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Abstracts

Sponsors and Supporters

Shear Stress-Induced Mechanobiological Changes in Epithelial Tissues

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Headline

Epithelial cells, forming the protective linings around internal organs, body cavities and the outer layer of the skin, endure diverse mechanical stresses in the body. We use a stress-generating device to study tissue's response to solid shear stress. Our research focuses on stress relaxation, analysing cell behaviour and morphological changes.

Abstract

Epithelial cells in the human body, serve vital functions such as respiration, nutrient absorption, and environmental sensing, thanks to their strategic organization. The epithelial tissue also has a critical role as a protective barrier, lining body cavities, organs, and blood vessels. To ensure effective protection, epithelial cells establish strong cohesion through tight junctions, adherens junctions, desmosomes, and gap junctions. These cell junctions undergo dynamic remodelling during growth, involving the creation of new junctions through cell divisions or the removal of junctions via live cell extrusions and cell death. [1,2]

Additionally, epithelial cells exhibit apico-basal polarity, vital for directional transport, sensory perception, and barrier function across various tissues such as intestinal linings, lung alveoli, and embryonic epithelia. Due to their protective function as a barrier, they are subjected to a wide range of mechanical pressures within the human body's environment, ranging from the rhythmic pulsations of blood circulation to the dynamic strains of physical activity. Understanding the impact of external mechanical stress on epithelial cells and their mechanical properties is essential. [1,3]

The structure and changes of epithelial monolayers and their response to physical forces are not entirely comprehended. It's crucial to understand how stress affects tissues to uncover how cells respond and how tissues function. Therefore, we utilize stress-inducing apparatus to methodically explore the impacts of various stress forms on the tissue. Although fluid shear and uniaxial stress have been thoroughly examined, the impact of solid shear stress transmitted via the extracellular matrix (ECM) remains relatively unexplored. This study utilizes a specially designed device capable of applying precise shear stress to the substrate that supports epithelial cell cultures.

To examine how epithelial tissue responds to shear stress, we culture MDCKII cells on polydimethylsiloxane (PDMS) substrate, which is attached directly to the shear device. The setup includes a special incubator with a water reservoir for added humidification and holes in the lid for CO2 exchange. Different clamp sizes adjust the pre-stretch to prevent substrate ruffling. A slider enables upper substrate movement, with a millimetre scale to control shear. [Figure 1] An adapter plate helps mounting the shear device on a microscope stage, and incubation conditions are maintained using a

custom adapter for live-cell incubation. After exposing the examined cluster to solid shear stress, we methodically record the tissue's reaction to the applied stress under the microscope. [Figure 2]

Our study explores the effects of solid shear stress on cellular behaviour, including the analysis of the short-term effects as cell area, elongation, perimeter, orientation, and long-term reactions such as proliferation, apoptosis, stress relaxation, and T1 transitions at the cell membrane, which involve neighbour exchanges.

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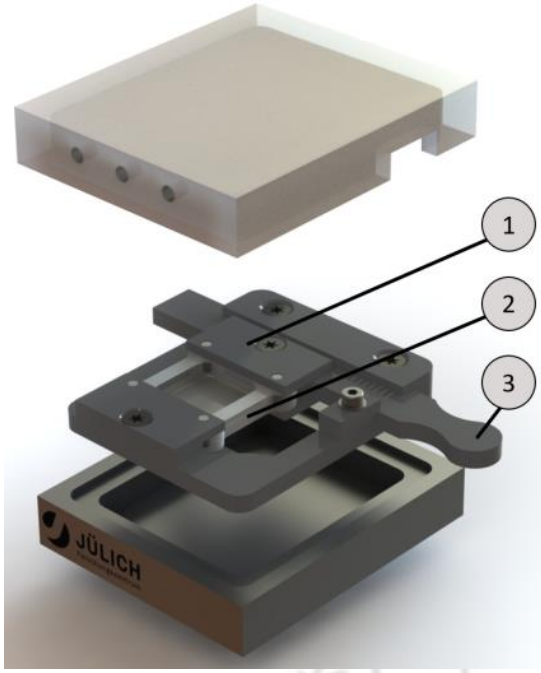

Figure 1. Design of the manual shear stretcher:

1-clamps, 2-substrate, 3-slider

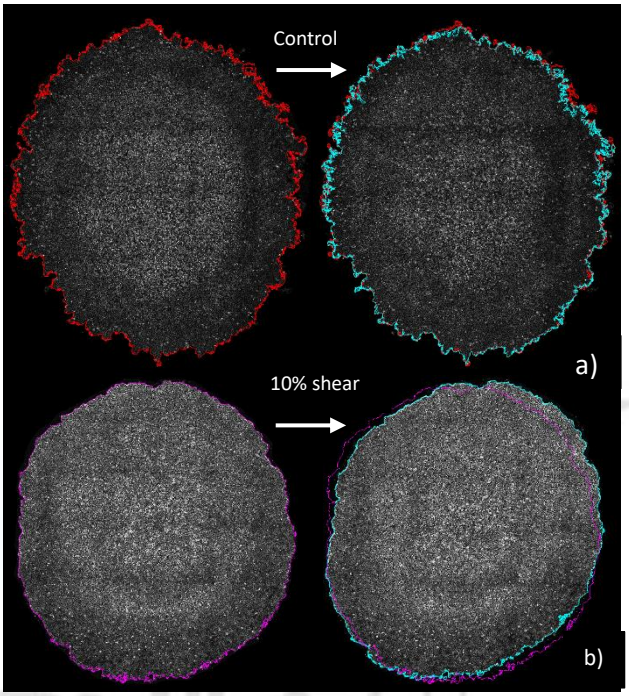

Figure 2. a) Control cluster b) 10% sheared cluster:

Engineering biomimetic multi-component fibrillar extracellular matrices to modulate tissue-specific cell response from a single cell adhesion to organoids

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Headline (max 400 characters including spaces)

Here, we used fibronectin as a building block to engineer multi-component fibrillar extracellular matrices (ECMs) in an easily tunable manner. By mimicking target ECM components, these matrices enabled to modulate tissue-specific cell responses from an integrin-mediated adhesion initiation, functional 3D tissue formation of neurons and epithelial cells, and to early-stage organoid morphogenesis.

Abstract (max 2 pages for the whole document)

Cells assemble the major extracellular matrix (ECM) protein fibronectin (FN) into fibrillar matrices to assemble tissue-specific 3D scaffolds together with other ECM proteins. However, although recent platforms enable to bioengineer 3D fibrillar FN matrices *in vitro*, it remains elusive how FN can be co-assembled with other ECM proteins into complex 3D fibrillar matrices that recapitulate tissue-specific compositions and promote cellular responses. Here, we introduce the engineering of fibrillar FN-templated 3D matrices that can be complemented with other ECM proteins, including vitronectin, collagen, and laminin, by using innate co-assembly mechanisms. Through recapitulating various tissue-specific ECM compositions and morphologies, the large scale multi-composite ECM matrices can guide fibroblast adhesion, tissue formation, or tissue morphogenesis of epithelial cells. In other examples, we customize multi-composite 3D fibrillar matrices to support the growth of signal propagating neuronal networks or of human brain organoids. We envision that these 3D ECM matrices can be tailored in scale and composition to modulate tissue-specific responses across various biological systems, and thus to considerably advance manyfold studies of cell biological systems.

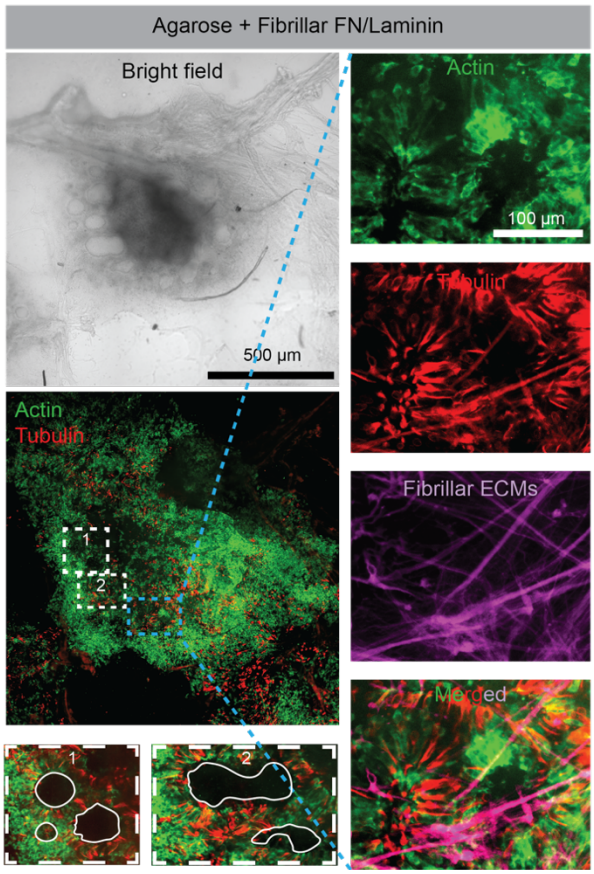

Figure 1. Brightfield and confocal images of brain organoids at day 10 (4 day after seeding embryonic body) within agarose + 3D fibrillar fibronectin/laminin matrices.

Collagen Microarchitecture Drives Breast Cancer Cell Fate Independently of Matrix Stiffness

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Headline (max 400 characters including spaces)

Tuneable 3D in vitro models were developed to monitor mechanobiological changes during breast cancer progression.

Abstract (max 2 pages for the whole document)

Breast cancer (BC) is the leading cause of cancer related mortality in women globally [1]. Breast cancer (BC) is the leading cause of cancer-related mortality in women globally [1]. Despite lacking hormonal targets, aggressive BCs display extracellular matrix (ECM) stiffening and abnormal collagen reorganization. These aberrant microenvironments potentiate epithelial-mesenchymal transition (EMT) and invasion [2-3].

To isolate the effects of ECM microarchitecture from mechanics, we developed tuneable collagen fibrillar scaffolds with healthy (porous) and cancer-like (dense) organizations with constant stiffness. Non-malignant (MCF10A) and BC lines (estrogen receptor+ MCF7; triple negative MDA-MB-231) were cultured on these networks for 7 days. Despite consistent collagen mechanics, BC cells demonstrated enhanced collagen bundling and realignment when interacting with dense (cancer-like) scaffolds, which increased with more invasive phenotypes. Additionally, BC cells acquired aggressive traits including elongated morphology, enhanced proliferation, and metabolism, and elevated EMT markers. Fibronectin deposition was also altered in cancer-like scaffolds. Our data underlines collagen architecture as an independent driver of BC aggression, enabling EMT and invasion. This novel platform elucidates specific cell-ECM interactions, receptor dynamics, and microenvironment mechanics exploited in cancer progression, unveiling new non-hormonal biomarkers.

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Hydrostatic pressure drives sprouting angiogenesis via adherens junction remodeling and YAP signaling

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Endothelial cell physiology is governed by its unique microenvironment at the interface between blood and tissue. One major contributor to endothelial biophysical environment is blood hydrostatic pressure, which in mechanical terms applies isotropic compressive stress on the cells. While other mechanical factors such as shear stress and circumferential stretch have been extensively studied, little is known about the role of hydrostatic pressure and the underlying mechanotransductive regulation of endothelial cell behavior. Here, for the first time, we show that hydrostatic pressure triggers partial endothelial-to-mesenchymal transition at values mimicking capillary pressure environments. Pressure promotes proliferative and migratory behavior and impairs barrier properties that are characteristic of a mesenchymal transition, resulting in increased sprouting angiogenesis in 3D organotypic model systems *ex vivo* and *in vitro*. Mechanistically, this response is linked to differential cadherin expression at the adherens junctions and to an increased YAP expression, nuclear localization, and transcriptional activity. Inhibition of YAP transcriptional activity prevents pressure-induced sprouting angiogenesis. Altogether, this work establishes hydrostatic pressure as a key modulator of endothelial homeostasis, and as a crucial component of the endothelial mechanical niche.

Compression force induces myosin II-dependent viscoelastic deformation and stiffening of single cells.

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Headline (max 400 characters including spaces)

In this work, we show how short and local compression induces a twofold increase in the elastic modulus of single cells. The transient change in mechanical properties is linked to the viscoelastic deformation of cells, as these remain temporarily compressed once the load is removed. Myosin II was found to be the leading regulator of this behavior.

Abstract (max 2 pages for the whole document)

In living tissues, single cells are continuously exposed to a plethora of physical stresses emerging from adjacent cells, the extra cellular matrix, pressure in interstitial spaces or vascular flow. The ability of cells to sense and adapt to these external mechanical inputs, referred in the field as mechanosensation and mechanotransduction, is essential for cells and tissue homeostasis [1, 2]. The response of cells to mechanical inputs is highly dependent on the frequency, duration and magnitude of the stimulus [3]. Several studies have highlighted the activation of different biochemical pathways in response to external mechanical compression in healthy and diseased conditions [4]. However, most of these reports focused on the compression of entire cells [5-8]. Consequently, we developed a protocol to investigate the effect of local compression on the mechanical properties of single cells. Also, compression parameters as frequency or duration, were separately modified to systematically discriminate their individual influence on cells behavior upon compression. Using a microcantilever mounted on an atomic force microscope, we quantified the elastic modulus of single Hela Kyoto cells before and after being compressed. 5 s after compression, cells presented a significant increase in elastic modulus which lasted up to 60 s after compression, when initial mechanical properties were restored. Similar observations were made when compressing on top of the nucleus or on top of the cytoplasm. Also, away from the location of compression the elastic modulus was found to decrease. Quantifying the height of single cells, we observed a viscoelastic deformation as cells would remain in a compressed state 5 s after compression and would recover their initial height only 65 s after compression. Performing inhibitory assays, we identified myosin as a primary regulator of the change in height and elastic modulus. In fact, when inhibiting or activating myosin II before compression we observed a decrease in compressive strain and no significant change in elastic modulus 5 s after compression. To differentiate the effect of different compression parameters, we modified them one at the time. Results showed that the change in elastic modulus was not dependent on the magnitude of the load, whereas it was influenced by the speed of compression to some extent. Increasing the time of compression to 300 s drastically reduced the change in elastic modulus whereas cells would maintain a higher elastic modulus upon cyclic compressions. In conclusion, we showed that short and local compression is enough to produce a change in the biophysical properties of single Hela cells. This mechanical input leads to a viscoelastic deformation in single cells which results in a transient yet significant increase in elastic modulus. This behavior is primarily regulated by myosin II in a compression-time dependent manner.

