractility increases and eventually transforms cells from a non motile to a highly migratory phenotype, termed stable bleb (Ruprecht et al, 2015). This amoeboid transformation is highly conserved in between different cell types (Liu et al, 2015). However, how single cells are able to sense a physical force and how Myosin II is activated under confinement is still unknown. Here, by combining quantitative imaging with an interference approach, we identified a conserved minimal set of proteins necessary for **cell mechanosensation**. These two proteins allow the cells to sense the mechanical confinement and to differentiate between compression and inflation exemplified by hypotonic stress.

The tight interplay between **nuclear membrane tension** and **intracellular calcium levels** controls the mechanosensitive machinery which further activates myosin II and leads to the stable bleb motility transformation. We show that mechanical confinement induces nuclear membrane (INM) unfolding followed by INM tension increase and, in the presence of high calcium levels, cytosolic phospholipase A2 ($cPLA_2$) translocation to the inner nuclear membrane. $cPLA_2$ cleaves fatty acids, releasing arachidonic acid (AA) which further regulates TRPV4 channels and Ca^{2+} entry. Inhibition of TRPV4 related Ca^{2+} entry and $cPLA_2$ functioning completely blocks the mechanosensitive cell transformation. Altogether, the interrelated function of nuclear membrane tension and Ca^{2+} levels specify **a novel mechanosensation module** capable of reading mechanical force and osmotic inflation from the cell's microenvironment, with important impacts for amoeboid migration in diseases and cancer.

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Active transport confers specific, tuneable, and reversible mechanosensitivity to nucleocytoplasmic shuttling.

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YAP is a transcriptional regulator with roles in development, cancer and regeneration, which has been described to be mechanosensitive. Forces applied to the nucleus increase the nuclear accumulation of YAP by enhancing its active import through nucleopores [1]. However, the mechanism by which forces to the nucleus affect the active import of YAP, or any other protein, is unknown. Here we studied how forces to the nucleus regulate the active transport of molecules through nucleopores. We combined static and dynamic measurements of nuclear import signals (NLS) and nuclear export signals (NES) of various binding constants to karyopherins. We plated mouse embryonic fibroblasts transfected with GFP-NLS on substrates of different rigidity, and observed that the nuclear to cytosolic ratio increased for high substrate rigidity (where the forces applied by cells to their nucleus are higher). However, the nuclear to cytosolic ratio decreased with rigidity for GFP-NES. When we exerted forces to the nucleus by a bead attached to an AFM cantilever, we measured that GFP-NLS and GFP-NES respectively increased or decreased their nuclear to cytosolic ratio, indicating that forces to the nucleus enhance active transport across nucleopores in both directions. We also measured the dynamics of active transport through the nucleopores on gels of different rigidities by optogenetically activating a basally caged NES within an NLS-containing construct. We observed that both import and export dynamics are increased with rigidity. Our study shows a general mechanism where rigidity increases active transport through nucleopores by exerting forces to the nucleus, which could explain how cells regulate the specificity of transcriptional program activation in response to mechanical cues.

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Effect of therapeutic agents on tumor cell compartments studied by local measurement of cell stiffness via Scanning Ion-Conductance Microscopy

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Stiffness measurement of single cell via Scanning Ion-conductance Microscopy (SICM) is a novel method of studying cell mechanical properties. Due to the work principle of SICM [Korchev et al., 2009], which is allow to topography mapping with lateral and vertical nanoscale resolution. Also, it's possible to provide simultaneously stiffness mapping, due to applying low stress on cell surface [Clarke et al., 2016], whose nature is intrinsic colloidal pressure between nanopipette tip and cell membrane. Nanoscale diameter of nanopipette tip allows to obtain cell stiffness distribution on different parts of single cell. We report cell stiffness measurement of drug-induced alterations in cancer cell compartments studied by SICM, specifically, we measured fibrosarcoma cells (HT1080) transfectioned with Progerin, which is integrate in protein structure of nucleus membrane. Progerin was modified with GFP fluorescence dye (GFP-Progerin). Also, we analysed human prostate cancer cell line PC3 subjected with Paclitaxel for microtubulin stabilization and Cytochalasin-D for actin depolymerization. Experiments with GFP-Progerin were provided in heterogeneous population of HT1080 with control and GFP-Progerin transfectioned cells. Control cell stiffness measurement shows ~1.7kPa and ~0.7kPa, when GFP-Progerin treated cells increased value only on nucleus area (~2kPa). In control and treated PC3 cells we measured cell stiffness upon the nucleus area and cytoplasm area, which are show two different values in control cells (~1.3kPa and ~0.8kPa, respectively). Measured stiffness after Paclitaxel treatment shows significantly increased stiffness value on nucleus area and cytoplasm area (~4kPa and ~1.8kPa), whereas Cytochalasin-D treatment reduced cell stiffness only on cytoplasm area (~0.5kPa). As we can see, SICM-base measurement of stiffness shows different effects Paclitaxel, Cytochalasin-D, Progerin on cancer cell compartments, including actin, microtubulin and nucleus membrane, respectively. Drug-induced disruptions of these cell compartments lead to cell mechanical properties alteration, depending on inhibition mechanism.

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Hierarchical biointerfaces as smart cellular mechanoselective surfaces

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The surface topography is crucial for biomedical implant effective implantation and tissue healing. This generally involves promoting cellular proliferation around the implant surface and simultaneously preventing possible infection by bacteria. Today this is commonly achieved by the co-administration of growth factors and antibiotics upon surgery. Nonetheless, a significant percentage of artificial implants still fail due primarily to aseptic loosening and infection [1]. The possibility to control the fate of both mammalian and bacterial cells simultaneously by topographical mechanoselective means have opened up new possibilities to solve these issues. Both mammalian cells [2] and bacteria [3] have seen previously to be responsive towards the surface topography, although at different micro and nano size ranges. This is due to the differences in physiology, morphology and size between bacteria and mammalian cells. As such, the design of effective topographical features, that not only prevent bacterial colonization, but also promote mammalian cell proliferation is extremely challenging. This work deals with this challenge by creating a convergent design of nano and micro hierarchical topographies. A novel fabrication process combines sequential nanoimprinting with optical lithography steps. This process allows for a simple and well-controlled hierarchical structures fabrication, where the nanofeatures cover the entire micropattern. These topographies have been fabricated onto biocompatible polymers. Hierarchical surfaces provide unique physical environments allowing for testing different biological scales at once. As a biological source, we employ mesenchymal stem cells because of their in vitro capacity to form differentiated cellular identities, which play a key role in tissue regenerative processes. Results have shown that bacteria are sensitive to the nano-scale where bacterial lysis is found. The impact of the hierarchical topography on mesenchymal stem cells surface topography in terms of morphology, cell growth and differentiation, is under study. Previous tests in mammalian cells are shown

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