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The impact of mechanical cues on axonal guidance and Piezo 2 channels in dorsal root ganglion

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Headline

How mechanical stimuli affect the development of DRG functionality

Abstract

Dorsal root ganglion (DRG) neurons play a central role in the somatosensory system and serve as crucial components in the pathophysiological alterations leading to neuropathic pain resulting from nerve injury or inflammation. One notable characteristic of DRG neurons lies in their distinctive morphology, where a singular process divides into both a peripheral and a central axonal branch. Each neurite possesses distinct functions and cellular characteristics, as for instance the presence of the mechanosensitive Piezo 2 channels in the afferent fibres. Piezo 2 channels are the main transducers of the mechanical stimulus for the sense of touch [1], and are involved also in pathological conditions as chronic pain [2].

In spite of the interest in the field, the factors that determine DRG neurons functionality and Piezo 2 specific localization and organization, and in particular what is the role played by mechanical forces has not been yet unveiled. The use of nano and micro-patterned substrates, including grooves and pillars, provides a controlled and reproducible tool for inducing neural cell polarization and axonal guidance. This tool proves valuable for exploring diverse aspects of DRG behavior, encompassing the morphology, the arrangement of Piezo 2 channels and the cellular responses to distinct microenvironmental cues. To investigate how topographical or mechanical stimuli can promote directional axonal elongation and the Piezo 2 channels' expression and organization in cells, we exploited directional patterned substrates. By solvent casting, we fabricated polydimethylsiloxane (PDMS) micro-grooved substrates, gratings (GRs), with a period of 2 µm and 20 µm (period= ridge + groove width). HEK cells transfected with Piezo 2-green lantern and DRG cells were cultured on standard and GR substrates. The Piezo 2-green lantern, a fluorescent chimera protein that enables to localize the Piezo 2 in the cell, was exploited to study the organization and expression of Piezo 2 channels. The impact of the topographical features on DRG and Piezo 2 arrangement was investigated by confocal microscopy.

This work is supported by PRIN 2020- Project Touch on a chip.

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Replacement of tumor xenograft mouse models by biophysically and molecularly defined 3D in vitro systems

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Headline (max 400 characters including spaces)

Development of a 3D scaffold-based spheroid tumor model to capture relevant physiological components of the tumor microenvironment in cutaneous squamous cell carcinoma: integrating tissue-specific adhesion-mimetic cues and stromal cells in alginate scaffolds to enhance physiological relevance of *in vitro* tumor spheroid model.

Abstract (max 2 pages for the whole document)

In vitro models mimicking pathophysiological characteristics of cutaneous squamous cell carcinoma (cSCC) are critical for basic and translational cancer research. 3D models serve as a bridge between 2D cell culture models and *in vivo* xenograft models. Here, spheroids have shown immense potential in replicating critical aspects of tumor architecture. Spheroid models are commonly utilized in a scaffold-free context, but these lack crucial interactions with the physical and cellular environment, recognized as critical factors in tumor growth and tumor invasion.

Here we present a microenvironment-inspired, 3D scaffold-based spheroid tumor model that captures relevant physiological components of the tumor microenvironment in cSCC (Figure 1). We initially tested natural (alginate (ALG)) and synthetic poly(ethylene-glycol) (PEG) polymers materials for the scaffold. Alginate was selected based on the ability to tune stiffness and stress relaxation properties of the scaffold, as well as the potential to conjugate adhesion-mimetic peptides by click chemistry to maleimide-functionalized alginate.

We encapsulated pre-formed A431 cancer cell spheroids in a mechanically and biochemically-defined ALG scaffold allowing for interactions with a physical and cellular environment. To capture the effect of interactions between cancer cells and stromal cells within the model we integrated activated fibroblasts called cancer associated fibroblasts (CAF) into the cancer cell spheroids as they are a crucial cell population in the tumor environment, remodeling surrounding ECM and secreting various factors favoring tumor growth and progression [1][2]. This resulted in an A431–CAF spheroid co-culture encapsulated in alginate hydrogels. The presence of CAF within the model induced visible effects on spheroid growth relative to cultures consisting solely of A431 cancer cells. The influence on spheroid growth is also more pronounced when compared to co-cultures with normal human fibroblasts (NHFs) (Figure 2).

To mimic the specific ECM of the cSCC environment, we wanted to reproduce features of the epidermal, basement membrane and dermal layers. These include the physical properties and especially the adhesive properties of the ECM, as these are known to significantly influence cancer cell growth and migration potential. Drawing insights on ECM composition from documented proteomic data of *in*

vivo cSCC [3][4][5], we propose to replicate these crucial cell–ECM interactions. We will screen for the essential adhesive cues on 2D hydrogels and then incorporate them in 3D hydrogel scaffolds.

To investigate the mechanobiological role of different adhesion-mimetic peptides, we will seed A431 cancer cells onto a hydrogel surface presenting different combinations of adhesion sequences. Identification of adhesive ECM components, favoring cancer cell growth and migration, will be done by evaluating the potential of cancer cell expansion in 2D with the help of a migratory assay. The actively engaged cell receptors (integrins) will be identified by immunostaining and will serve as validation for the selected adhesive cues. This will enable the replication of adhesive cues in 3D ALG hydrogel scaffold present in the *in vivo* tumor microenvironment, and help us design biophysically and molecularly relevant tumor cancer models.

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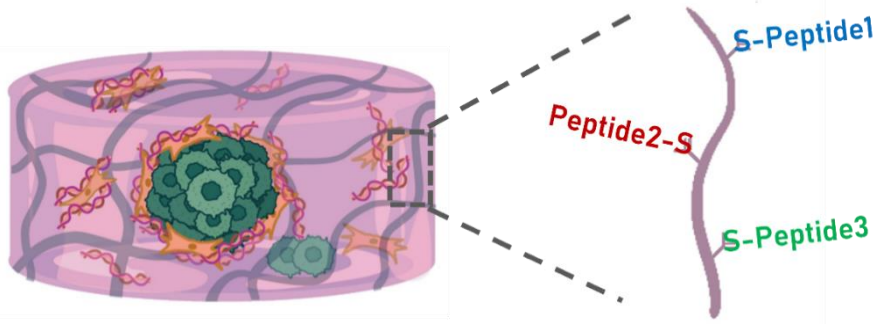

Figure 1. Representative illustration of a microenvironment-inspired 3D scaffold-based spheroid tumor model. The thiol group 'S-' is used to attach the peptide to maleimide-functionalized alginate polymers.

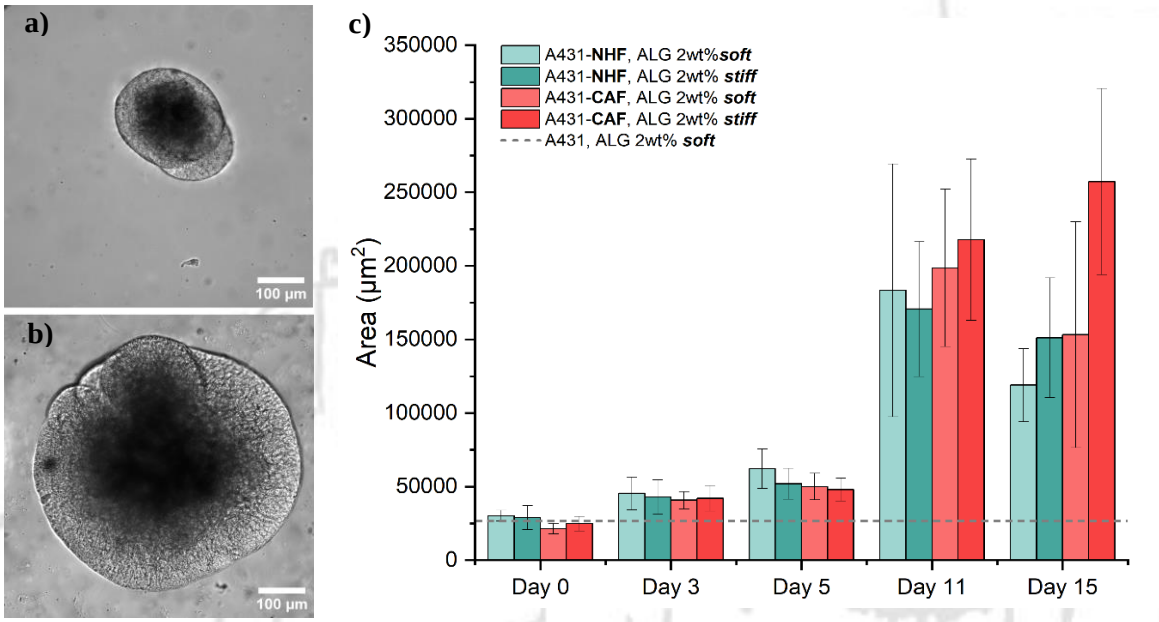

Figure 2. (a) A431 cancer cell spheroid in alginate hydrogels (2wt%), Day 11; (b) A431-CAF spheroid co-cultures in alginate hydrogels (2wt%), Day 11; (c) Growth rate of cancer cell-fibroblast spheroid co-cultures in alginate hydrogels (2wt%).

Optimizing cell-based processes through ultrasounds: a focus on yeast and microalgae

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Headline

The use of a sono-(photo)bioreactor, able to transduce highly characterised ultrasound signals (US) in standard reactors, allowed the study of the effect of tailored stimulation for the optimization of yeast and microalgae-based processes. Data show a US-mediated increase in yeast growth, while in microalgae US induce power-selective enhancement of biomass and a shift in macromolecular content.

Abstract

Yeast biomass plays a fundamental role in the brewing industry, most brewers inoculate newly propagated yeast from master cultures into each fermentation batch. Moreover, industrial breweries have implemented a standard 'in house' biomass production process to avoid handling and transport. A critical point of this system is the poor fermentative efficiency of the newly propagated biomass during initial wort fermentation, which is probably due to lack of synchronicity in the cell population and the small cell size obtained in aerobically grown cells. The use of dried yeast, already a common practice in wineries, has been tested as an alternative for brewing yeast. Hence, research and industry require technologies to increase the yield without impacting the sustainability of the overall process[1]. The use of ultrasound (US) represents a valid alternative to increase yeast biomass production. O. Schläfer and colleagues showed that intermittent sonication leads to final biomass concentration increase from 0.12 to 0.4 g/L [2]. In microalgae, few papers underline US capability to influence cultures in terms of biomass productivity and accumulation of highly valuable compounds. In *Tetradesmus obliquus,* intermittent US treatment induced an enhancement of lipid and β-carotene yield, by 34.5 and 31.5%, respectively [3].Literature shows promising, but sometimes elusive data regarding US-induced bioeffects, underlying the need of US waves to be highly characterised in terms of power, frequency and application mode (continuous or intermittent). US application mode emerges as a pivotal aspect to consider, since this mode affects growth and production of highly valuable compounds.

In this study, a novel platform for lab scale US irradiation is exploited, the sono-(photo)bioreactor (SN(P)BR, (Fig. 1a), developed and presented in the Chicarella et al contribution. The system consists of a transducer glued to a 2L flask, connected to a device that generates an electrical impulse to be converted into a mechanical impulse. The SNBR was exploited to test the effects of US on processes based on yeast and on microalgae cultures. In detail, two different algal strains were tested, *Chlorella vulgaris CCAP 211/12* and *Desmodesmus* sp. *VRUC 281* along with one strain of the yeast *Saccharomyces cerevisiae*, *US-05* (SafAle™). In algae, the SNPBR was exploited with light supplementation, due to the autotrophic metabolism of algae. Tests were carried out at three US intensity levels (1x, 2x and 4x) for 96h stimulation to investigate the culture response at the stationary phase, in terms of biomass productivity and macromolecule content (as lipids, carbohydrates and proteins). In microalgae, US correlates positively to biomass production, reaching 2.25 g/L in *Desmodesmus* sp., up to 30% more biomass vs control (1.72 g/L), whereas in *C. vulgaris* lower levels were obtained. It is observed that *Desmodesmus* sp. stimulated by US intensity 2x reaches protein concentration of 2.23 µg/ml, with control value of 1.80 µg/ml (Fig 1b). SNBR was employed for anaerobic growth of *Saccharomyces cerevisae* US-05, to test the effect of US treatment on cell growth and biomass productivity. Time course optical density monitoring shows US treatment to increase cell concentration, confirming the potential of US in stimulating cell growth (as shown in Fig.1c). US treatment emerges as a tool to increase biomass productivity and bioproduct yield; this technology may contribute to reduce production and environmental costs related to cell-based processes. Further studies will be focused on studying the signal transduction, with a focus on cellular mechanoreceptors. Light on their role in mechanical signal transduction, will pave the way to further understand the mechanism responsible for cell response to US.

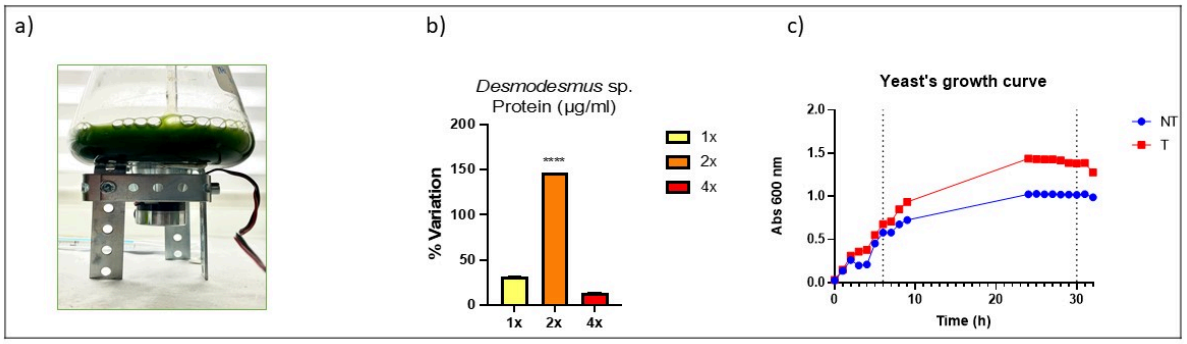

Figure 1. Ultrasound-mediated optimization of green microalgae and yeast processes. A detail of the sono-bioreactor (a). The effectiveness of the system represented with an increase in the macromolecular content, in particular protein content (b). A *Saccharomyces cereviasi*e US-05 strain growth curve: in the treated sample, values are higher than in the control (c).

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Chemical and mechanical stressors impact on astrocytic phenotype and function: Insights from a 3D alginate-based model

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Headline (max 400 characters including spaces)

Astrocytes, one of the most abundant glial cells in the brain, have been shown to participate in neurological disease onset and progression. Developing pioneering *in vitro* models that better recapitulate the brain's physiological environment, is crucial for the study of pathophysiological mechanisms and the discovery of new drugs for CNS disorders.

Abstract (max 2 pages for the whole document)

A great number of neurological diseases lack effective treatments and the study of the pathological mechanisms involved remains an important research target. In the past years, the involvement of glial cells in the onset and progression of neurological disease has been highlighted. Astrocytes, which belong to the glial cells of the brain have been shown to actively participate in several diseases by maintaining both neuroprotective and neurotoxic traits. Due to the dynamic changes they undergo upon an insult, a process often termed as "astrogliosis", astrocytes have been considered targets for the discovery of new therapeutic approaches for several central nervous system (CNS) neuropathologies, such as stroke [1]. The changes in gene and protein expression are often accompanied by changes in the extracellular environment, and the extracellular matrix (ECM) biomechanical cues are being investigated as possible mediators of neurological processes [2]. Nevertheless, the link between astrocytes' phenotypic and functional changes with the mechanical properties of the ECM have not yet been elucidated.

Here we propose the development of a 3D *in vitro* model to explore astrocyte behaviour in response to different stimuli: chemical, by subjecting the cells to oxygen and glucose deprivation, and mechanical, by changing the extracellular environment mechanical properties.

Primary rat astrocytes were encapsulated in modified alginate hydrogels containing the cell adhesive peptide RGD, incorporated via carbodiimide chemistry, and the matrix metalloproteinase sensitive peptide PVGLIG, engrafted through reductive amination, on partially oxidized alginate [3]. For the chemical stimulus, astrocytes were embedded in 1% (w/v) modified alginate hydrogels and kept in a media lacking glucose and in a hypoxic chamber (<1% O2) for 6h, 24h or 48h. The cell response was assessed by measuring metabolic activity and lactate dehydrogenase (LDH) release, immunofluorescence for glial fibrillary acidic protein (GFAP) and gene expression for astrogliosis marker genes, in different timepoints after reestablishment (R) of oxygenation and nutrition. For

assessing the impact of mechanical properties on astrocytes phenotype and function the cells were cultured in 1% (w/v) modified alginate hydrogels and kept either in control conditions or subjected to a barium chloride ($BaCl_2$) bath for inducing an increase in hydrogel's stiffness. Alternatively, modified alginate matrices with different polymer concentration and oxidation degree were produced (1%, 2%, 3%) (w/v) to evaluate the astrocytic response in a gradient of different stiffnesses, in normoxia and hypoxia. Cell response was assessed by measurements of metabolic activity, immunostaining for GFAP and calcium imaging.

The metabolic activity of astrocytes decreased with increased OGD/R time while LDH release increased with prolonged OGD duration, showing a more pronounced effect after 48h of OGD. From the gene expression analysis, a significant decrease in GFAP expression after 24h of OGD was observed, indicating the impact of prolonged OGD challenge on the cells. Calcium imaging showed that embedded astrocytes exhibit spontaneous activity indicating maintenance of physiological function within the modified alginate matrices. Astrocytes exhibited spontaneous activity in control and stiffer hydrogels while increasing the stiffness of the matrices led to phenotypical changes, as shown from altered GFAP protein expression.

An OGD protocol was successfully implemented in a 3D *in vitro* glial tissue engineered hydrogel-based system. In the future, we will aim at combining the different chemical and mechanical stimuli to explore how matrix alterations impact astrocytes' phenotype in the context of ischemic conditions and possibly reveal new avenues to tackle this CNS pathology.

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Acknowledgments

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Piezo1 is a key mechanosensor of soft tissues viscoelasticity

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Headline

We have established a system in which to investigate the role of Piezo1 in mediating mesenchymal stem cell behaviour in response to physiologically emulating viscoelastic cues at different stiffness scales; describing for the first time the importance of Piezo1 in transducing soft tissues viscoelasticity.

Abstract

In the last 30 years, it has been shown that cells exert and perceive mechanical forces as part of physiological (e.g., tissue formation) and pathological (e.g., cancer progression) processes; leading to what is now known as the field of mechanobiology. The most common approach in the field has been to study cellular processes in response to substrates of defined elasticity (commonly referred to as stiffness); however, tissues behave as viscoelastic solids, and energy dissipation (e.g., stress relaxation) has recently shown to strongly influence cell behaviour[1]. Mechanosensitive ion channels have emerged as fundamental proteins in sensing substrate stiffness. Among those, Piezo1 has been proposed as a key mechanosensor in cells, with its discovery being awarded the 2021 Nobel Prize in Physiology and Medicine[2]. Despite this, the exact mechanism by which this channel perceives mechanical cues and transduces them to affect cellular behaviour is not fully understood. To address this gap, the mechanisms by which Piezo1 is involved in transducing physiologically relevant viscoelastic cues were investigated.

For this purpose, three pairs of 2D polyacrylamide hydrogel groups were initially designed. Each pair was comprised of a traditionally elastic (slow stress relaxation) and viscoelastic (fast stress relaxation) counterpart with the same initial Young's modulus, to decouple stiffness and viscoelastic cues at three different stiffness scales (E=0.3 – 25 kPa). Secondly, mechanosensitive mesenchymal stem cells (Y201 MSCs)[3] were used to investigate how Piezo1 expression affects cellular behaviour when cultured on the polyacrylamide substrates of controlled viscoelastic properties. Channel expression was transiently suppressed in Y201 MSCs using siRNA.

We demonstrated that cytoskeletal dynamics were controlled by Piezo1 expression (reduced cell spreading area, focal adhesion formation, increased actin retrograde flow and low traction force generation) in response to viscoelasticity. Increasing viscosity showed a stiffness-dependent response: at low stiffness, increasing substrate stress relaxation enhanced cell spreading area, focal adhesion formation, reduced actin retrograde flow and increased traction force generation. At medium and stiff ranges, the opposite happened. RNA sequencing supported observed phenotype, and proposed stiffness-dependent mechanisms for cell response to substrate viscoelasticity

We have established a system in which to investigate the function of Piezo1 in mediating cellular behaviour in response to physiologically emulating mechanical cues at different stiffness scales. Furthermore, the Y201 MSCs offer a flexible and tuneable cell model in which to investigate how protein or gene expression changes can alter cell response to substrate viscoelasticity. In this case, Piezo1 was transiently knocked down to assess how the mechanosensitive ion channel was involved in sensitively sensing and relaying the engineered mechanical cues. This project described for the

first time the cell phenotype and transcriptome in response to varying substrate viscoelasticity and Piezo1-expression.

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The cell-surface metalloproteinase ADAM17 is involved in immediate epithelial cell responses to mechanical signals transduced by Piezo1

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Headline

ADAM17 is induced upon activation of Piezo1 and mediates proteolytical release of the growth factor amphiregulin and the junctional adhesion molecule A. This can be one reason for mechanically induced tissue damage and altered tissue repair. Here we provide evidence for a role of this mechanism in ventilator-induced lung injury and dermal wound healing.

Abstract

Epithelial cells are constantly exposed to various mechanical forces, such as stretching during respiration or compression of the skin. The physiological mechanical exposure of the cells is important for the functional maintenance of the tissue, such as structural integrity, a proper inflammatory response and wound healing. This involves a regulation of the proliferative and migratory potential of cells, the permeability, and the expression profile of inflammatory proteins. Therefore, a chronic pathological alteration of these mechanical stimuli can also contribute to the development or progression of diseases due to dysregulation of the aforementioned processes. Mechanical stimuli are sensed, among others, by mechanosensitive (MS) ion channels, such as Piezo1, which rapidly convert the stimuli into an intracellular signal and, thus, influence the cellular functionality. However, this intracellular signal must be further processed and converted to achieve a functional response. The cell-surface metalloproteinases of the a disintegrin and metalloproteinase (ADAM) family are possible candidates how this could be done. With their limited proteolytic cleavage of chemokines, adhesion molecules, receptors, growth factors, and signalling molecules, they can be important mediators of immediate functional reactions to mechanical signals. In this study we demonstrate that activation of the MS ion channel Piezo1 by chemical and mechanical stimuli, such as stretching, leads to increased proteolytic activity of ADAM10 and ADAM17 in lung and skin epithelial cells. For the lung epithelial cell line H441 we could show that increased ADAM activity results in the release of the growth factor amphiregulin (AREG) as well as the adhesion molecule JAM-A which was measured via ELISA or immunofluorescence (IF). The increased shedding activity in response to stimulation of Piezo1 is strongly reduced in *PIEZO1* knockdown cells. Furthermore, we could confirm with the preferential ADAM17 inhibitor TAPI-1 that AREG release is due to ADAM17 activity. IF staining of JAM-A reveals an overall reduction of JAM-A and a thinning of the cell adhesion width which indicates a functional implication in permeability regulation. In addition, we studied a possible clinical relevance for the Piezo1-ADAM axis using the murine ex vivo model of isolated perfused lung (IPL). High-pressure ventilation of IPLs leads to increased levels of AREG and the murine CXCL8/IL8 homologue KC which can be counteracted by pre-treatment of the newly described Piezo1 inhibitor salvianolic acid B (SalB). This indicates that Piezo1 might be a promising pharmacological target to prevent ventilator-induced lung injury (VILI). In parallel to our experiments with lung epithelial cells, we could show that

the Piezo1-ADAM axis is also relevant in keratinocytes. We observed an increased AREG and JAM-A release in the immortalised keratinocyte cell line HaCaT as well as in primary keratinocytes. This was further studied by *PIEZO1* knockdown and specific inhibition of ADAM10 and ADAM17 which revealed that Piezo1-mediated JAM-A cleavage is mediated by ADAM10 and ADAM17. Functional experiments indicated an important role of the Piezo1-ADAM axis in proliferation and migration in keratinocytes. Activation of Piezo1 leads to a drastic decrease in proliferation and migration which can be attenuated by a *PIEZO1* knockdown. Finally, we found increased AREG release of primary skin equivalents in response to wounding which highlights that the Piezo1-ADAM axis is important in dermal wound healing. In summary we could show that distinct metalloproteinases can be activated via the mechanosensitive ion channel Piezo1 in epithelial cells of the lung and the skin. Due to the versatile nature of the investigated metalloproteinases, these findings should be important for the understanding of many physiological and pathophysiological processes in epithelial cells, such as tissue development, maintenance of tissue functions, inflammatory response, fibrosis, and cancer. Furthermore, we provided evidence that the Piezo1-ADAM axis might be a promising target for the prevention of VILI and improved wound healing.

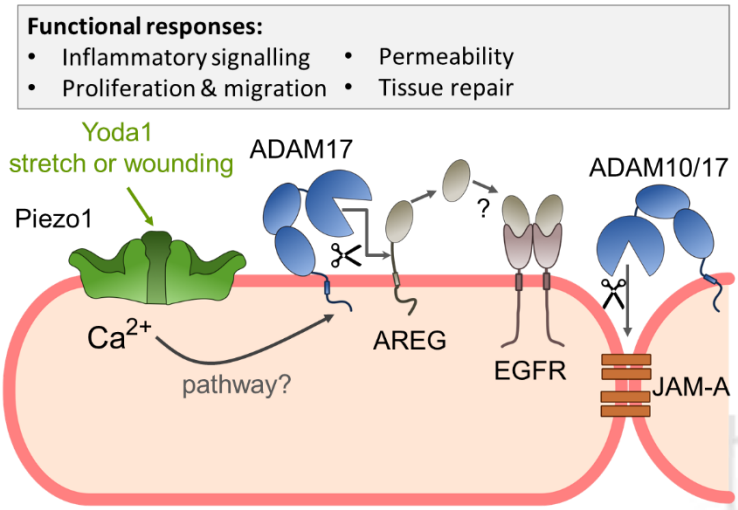

Figure 1: Activation of Piezo1 by Yoda1 or mechanical stimuli induces ADAM17 activity through a yet unknown pathway. This leads to the proteolytic cleavage of amphiregulin (AREG) and the junctional adhesion molecule A (JAM-A). By this, the Piezo1-ADAM17 axis can influence functional responses of cells to mechanical stimuli.

Tissue fluidification in pathophysiology: contact percolation sets phase transition and genetic rewiring in heterogeneous breast cancers

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Headline

In a breast cancer model, we uncover a chemo-mechanical axis that drives collective migration and induces a pro-inflammatory response in mechanically diverse mixed cell populations. Specifically, contact percolation of fluid-like and highly mobile RAB5A-overexpressing cells induces flocking migration of 2D monolayers and a morpho-phenotypic switch in otherwise solid-like and immotile control cells.

Abstract

The transition from ductal carcinoma in situ (DCIS) to invasive ductal carcinoma (IDC) is marked by increased cell motility and a transformative phase transition (PT) from a solid to a liquid-like state at the tissue level. Our recent study revealed the role of RAB5A, a key endocytic small GTPase overexpressed in DCIS, in promoting tissue fluidification and breast cancer progression [1,2]. This PT also activates an inflammatory gene response through the cGAS/STING pathway, coinciding with the acquisition of malignant characteristics [3]. While previous research mainly used genetically identical cell models, breast carcinoma comprises heterogeneous cell populations with variations in genetics and mechanics. The interplay among mechanical, genetic, and phenotypic diversity, characteristic of solid malignancies, and its interaction with carcinoma collective dynamics, particularly its impact on PT and cancer progression, remains inadequately explored. To address this gap, we employed densely packed epithelial monolayers composed of a mixture of control and RAB5A-expressing MCF10DCIS.com breast tumorigenic cells, mimicking the genetic and mechanical diversity seen in DCIS. Using time-lapse microscopy, we observed a transition to a flocking fluid mode of motion, characterized by collective and coordinated cell migration, occurring at a crucial tissue composition state. This transition takes place when RAB5A-expressing cells reach a critical level of cell connectivity, known as the contact percolation threshold, that coincides with the formation of an interconnected cell network throughout the system. By using mixed cell populations in which percolation is locally hindered, we established a causal relationship between the percolation threshold and the initiation of the fluid-like transition through flocking. Remarkably, numerical simulations using an agent-based biophysical model revealed that this transition induces a phenotypic shift in control cells. Specifically, we experimentally verified that control cells acquire elongated cell shapes and they develop cryptic lamellipodia oriented in the direction of collective motion, similar to what is observed in uniformly RAB5A-expressing cells. This morphological transition is also accompanied by transcriptional reprogramming toward a pro-inflammatory profile, suggesting that a mechano-chemical communication between fluidized and solid-like cells influences the fate and behaviors of heterogeneous malignant model tissues. Consistently, we found that RAB5A cells secrete pro-inflammatory factors, which, along with their direct intercellular physical contacts, induce an inflammatory phenotype in control cells. In summary, our study underscores the impact of contact

percolation, a geometric feature, on the collective migratory behavior of heterogeneous tumoral tissues. This phenomenon also initiates an inflammatory gene transcription program in mixed models of breast carcinoma cells. We propose that chemo-mechanical feedback mechanisms related to contact percolation might be exploited by tumors to enhance local invasion and acquire pro-metastatic traits.

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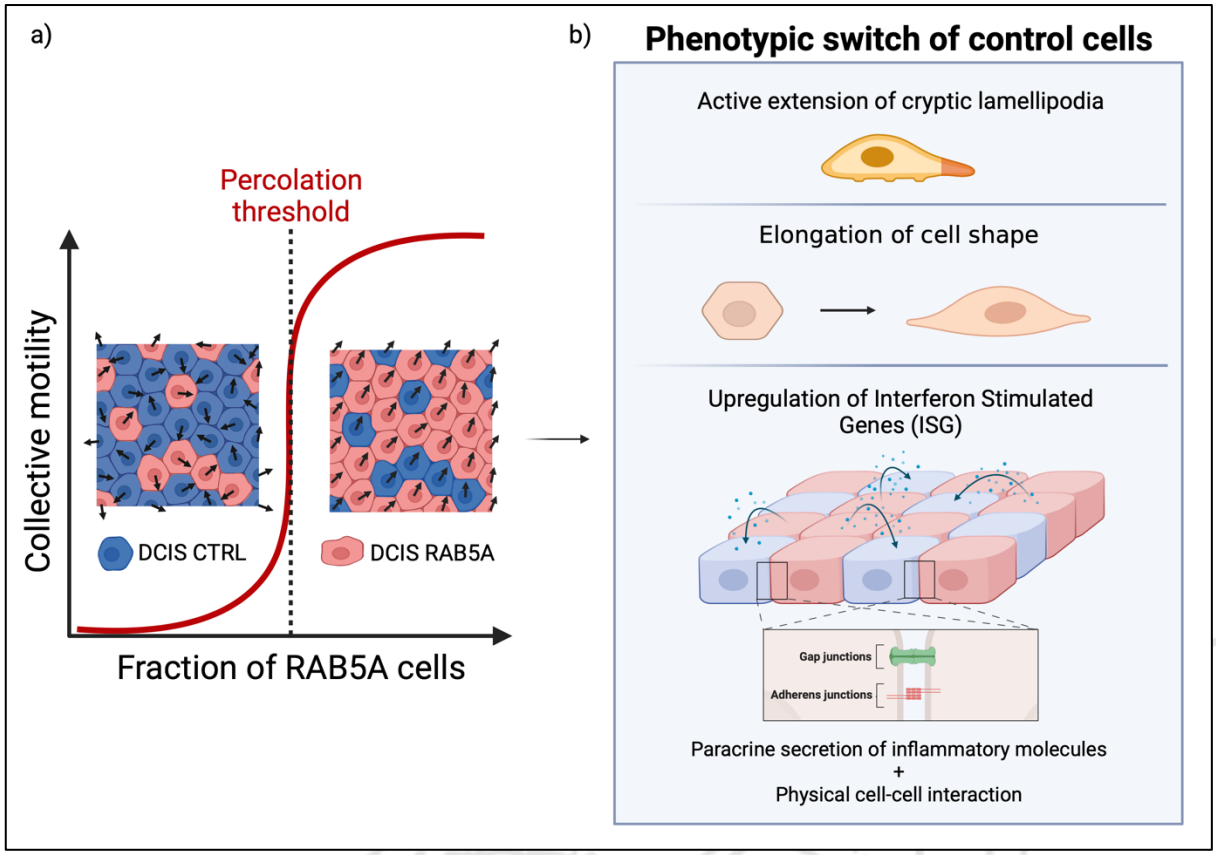

Figure 1. (a) In breast cancer epithelial monolayers composed of MCF10DCIS.com (DCIS) cells control (CTRL) or overexpressing RAB5A (RAB5A) mixed at different relative fractions, contact percolation of RAB5A cells sets the activation of collective flocking motility; (b) flocking transition induces a phenotypic switch in control cells, which acquire a phenotype similar to RAB5A DCIS: active extension of cryptic lamellipodia oriented in the direction of motion, elongation of cell shape and upregulation of pro-inflammatory Interferon-Stimulated Genes (ISG). This is mediated by paracrine secretion of inflammatory factors from RAB5A cells and direct cell-cell interaction between the two cell populations.

Peritoneal Metastasis-derived Extracellular Matrix Proteins Regulate the Tumor Microenvironment Leading to an Impairment of the Immune Cells Antitumor Activity

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Headline

Changes in mechanical properties and biological cues of the ECM favor the development of the metastatic environment leading to immune surveillance escape. The comprehension of the mechanisms that sustain these processes could help develop novel therapeutic strategies based on awakening the immune response.

Abstract: peritoneal metastasis (PM) results from complex molecular and biomechanical interactions between cancer cells and the tumor microenvironment (TME), including remodeling of the extracellular matrix (ECM). The impact of the immune TME on cancer response to current therapeutic strategies has been clearly demonstrated. Current treatments could generate a permissive environment for tumor growth that supports remodeling of the ECM and impairment of immune surveillance, resulting in disease relapse.

Objectives: is to understand the composition and changes of immune TME and PM-derived ECM induced by clinical and surgical interventions. Mechanical changes were also investigated.

Methods: Tissue samples were analyzed by immunohistochemical (IHC) analysis of key lymphoid and myeloid markers. Fresh PM tissues were also characterized using panels of antibody matrices based on FACS analysis. PM-derived samples were decellularized and their ECMs were analyzed by LC-MS/MS. Changes in the relative stiffness were investigated by atomic force microscopy (AFM).

Results: IHC and FACS analysis demonstrated changes in the composition of the immune TME, characterized by a shift to a myeloid-derived immunosuppressive phenotype. Proteomic analysis

revealed the structural protein composition and abundance of the PM ECM and the presence of specific cytokines/chemokines, growth factors, and specific matrix-bound vesicles released earlier by the resident cells and involved in the development of the metastatic PM niche and I-TME-associated changes. AFM analysis highlighted differences in tissue stiffness caused by clinical and surgical interventions.

Conclusion: Because immune composition and ECM mechanical properties in PM diseases remain largely unexplored, understanding the biological mechanisms responsible for altering immune surveillance and ECM modifications could help develop innovative therapeutic strategies based on awakening the immune response.

Unraveling transmission of mechanical signals in living cells: Insights from Optical Manipulation and Force Imaging

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Headline

Cells generate and encounter mechanical forces that influence the structure of tissues and regulate signaling pathways. Cell mechanical stimulation combined with the FRET tension sensors inserted into crucial proteins allow unravelling the molecular mechanisms through which forces propagate and transduce signals.

Abstract

Cells generate and encounter mechanical forces that influence the structure of tissues and regulate signalling pathways in various physiological or pathological scenarios. However, the molecular mechanisms through which mechanical signals propagate within cells and between cells in tissues remain elusive. On one hand, cells exert forces through non-muscle myosin, which propagate to the actin cytoskeleton and other intracellular structures, influenced by the mechanical properties of the extracellular matrix (ECM). Conversely, forces exerted by adjacent cells propagate within the cell through the cell membrane and cytoskeleton. However, how these forces propagate with changing cell type and state, ECM properties, and maturation of their adhesion complexes is not fully understood.

To investigate these phenomena, we have developed a setup that combines optical manipulation and force imaging using Förster resonance energy transfer (FRET)-based force sensors. The method exploits genetically encoded FRET tension sensors inserted into crucial proteins (e.g. F-actin, adherens junction's proteins and nuclear proteins) in different cell lines. The mechanical forces were investigated in healthy and diseased conditions, in cells grown on substrates characterized by different stiffness and differentiated cells in a non-damaging and minimally invasive way. Moreover, by employing a combination of optical tweezers and FRET-based force microscopy, we aim to examine how mechanical signals propagate from the other cell membrane into different cell types.

Our approach promises to shed light on the intricate dynamics of cellular force transmission, offering insights into the mechanobiological processes underlying cellular behavior and tissue function.

Towards accessible mechanobiology experiments:

a DIY cell stretcher with time-lapse imaging

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Headline (max 400 characters including spaces)

We present a series of custom-made cell stretcher devices designed and realised in our laboratory, with a do-it-yourself approach, exploiting Arduino microprocessors, dedicated Python codes and LabVIEW interfaces. The devices apply cyclic stretching to *in vitro* cells on deformable PDMS substrates while performing imaging with an optical microscope. Some examples of their applications are reported.

Abstract (max 2 pages for the whole document)

Cell stretcher devices are raising an increasing interest in the field of mechanobiology, as they represent a practical platform to investigate cellular response to external physical stimuli by simply imposing a deformation to elastomeric substrates on which cells are cultured. Owing to its optical transparency, tuneable elastic moduli, and permeability to gases (i.e. air/CO_2 mixtures), polydimethylsiloxane (PDMS) is typically the polymer of choice, for the crafting of deformable substrates for such applications.

In this work, a series of cell stretchers designed and built in the NanoBioLab at the University of Modena and Reggio Emilia (Italy) is presented. These devices rely on a fully integrated Arduino-based system for the application of stretching cues to living cells. Cells are maintained inside an on-stage incubator, allowing to perform time-lapse observation over a considerable period of time with an inverted optical microscope [1], [2]. An Arduino Uno microprocessor is responsible for the maintenance of the proper conditions for cells to grow and survive. It keeps the temperature of the device close to 37°C and the relative humidity approximately at 95-100% by heating water reservoirs and feeding current to an ITO glass window that prevents condensation of water on the cover lid of the device. In addition, the microprocessor regulates the opening/closing of a solenoid valve that regulate the CO_2 flow from a pressurized bottle so that CO_2 concentration inside the incubator is 5% (as measured by an Infrared sensor). In order to provide cells with different stretching stimuli, the devices come in different geometries (as shown in figure 1), thus allowing for uniaxial, biaxial or isotropic deformation.

Time-lapse imaging and stretching processes are governed by an Arduino Mega microprocessor. On the imaging side, Arduino Mega moves a motorized stage, acquires z-stacks by adjusting the focus knob and select the focussed slice; while on the stretching side, it controls the stepper motors by imposing the deformation to the PDMS substrate. To do so, the Arduino Mega is capable to control the stretching in terms of duration, waveform, amplitude and frequency.

Cells are cultured on stretchable fibronectin-coated PDMS 1:20 [3] that is placed in correspondence of a transparent central lodge featured in the stretchers. The stretcher is then placed on top of the motorized stage of the microscope to allow imaging.

Prior to application, the devices are calibrated by stretching PDMS substrates coated with fluorescent nanobeads covalently bound to the surface and used as spatial markers. Nanobeads displacement is analysed by a custom-developed Python code that calculates the strain map along the principal and secondary directions (ε_{xx} and ε_{yy},) and returns the direction along which the strain is minimized ($\theta_{minimum\ strain}$). To validate the experimental calibration, Finite Element Measurement (FEM) simulations are performed, mimicking the deformation of the PDMS substrate. Once experimentally relevant conditions are met – usually with a strain value close to 10% – the devices are used to study the response of cells to the application of stretching stimuli, namely migration, orientation and reaction to changes of the direction of the stretching stimulus.

Human Cardiac Fibroblasts (HCF) cells were chosen as model since they experience cyclic stretching in their native physiological environment. Moreover, HCF are known to be quiescent cells that upon damage to the cardiac tissue activate, transitioning to myofibroblasts [3]. HCF migrate to the location of the damaged tissue and start excreting Extracellular Matrix proteins (ECM) in order to help the recovery process. Paradoxically, if dysregulated, this process can become pro-fibrotic, leading to the onset of pathologies such as cardiac fibrosis, arrhythmias and heart failure [4]. Release of chemical factors, angiogenesis and mechanical stimuli have been addressed as potential factors capable of triggering the failure of the repairing mechanism and, consequently, HCF differentiation into myofibroblast. In particular, mechanical stimuli are likely to arise from the increased pressure load on the heart due to hypertension. As such, understanding which are the biological mechanisms triggered by mechanical cues would represent an important advance in our understanding of related cardiac pathologies and in establishing effective pharmacological and clinical treatments.

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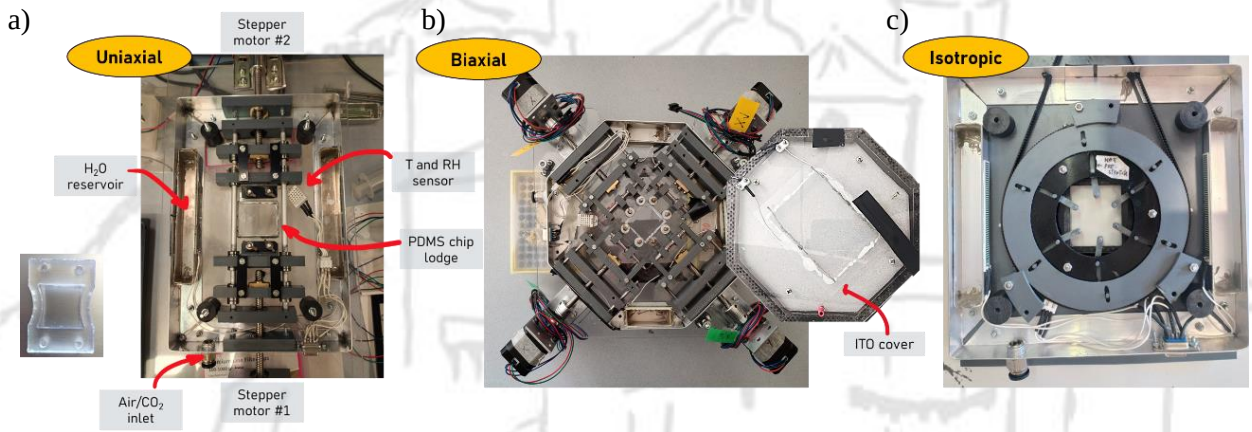

Figure 1: Different geometries of the cell stretcher devices developed in the NanoBioLab: a) the uniaxial stretcher with a PDMS substrate at the side; b) the biaxial stretcher and c) the isotropic stretcher.

Mechano-electrical feedback in a dish: setting up a cardioid approach.

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Headline

The experimental approach to mechano-electrical feedback has been mainly through *in-vivo* models, yet few *in-vitro* solutions were tempted. This study combines Fluid Force Microscopy, controlling stimuli and force applied, and cardioids to offer a new gateway for personalized and precision medicine.

Abstract

Intimate correlation interlaces the electrical and mechanical cardiac machinery, urging failure when one of these actors lacks. Mechano-electrical feedback is one of the expressions of this relation, which may occur in arrhythmias and even sudden cardiac death (SCD), as reported in the literature. This phenomenon mainly occurs when a mechanical wave impacts on the precordial region, rising life-threatening reaction chain, mainly causing the activation of the stretch-activated ion channels (SACs), altering tissue excitability and heart rate. The advent of human induced pluripotent stem cells (hiPSCs) [1] and their reprogramming may give an interesting and ethic approach to this peculiar and sometimes lethal occurrence. Evidences in the literature [2,3] support the presence of mechano-sensing machinery present in the hiPSCs differentiated in cardiomyocytes (hiPSCs-CM), in both physiological and Dilated Cardiomyopathy-affected cells (DCM). In this work, we combined the hiPSCs-CM, isogenic control and DCM (iCell Cardiomyocytes, Cellular Dynamics®, cat. #R1153, #R1154), together with confocal-Traction Force Microscopy (cTFM) and Fluid Force Microscopy (FFM), to inspect possible mechano-electrical feedback through an *in-vitro* approach and by tissue tapping stimulation[4,5]. The biological model adopted in this study was the cardiac spheroid or cardioid, which can be highly reproducible and mimicks 3D tissue organization[6]. The spheroids were seeded on PDMS layers with known Young's modulus and imaged with confocal laser scanning microscopy (CLSM, Olympus, FV3000), offering the simultaneous registration of bright field images and fluorescence. First, we assessed the viability (Calcein-AM/Ethidium homomer-1), the calcium-machinery (Fluo4-AM) and electrophysiological differences (FluoVolt) between the samples. Overall, the viability of the constructs was above 70% for both cell types. Concerning the Fluo-4AM and FluoVolt charged samples, 20-s videos were acquired with a 30 frames/s rate in both fluorescent and bright-field (BF) channels. This simultaneous acquisition led to real-time comparison of contractility (BF) and calcium/voltage fluorescence changes (fluorescence, $\lambda = 405$ nm). The bright field registration was analyzed with a new algorithm, OptTrack, validated through a comparison with the Cellogram algorithm[7], to evaluate the contractility differences. The measurements were performed in spontaneous beating regimen. A significant increase

in the latency time was observed between the calcium maximum outflow and the peak of contraction in the DCM-affected cardioids compared to the control, whilst in the action potential measurements, the data revealed no differences. Successively, the samples were transferred to the FFM setup to determine the possible evidence of mechano-electrical feedback. The experimental protocol was adapted starting from the literature [4,5,8]. The force applied in isogenic control cardioids to urge an apparent response was 10 nN with a maximum speed of 100 µm/s (Fig.1). The time span between the collision and the response does not prove the presence of an immediate contraction after the mechanical stimulation. Interestingly, the samples did not respond to higher forces and in most of the spheroids tapped with forces higher than 50 nN apoptosis occurred. For the DCM cardioids, as indenting forces of 10 nN and 30nN were not eliciting any response, the force of stimulation was increased up to 100 nN. Once again, contraction activity was observed only after more than 1 s from the collision time, so that it must be determined whether the mechanical response observed is indeed stimulated by the tapping from the probe or is a spontaneous beating activity of the cardioid.

Overall, the presented work lies the first steps for the establishment and optimization of a new methodology which combines high quality and reliable biological samples and cutting-edge nanoengineering approach.

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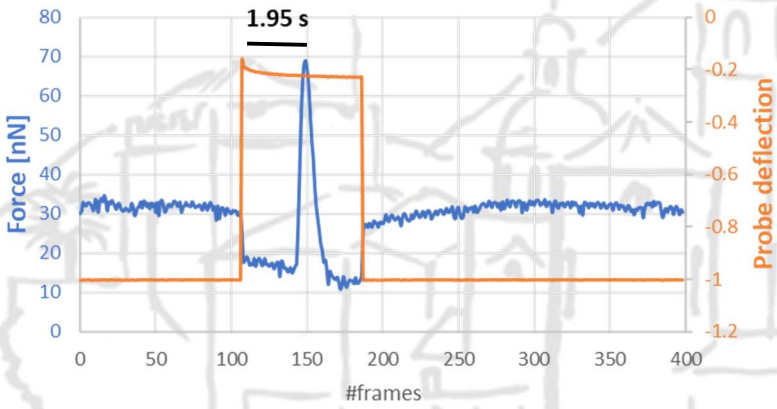

Figure 1. Example of response to FluidFM tip mechanical stimulation in isogenic control cells.

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Magnetic nanoparticles as a tool to target and study mechanoreceptors

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Headline (max 400 characters including spaces)

Magnetic particles coupled to magnetic fields are a tool to precisely manipulate mechanical forces, providing an approach to study mechanoreceptors. Cadherin fragments immobilized on magnetic nanoparticles can be used to selectively target cadherin expressing cells and to activate intracellular pathways if the fragments are correctly oriented on the nanoparticle surface.

Abstract (max 2 pages for the whole document)

Magnetic particles specifically anchored to membrane receptors can be used to exert traction forces on these receptors when a magnetic field is applied. Indeed, over the past decades magnetic microparticles and magnetic tweezers have been widely used to highlight how mechanical cues can impact biological processes. The use of magnetic fields offers some advantages such as deep-tissue penetration, the possibility to apply a wide range of stresses and forces (fN to nN) without damaging the sample and the possibility to manipulate the receptors in a remote fashion.

Althoug magnetic microparticles are widely used for this purpose, their large size might result in multivalent binding, causing clustering of receptors and activation of intracellular signalling even in the absence of a magnetic field. Alternatively, smaller magnetic nanoparticles (MNPs) offer undeniable advantages for manipulating mechanoreceptors as they show spatial control at the molecular level. However, MNPs exert smaller forces (in the range of fN or pN), that might be low to activate a mechanoreceptor. Therefore, a full optimization of the magnetic properties of the MNPs (through control of their size, shape and composition) and a correct design of the magnetic field applicator are crucial to reach this threshold.

We are interested in using MNP to stimulate cellular E-cadherins in order to understand and modulate remotelly important intracellular signals connected with them. To do so, we functionalize MNPs with cadherin fragments, so that they can further interact with cellular cadherins. Critical factors that can ultimately affect cellular recognition are the orientation and density of the biomolecule on the MNP surface. Indeed, as the interaction between two E-cadherins depends on a correct spatial position, the orientation of the protein domains on the MNP is crucial for a correct attachment to the cell membrane. To ensure this orientation, we have optimized the synthesis of MNPs and their smart functionalization with cadherin fragments modified with a histidine tag through metal affinity binding, assuring both functionality and stability [1]. By controlling the number and orientation of cadherins over the MNPs surface, the specific interaction of these MNPs with cells expressing E-cadherin can be obtained. Once

attached to the cellular membrane, these MNPs can be used to stimulate intracellular pathways *per se* or using different configurations of magnets.

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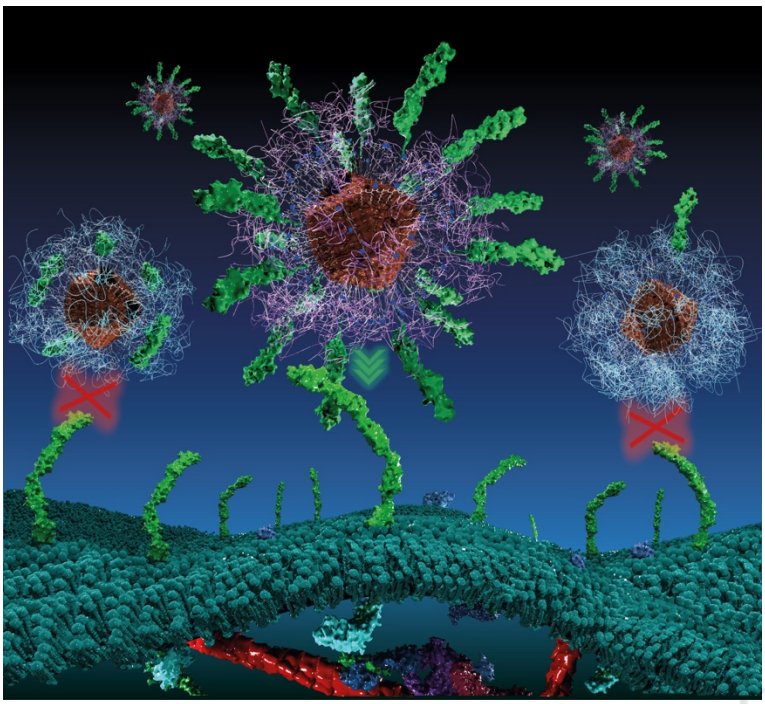

Figure 1. (a) Magnetic nanoparticles functionalized with E-cadherin fragments in an oriented manner selectively target cellular cadherins only when the density of cadherin fragments is high.

Revealing mechanosensitivity of cellular adhesion complexes by high-speed optical manipulation and imaging

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Headline (max 400 characters including spaces)

A combination of high-speed laser trapping and fluorescence microscopy is optimised to investigate interactions between single adhesion complexes and F-actin and unveil their mechanoregulation.

Abstract (max 2 pages for the whole document)

At cellular adhesions, a vast array of proteins interacts with the cell cytoskeleton and regulates its structure, impinging on focal adhesions and adherens junctions, on cell motility and morphogenesis. Mechanical forces play a pivotal role in these processes, but the molecular mechanisms are still unclear. Single molecule methods are ideally suited to directly observe such events, but face challenges related to the complexity of the multi-molecular interactions involved and their broad time scales, and to difficulties in controlling force and directionality in *in-vitro* reconstituted assays.

To overcome these challenges, we have been working on an experimental assay that combines several advanced single molecule techniques. Here, a single actin filament is suspended between two trapped microspheres and can interact with binding proteins attached on a pedestal bead fixed to the coverslip. An ultrafast force-clamp allows us to directly probe the force-dependence of molecular interactions with sub-millisecond time resolution [1]. Sub-nm stabilization of the microscope through local gradient localization allows resolving protein conformational changes and protein binding position on single actin monomers [2]. An experimental arrangement (termed oriented dumbbell) allows us to suspend the actin filament with known polarity between the two trapped microspheres and determine asymmetries in the force response of the interacting proteins. We applied our methodology to reconstitute *in-vitro* the interaction between α- catenin and F-actin, unveiling a molecular switch between a slip and an asymmetric cooperative catch-bond with F-actin [3]. Finally, we introduce synchronized fluorescence detection with single molecule sensitivity and ms time resolution that allows us to probe multimolecular interactions, such as vinculin binding to α-catenin during its interaction with F-actin.

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An application of ultrasounds in cell culturing: Prototyping a Sonobioreactor for optimizing fermentation process

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Headline

In this study, we developed a tailored system to irradiate characterised mechanical signal into standard cell culturing reactors. This enabled the study of the effect of ultrasounds in yeast-based fermentation. We found ultrasounds can optimize fermentation time duration and yeast biomass productivity without affecting yeast vitality.

Abstract

Ultrasounds (US) are mechanical waves that propagate into a medium at a frequency greater than 20KHz and can generate inside it a local pressure variation [1]. US-based technologies gathered much research and industrial interest, and are currently applied in diverse fields, from engineering to medicine and biology. A particularly interesting application exploits the US as a tool to influence cell functioning, particularly relevant for bioproduction. Indeed, the US was demonstrated to enhance cell-based processes in terms of biomass productivity and valuable products yield [2]. It was proposed that ultrasounds can induce transient pores on the membrane, increase nutrient flow throughout the cell membrane, remove inhibitory metabolites, that overall results in increased bioconversion rate [3]. It has been observed that ultrasounds can influence the metabolism of different organisms by triggering mechanoresponsive mechanisms. This aspect, along with promising papers showing a reduction of bioprocess time duration, an increase of biomass productivity and the yield of highly valuable biomolecules, paves the way for the usage of highly precise systems to optimize industrially relevant processes [4].

In this study, we designed and realised an *ad hoc* growth system, the sono-bioreactor (SNBR), to study the effect of low-power ultrasounds on liquid cell culturing. The developed SNBR is made of two modules: an electric signal generator and a wave transducer. The latter is applied externally to existing reactors. To ensure effective and characterised signal propagation, COMSOL software was exploited to study the distributions of acoustic pressure (Pascal) (Fig.1) thus guiding the proper installation of transducers into the outer layer of reactors. Furthermore, the propagation of the ultrasonic wave inside the bioreactor is monitored using an hydrophone and SPECTRA software, validating the computational findings. SNBR in the form of a cylindrical reactor of 30L volume, was used to grow different industrially relevant strains of the yeast *Saccharomyces cerevisiae*, *USO5* and *M84*. A novel protocol for mechanical treatment of yeast cultures was conceived and used to study the effect of ultrasounds on main biotic parameters, such as biomass productivity, dry weight and cell death, while abiotic parameters such as pH, medium density and temperature were monitored.

Analysis conducted during brewing fermentation, showed an increase in cell number, an overall increase in cell biomass, as indicated by optical density in Fig.2. Interestingly, it was found a significant

reduction in the fermentation process from 15% up to 37% for treated samples with respect to control, as indicated by the evolution of density of the medium during time. pH and temperature were monitored to understand if the US signal could affect these abiotic parameters, and no alteration was detected. Intracellular calcium was monitored throughout the fermentation process. It was observed that there was an increase in the treated sample compared to the control. This could be attributed to a heightened stimulation, induced by the ultrasonic waves, of transmembrane calcium channels. Overall these results suggest that ultrasound may stimulate various receptors and metabolic pathways involved in the expression of different genes implicated in the metabolism of the yeast or in the cell wall integrity.

The SNBR emerges as a tool to irradiate finely characterised US signals and understand the effect of US on diverse microbial cultures, from bacteria to yeasts and other microorganisms. We propose the use of SNBR as a standard platform for reproducible studies on the effects on growth, metabolism and composition of cell-based processes and to assess the potentiality of mechanical stress on bioprocess optimization.

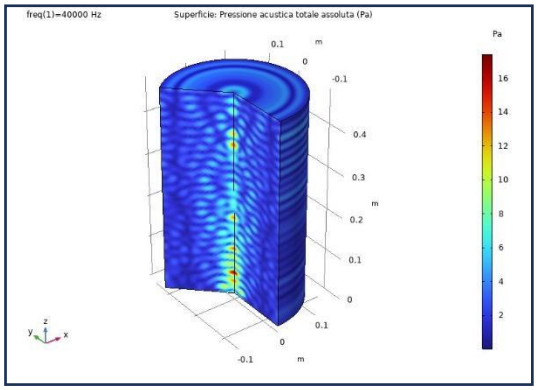

Fig.1. Simulation of ultrasound propagation by COMSOL software. Wave pressure areas in fermenter as Pascal.

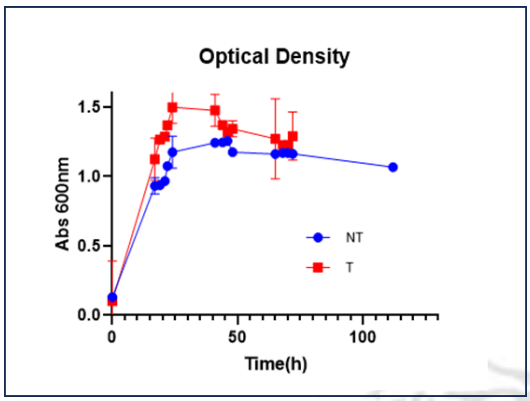

Fig.2. Effect of the ultrasound on *S.cerevisiae*, monitoring the optical density during fermentation process.

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Chromatin density influence nuclear mechanics and cell migration

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Headline

Two parameters are of paramount importance in the context of nuclear damage during cell migration: the extracellular matrix's density and stiffness, and the nucleus's density and stiffness. Our research focus on the properties of the nuclear content, the chromatin density and found it to be critical in defining its mechanics and the consequent cell ability to migrate through micro-sized pores.

Abstract

Cell migration in tissues takes place within a dense microenvironment consisting of other mobile and immobile cells and the extracellular matrix. Therefore, cells are forced to pass through holes and crevices that are smaller than their size. Cell nuclei are large and fairly stiff organelles, and they represent the limiting factor in cell migration in physically constrained microenvironments, since they hamper the squeezing of cells [1] Regarding nuclear stiffness, it is well known that lamins, the microfilaments that make up the nuclear envelope, play a key role [2]; nevertheless, also the mechanical properties of the nuclear chromatin, i.e DNA and histones, are likely to be critical. For example, it has been demonstrated that the nuclear deformation that occurs during cell migration could causes localized loss of the integrity of the nuclear envelope [1], or that activated macrophage loses their histones when activated with bacterial lipopolysaccharide (LPS) [3,4]. Our study is aimed at characterizing the changes of the mechanical properties of cell nuclei with lowered chromatin density in primary Mouse Embryonic Fibroblast (MEF) with histones decreased through the KO of HMGB1 protein and in fibroblast cell line 3T3-L1with CRISPR edited DNA. To this purpose, we have developed novel approaches to perform nanomechanical measurements with specific nuclear sensitivity in living cells by atomic force microscopy [5,6]. Our results demonstrate that cells with lower amount of histones, obtained by a described molecular process [7], shows a decondensed DNA, making their nuclei bigger, softer, and less of a hindrance during cell migration in a constrained environment. This result will be used to investigate the role of chromatin density in a biologically and clinically relevant system like cancer cell migration that undergoes to EMT (Epithelial to Mesenchimal Transition) during metastatization.

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Stimulation of vibrational mechanotransduction at industrially relevant scales

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Headline (max 400 characters including spaces)

Cell therapies require large numbers of cells, necessitating the use of complex culture systems. Here we demonstrate that vibrational phenotypic control, can be applied at scale to meet this challenge. We show that specific vibration conditions can be delivered in cell stack, hollow fibre and stirred tank culture environments. Further, we show derivation of osteogenic cells within these systems.

Abstract (max 2 pages for the whole document)

A growing number of in vitro vibration studies have shown mechanotransductive effects, with some having dependence on frequency and amplitude[1]. A variety of cellular responses have been seen due to vibration, ranging from changes in proliferation to phenotypic alterations. These studies highlight the potential for vibration to be exploited for cell and tissue engineering to generate highly specific cell products. Yet most of these studies occur at a small-scale, utilising culture plates and petri dishes. The reality of clinical cell therapy production is that tens of millions, if not billions, of cells are needed to treat a single patient[2]. Allogeneic products can produce cells for multiple recipients in the same batch, reducing the high costs of cellular products, but this also increases the demands on cell number even further. Can mechanotransductive techniques, such as vibration, be deployed in a scalable manner for industrial production of real-world, phenotypically defined, cell therapies?

We have previously demonstrated that nano-amplitude vibration (1 kHz, 30 nm) can stimulate mesenchymal stem cell (MSC) osteogenesis by driving adhesion related signalling via focal adhesion kinase and extracellular related kinase 1/2[3,4]. There have also been hints that mechanosensitive ion channels such as TRPV1 may be involved in signal transduction[5]. These prior studies were carried out in small scale well-plate culture, using a custom designed nanometrically accurate vibration plate[6]. Here we provide data to demonstrate the application of this same stimuli to three industrially relevant culture systems, multi-layer cell stacks, hollow fibre bioreactors and stirred tank microcarrier culture (Fig 1).

We present finite element simulation, and scanning laser vibrometry data to predict and quantify the vibrational response of multi-layer cell stacks and hollow fibre cartridges. Using these methods, we confirm that 30 nm vibration is achievable at the cell growth surface of these vessels, albeit with potential phase differences. Further we present phenotypic data relating to the osteogenic commitment of MSCs grown in these vessels. Preliminary data is also presented for the vibrational stimulation of MSC-laden microcarriers in suspension culture and impact on cell growth.

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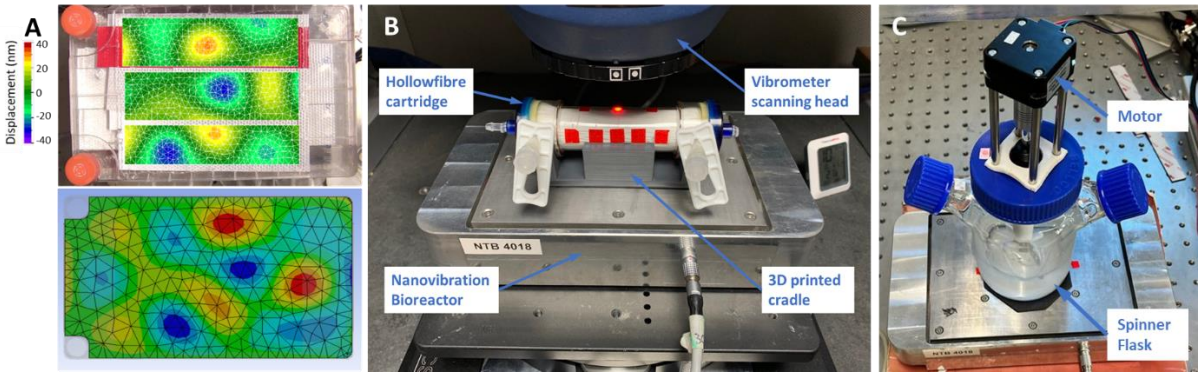

Figure 1. (a) Vibrometry and FE modelling of vibration in a multi-layer cell stack; (b) Vibrometry set up for a hollow fibre cartridge; c) vibration set up for 200 ml stirred tank bioreactor.

Mechanosensing at endothelial cell junctions

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PIEZO1 interaction with PECAM1 and CDH5 at cell-cell junctions

Vascular deterioration occurs physiologically with ageing but accelerates with cardiovascular disease and diabetes. Critical to blood vessel integrity is the inner lining formed by a patchwork of endothelial cells zipped together through special cell-cell junctions. Mechanical forces, for example from blood flow, activate signalling mechanisms that cause cell-cell junctions to tighten, loosen or open. We have recently shown that PIEZO1 is an integral part of this biochemical zipper [1] between cells, merging two prominent but seemingly opposed ideas for force sensing [2, 3, 4].

Abstract

The endothelium is a monolayer of cells forming the inner lining of all arteries, veins, capillaries and lymphatics. These cells form a crucial interface between blood and tissues to regulate exchange of substances and cells, partly via specialised cell-cell junctions[5]. Key junctional proteins involved in mechanosensation are the adhesion molecules CDH5 and PECAM1[4]. Subsequently, came the discovery of PIEZO1 mechanosensitive ion channel that was found to be a blood flow sensor in endothelial cells[2, 3]. We were interested in how these two mechanisms intersect.

In this study, we have used super-resolution (STED) and Förster resonance energy transfer measured by fluorescence lifetime imaging microscopy (FRET/FLIM) to identify interactions of PIEZO1 with PECAM1 and CDH5. Endogenous proteins were examined in tissue using our CRISPR modified HA-tagged PIEZO1 mouse. We used patch clamp and fluorescent calcium recordings to determine ion channel activity and calcium switch assays to recapitulate junction formation on endothelial monolayers.

We identified a pool of PIEZO1 located at cell junctions where PECAM1 and CDH5 are in complex with PIEZO1. PECAM1 formed a stable complex that leads to dampening of PIEZO1 activity. Shear activation of PIEZO1 showed dynamic recruitment of junctional CDH5 and PIEZO1 knockdown impaired CDH5 junction formation. PIEZO1 is required in Ca^{2+}-dependent formation of adherens junctions and associated cytoskeleton, consistent with it conferring force-dependent Ca^{2+} entry for junctional remodelling.

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Matrix viscoelasticity coupled with dimensionality controls epithelial cell migration

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Headline

By engineering micropatterned viscoelastic hydrogels, we show how matrix viscoelasticity and confinement regulate MCF10A breast epithelial cell migration. Our results reveal the intricate interplay between stiffness, viscoelasticity and spatial confinement in initiating cancer cell migration during metastatic dissemination.

Abstract

The viscoelastic properties of the extracellular matrix (ECM) are emerging as crucial determinants of cell fate, influencing processes such as cell spreading, differentiation, and cancer cell migration. However, our understanding of these complex processes predominantly relies on purely elastic matrices, leaving the cellular response to changes in ECM viscoelasticity largely unexplored.

To address this gap, we engineered ultra-soft (E=0.3 kPa Pa) and soft (E=3 kPa) polyacrylamide (PAAm) hydrogels, encompassing both elastic ($\tan(\delta) < 0.1$) and viscoelastic ($\tan(\delta) > 0.1$) characteristics, mimicking the mechanical microenvironment of normal and tumoral breast tissues. Time lapse microscopy revealed that matrix energy dissipation governs MCF10A breast epithelial cell migration through actin polymerization at the cell leading edge. Notably, MCF10A cells exhibited robust migration on ultra-soft viscoelastic matrices, contrasting with their limited migration on soft viscoelastic substrates. Conversely, increased stiffness had no impact on cell migration when matrices were purely elastic. To better recapitulate *in-vivo* 3D cell migration, incorporating spatial confinement typical of neighbouring cells or the ECM, we micropatterned 1D fibronectin adhesive lines on viscoelastic PAAm gels. Confined MCF10A cells displayed impaired migration on ultra-soft matrices, regardless of their viscoelasticity, while migration speed on soft elastic and viscoelastic matrices was significantly increased compared to unconfined conditions.

Our findings underscore the influence of increased stiffness and viscoelasticity on cell migration in a 3D environment, particularly under spatial confinement. These results shed light on the intricate interplay between stiffness, viscoelasticity and spatial confinement in initiating cancer cell migration during metastatic dissemination.

Regulation of Tight Junction and Apical Membrane Mechanics: The Role the ZO-1-Cingulin Cytoskeletal Tether and γ-actin

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Abstract

We are investigating the molecular mechanisms through which the apical junctional complex (AJC) of epithelial cells, which comprises tight junctions (TJ) and adherens junctions (AJ), and its associated actomyosin cytoskeleton controls the mechanical properties of the junctional and apical plasma membrane. We previously showed that ZO-1 is a mechanosensing protein, and we will present evidence the conformation of additional TJ plaque proteins may be regulated by actomyosin-dependent force and microtubule cytoskeleton integrity in epithelial cells. Cingulin (CGN) tethers nonmuscle myosin2B (NM2B) to ZO-1 to regulate the tortuosity of the TJ membrane and apical membrane stiffness in MDCK cells. In addition, the KO of CGN affects the localization of γ-actin, one of the two isoforms of cytoplasmic actin. To examine the role of g actin in the phenotypes of CGN-knock-out (KO) cells and further understand the role of γ-actin in epithelial membrane mechanics, we generated MDCKII cells KO for γ-actin. Among several interesting phenotypes of γ-actin-KO cells, we show increased expression and junctional accumulation of both β-actin and NM2A, and increased TJ membrane tortuosity, although the expression of NM2B is not affected. Through depletion, rescue and pharmacological approaches, we show that both increased TJ tortuosity and up regulation of junctional β-actin depend on NM2A, whereas other phenotypes of γ-actin-KO cells are NM2A-independent. We are currently investigating the role of g-actin in the dynamics of cytoplasmic TJ proteins, and the stiffness of the apical membrane. These, results reveal a previously unknown interplay between actin isoform expression and NM2A in the regulation of TJ membrane mechanics.

A controlled sub-cellular mechanical stimulus to study Piezo2 channel activation

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Headline

Piezo2 channel mechanical gating: localized vertical activation of cells that are Piezo 2 transfected and those that express Piezo2 endogenously.

Abstract

The mechanosensitive Piezo 2 channel is the main transducer of mechanical stimulus in physiological (i.e touch, proprioception) [2] and pathological conditions as cancer cells [3]. While some insights into the gating mechanism of Piezo channels have been gained, our understanding of how mechanical stimuli can modulate the gating of the Piezo 2 channel is still unclear. For this reason, we over-transfected in Hek-293 cells Piezo 2-green lantern, a chimera protein that enables also to localize the Piezo 2 channels in the cell. To mechanically stimulate Piezo2 ion channel on Hek-293 cells we exploited an atomic force microscopy (AFM)-based approach, while monitoring channel activation in real-time using functional calcium imaging. Using a standard pyramidal AFM probe, single Piezo 2-transfected cells were vertically stimulated on the nuclear region (see Figure 1) using different forces (5nN, 10nN and 50 nN) with a short duration time (500 ms). A significant increase in cellular response for Piezo 2-transfected cells stimulated with 5nN compared to the control was observed. From 10nN to 50nN we observed an increasing percentage of responsive cells both in transfected and control cells, likely associated with the lower occurrence of Piezo 1 mechanosensitive channels in the Hek cells. Based on these results, we extended our study to Dorsal Root Ganglion (DRG) neurons which express Piezo 2 endogenously. We delivered a regulated force to the soma of DRG using a standard pyramidal probe and a bead modified cantilever, and we examined how the cells responded to different pressure and membrane deformation. Furthermore, early findings demonstrate the transmission of calcium signalling in nearby DRGs that have been mechanically stimulated. This study contributes to our understanding of Piezo2 channel gating mechanisms in physiological conditions and on its role in signal propagation and cell communication.

This work is supported by PRIN 2020- Project Touch on a chip.

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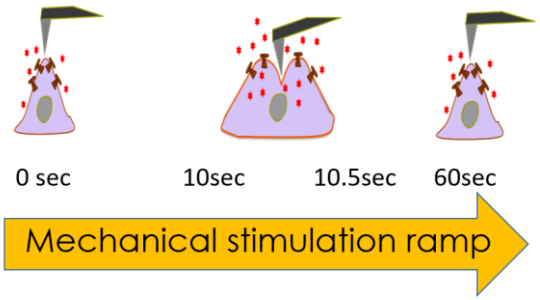

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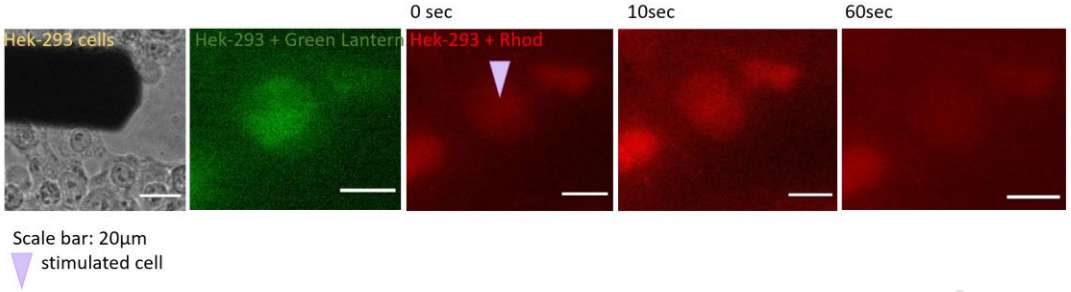

Figure 1: (a) Scheme illustrating vertical localized mechanical stimulation of Piezo2-transfected cells by a standard AFM probe; (b) Time-lapse of Hek-293 cells transfected with Piezo2 showing changes in calcium intensity after the mechanical stimulus.

Interest of Brillouin microscopy based on VIPA for mechanical mapping of biological samples

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Abstract

The need to characterize the mechanical properties of cells and tissu led biologists to promote spectroscopy based on Brillouin scattering. Indeed, microscopy based on Brillouin scattering could reconstruct images of biological sample stiffness [1]. This rapidly required a balance between two important criteria: on the one hand, the acquisition time for individual spectra, and on the other, sufficient contrast to observe low-intensity signals. Contrast is the property that enables us to measure both a high-intensity signal and a low-intensity signal at the same time. It is expressed in decibels. As for acquisition time, this is of particular importance in the case of biological samples, whose properties evolve on very rapid scales compared with the time scales of most materials under the same conditions. Acquisition time is important for mapping, where several thousand where several thousand spectra need to be collected. Technological developments in recent years have led to the emergence of so-called Brillouin spectrometers based on Virtual Image Phased Array (VIPA) [2], as an alternative to Fabry-Perot cavities based spectrometer, which have the disadvantage of being much slower, albeit with better contrast. These advances have made it possible to discover cell properties using a non-contact, non-labelling method. Indeed, human cells are distinguished by their absence of a protective wall, but have a membrane consisting of a phospho-lipidic double layer as well as surface proteins. It also contains a cytoskeleton, composed of actin filaments, microtubules and intermediate filaments, which provide mechanical resistance to stress. The rigidity of human cells evolves with age, in the sense of an increase in cellular rigidity and a loss of the capacity to undergo strong reversible deformation [3]. Similarly, these properties can evolve in response to external aggressions such as inflammatory reactions [4] or pathologies such as advanced stages of cancerous lesions [5] .

The conditioning of cells by the extracellular matrix is also a source of modification of mechanical properties. Cells have the ability to sense and respond to mechanical stimuli by mechanotransduction.

In order to measure or simulate the mechanical behavior of cells, several techniques have been developed in the last decades. At the same time, spectrometers have been developed to study Brillouin scattering. This particular scattering, based on the interaction between light and acoustic phonons of the material, allows to obtain a signal close to the exciting line, linked to the mechanical properties of the sample. The first spectrometers were built from Fabry-Perot interferometer. Sandercock was one of the inventors of highly resolved spectrometers with a high contrast (~100 dB) by multiple passages of the field within the cavity. A notable evolution has been achieved from spectrometers allowing much shorter acquisition times and thus, to perform scans of biological samples. This shortening of the integration time has been made possible by the simultaneous acquisition of the whole spectrum, containing the Brillouin signal, using a component very close in its design to a Fabry Perot cavity: the VIPA (Virtual image Phased Array).

This component has a totally transparent entrance window to inject the beam, as well as two reflecting faces, one at 95%, the other at 100%, in order to create a set of virtual images, all having an optical path difference as for the Fabry-Perot cavity [2]. This allows to partially

separate the two components of the beam, one at the Rayleigh frequency, identical to the incident light, the other at the Brillouin frequency of interest. The measured Brillouin frequency shift ν_B is generally related to the longitudinal sound velocity V_L and to the real part of the longitudinal elastic modulus M' at GHz frequencies through:

$$M' = \rho V_L^2 = \rho \left(\frac{\lambda_0}{2n}\right)^2 \nu_B^2$$

where n is the real part of the refractive index of the medium and ρ is its density. Both n and ρ, or at least ρ/n^2, must be known to obtain M'. The Brillouin linewidth Γ_B, actually the FWHM of the peak, is related to the dynamic longitudinal viscosity η_L and to the imaginary part of the longitudinal modulus M'':

$$M'' = 2\pi\nu_B\eta_L = \rho \left(\frac{\lambda_0}{2n}\right)^2 \nu_B\Gamma_B$$

A spectrograph based on 2 crossed VIPA was combined with a diffractive mask, inspired from Lyot coronograph, reach a contrast of 80 dB (Fig 1). These setting could allow scanning cells with an optical resolution in one hour (Fig 2). The development of such home-made instrument paves the way for a new field of data accumulation and exploration of biological tissues.

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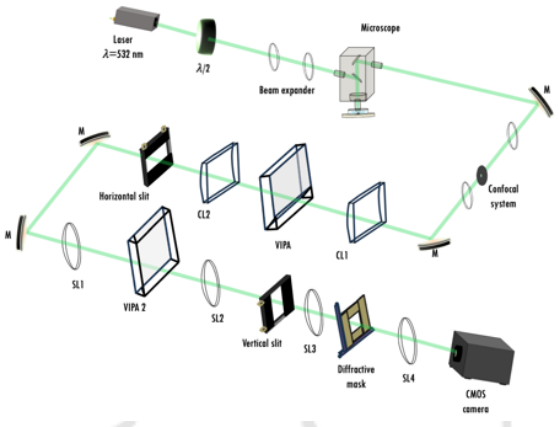

Figure 1. Brillouin Microscope setting. M: mirror; SL : spherical lens; CL: cylindrical lens; VIPA : virtually imaged phased array

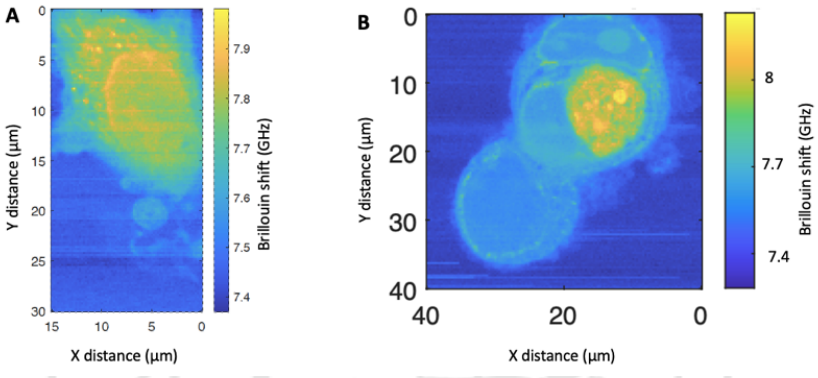

Figure 2. A) scan of dental pulp stem cell (DPSC) and B) Michigan Cancer Foundation-7 (MCF7) with Brillouin microscope. Each point corresponds to a frequency shift of scattered light, connected to the look up table.

Mesoscale Epithelial Mechanobiology and Cellular Interfaces

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Headline

My research aims to dissect the role of cell adhesion and intercellular communication in epithelial mechanobiology starting from the medically relevant retinal epithelium biology. In my group, we use engineered hydrogel systems to control the mechanobiology of cell adhesion, reducing the degree of freedom in tissue systems and thus revealing phenotypical tissue plasticity and molecular function.

Abstract

Mechanical properties regulate tissue functions at a multicellular length scale or mesoscale. These properties depend on the interaction of cells with their interfaces, hence on the balance between intercellular tension and the extracellular matrix (ECM) adhesion forces.

My group aims to dissect the role of adhesion types and their integration with the different cytoskeleton elements (e.g., actin and keratin networks) that define epithelial biomechanics. In contrast to the experimental investigation of traditional biological sciences, my laboratory uses cross-disciplinary approaches combining synthetic hydrogels with stem cell-based models to address the medically relevant retinal pigment epithelium (Fig. 1). We particularly develop and adapt biohybrid systems where cells interact with hydrogels that are designed to control cell-cell or cell-ECM adhesion. Synthetic material allows the unique reduction of the degree of freedom in the cellular/tissue system, thus helping us to reveal phenotypical tissue plasticity and molecular function.

I will present an overview of published work[1,2] on understanding how ECM physical (elasticity) and biochemical cues (receptor density) impact epithelial system properties, namely stress heterogeneity and intercellular force coordination. I will show that these properties are not only *in vitro* observations but play pivotal roles in controlling our vision. A density gradient of ECM characterizes the contractility of the retinal epithelium *in vivo* and modulates its efficiency in supporting photoreceptor cells' homeostasis. Furthermore, I will show data from the ongoing work which addresses different aspects of the mechanobiology of tissue aging. We optimized phototunable hydrogels (Fig. 1) as substrates for epithelia to model ECM local remodeling on demand. Moreover, we developed microgels used as phototunable phantom cells to simulate age-related mechanical anisotropy in tissue. Altogether, we can dissect the relationship between tissue mechanics and function by controlling cellular interfaces' temporal and spatial properties.

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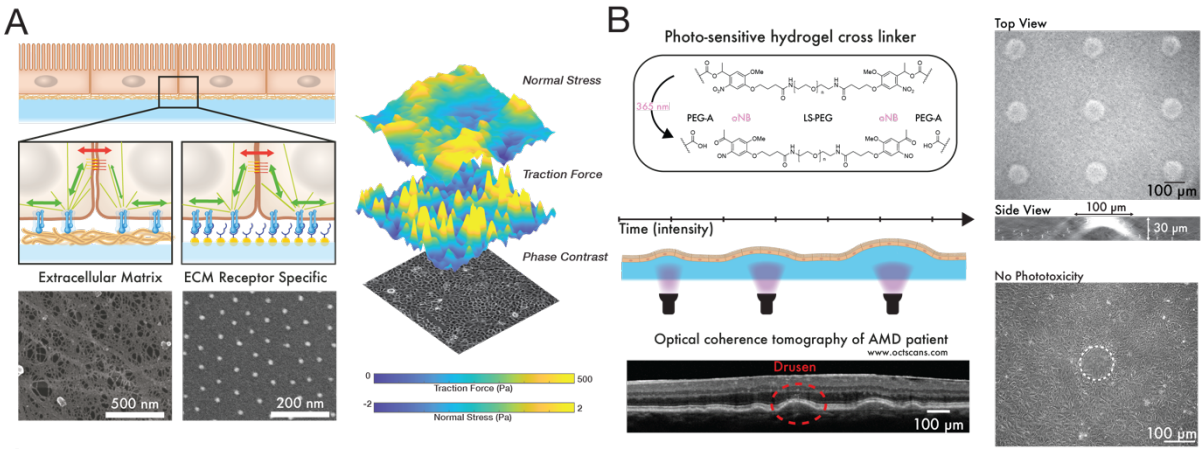

Figure 1. ***(A)*** *Schematic summary of the different hydrogels' functionalisation strategies available in my group. Hydrogels can be either coated with control density of full-length ECM proteins or nanopatterned with peptides to control the density of ECM receptors. Biomechanical characterization of epithelial monolayers obtained on hydrogels can be performed by traction force and monolayer stress microscopy.* ***(B)*** *A self-synthetized photosensitive crosslinker can be used to create phototunable hydrogels. oNB = ortho-nitrobenzyl group; PEG-A and LS-PEG = PEG acylate and light-sensitive PEG, respectively. The control of time and space of illumination allows to reproduce of in vitro artificial drusen, the hallmark of ECM aging in the outer retina. AMD = age-related macular degeneration*

Structure, mechanics, and dynamics of biological systems studied by AFM

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Abstract

Mechanobiology encompasses a range of studies on the mechanotransduction between growing cells and their microenvironment. Active forces in biological systems define the interactions between single molecules, growing cells and developing tissues. Over the last three decades atomic force microscopy (AFM) has become an indispensable tool for characterisation of samples from single molecules to complex living systems, featuring cells and tissues.

We will further introduce the concept of automated large area multiparametric characterization of densely packed cell layers and highly corrugated tissue samples, where full automation, smart mechanical sample analysis, multiple scanner technology, and optical integration is critical for data throughput and reliable correlative microscopy. We will discuss how these developments, in combination with advanced optical microscopy techniques, can overcome the inherent drawbacks of traditional AFM systems for characterizing challenging biological samples.

We will also introduce the most recent high-speed tip-scanning atomic force microscopy (AFM) developments that enable kilohertz linerate imaging and visualization of molecular dynamics by enabling temporal resolution on the sub-100-milisecond scale. Such developments are critical for studying of cells which adapt their shape and react to the surrounding environment by a dynamic reorganization of the F-actin cytoskeleton. We will demonstrate how cell spreading and migration in living KPG-7 fibroblasts and CHO cells, can be studied with high-speed AFM and associated with spatially resolved cytoskeletal reorganization events.

Tuning the force for remote magnetomechanical gating of Piezo1 channels

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Headline

The work aims to develop a multifunctional magnetic tool that is: **i) specific:** MNPs bind to Piezo1 receptor selectively; **ii) precise:** small MNPs to gain control at the molecular level; **iii) with remote and fast spatiotemporal response:** digitized output signals in response to magnetic input cues and **iv) real-time monitoring:** magnetic applicators integrated in a fluorescence microscope.

Abstract

In a process called mechanotransduction, our cells use a set of receptors capable of sensing mechanical forces from their environment and convert them into electrochemical signals, triggering cellular responses. Among these receptors, Piezos (Piezo1 and 2) have emerged as crucial mechanosensitive ion channels, highly implied in a variety of normal and pathological processes. Piezos are non-selective ionic channels that can open in response to pressure stimulation or shear stress, allowing cell depolarization and Ca^{2+} influx [1]. However, and despite their great importance, the full mechanism of how Piezo responds to a force and transduces it into pore opening remains largely unknown. Therefore**,** developing new tools for the study and remote control of these mechanosensitive channels is a crucial current challenge.

This work aims to develop and validate a novel platform that uses small magnetic nanoparticles (MNPs) to study mechanotransduction linked to Piezo1 channels in endothelial cells through remote magnetic stimulation, obtaining real-time responses. Two specific objectives will accomplish this goal: **1)** To develop a toolkit including **i)** a surface engineered MNPs library (different size, shape and composition) with tuneable magnetism to exert high mechanical forces and able to selectively target Piezo1 channel and **ii)** a dedicated magnetic applicator able to deliver diverse magnetic cues. **2)** To investigate the possibility of opening Piezo1 upon magnetic switching and to activate important intracellular pathways connected with proliferation in endothelial cells. As Piezo1 is highly expressed in endothelial cells and its gating mediates Ca^{2+} entrance, it could be a promising target for the treatment of ischemic disease [2-4].

Cubic-shaped manganese iron oxide nanoparticles ($Mn_xFe_{3-x}O_4$) and octahedral mixed ferrites ($ZnMnFe_2O_4$) were synthesized in organic solvents following the one-step thermal decomposition. Further, MNPs were transferred into the water using a polymer coating, and a fluorophore was attached in order to track them once inside the cells. The obtained MNPs were fully characterized with a diversity of spectroscopies and physico-chemical techniques. Theoretical calculations were performed to assess the force exerted by MNPs upon simulation with different kinds of magnetic fields. Moreover, direct oriented Piezo1 antibody conjugation on optimized MNPs surface was achieved by a two-step process: **1)** ionic reversible interaction between the antibody and the MNP surface, and **2)** irreversible covalent attaching via carbodiimide chemistry. The amount of immobilized Piezo1 antibody on the MNPs surface was measured by Dot blot technique.

On the other side, the study of Piezo1 receptor expression in HUVEC cells was performed by several techniques, including immunofluorescence (IF), flow cytometry (FC), and quantitative real-time PCR (qRT-PCR). By changing several parameters, such as MNP concentration and incubation time, we established the optimal conditions for the colocalization of the MNPs with Piezo1 for each MNP type.

Last, the in-situ effect of magnetic mechanotransduction on the modulation of Ca^{2+} influx in HUVEC cells without modifications was followed by time-lapse fluorescence microscopy. Fluo-4 Ca^{2+} indicator dye was used to detect small changes in intracellular Ca^{2+}. Different experimental conditions were explored, to elucidate if small MNPs targeting Piezo1 can effectively produce Ca^{2+} influx in HUVEC cells.

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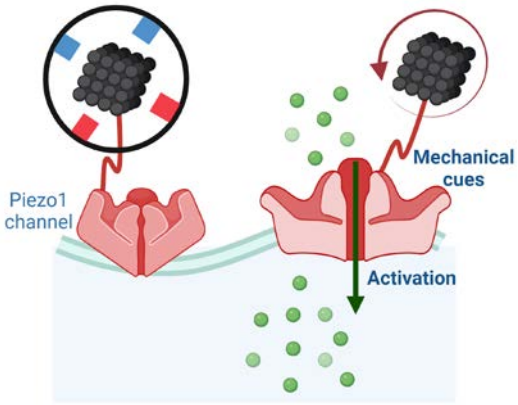

Figure 1. Schematic representation of the aim of the work based on the magnetomechanical activation of Piezo1 channel *in vitro*.

N4M

Nanotopography modulates cell identity in mouse Embryonic Stem Cells by rewiring chromatin structure.

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Headline

What shapes the life of a cell and its identity, from the outside (biophysical cues of the cellular microenvironment) and from the inside (nuclear and chromatin organization) and how is it connected (mechanotransduction and epigenetics)? Here we present the dynamic changes in chromatin structure and gene expression during mESCs fate transition in response to nanotopography.

Abstract

In recent years, it became evident how much the biophysical aspects of the microenvironments have profound impact on the cell both in physiological as well as in pathological conditions. Physical features of the extracellular matrix (ECM) modulate mechanical response which, in turn, can be transferred to the nucleus, influencing chromatin regulation, gene expression patterns and ultimately the cellular identity. However, many details of the mechanotransduction-induced impact on 3D chromatin structure in stem cell fate transition remains largely unknown. Our study aims at identifying and functionally characterize the mESCs response to the nanotopography of the cellular microenvironment, in relation to the 3D nuclear architecture and epigenetic chromatin landscape. The results show that nanotopography affects the stiffness of the cells and induces morphological alterations of the cell shape and their nuclei. The nuclear deformation is associated with a significant reduction of histone repressive marks together with their redistribution in the nuclear compartment. Importantly, we find that the mESCs functionally respond to the nanotopography by converting mESCs in the presence of LIF towards cells that have a transcriptional profile typical of the ground state and by upregulating pluripotency markers like Nanog and Esrrb. Functional experiment perturbing the 3D chromatin folding reverts this phenotype. The results show that 3D chromatin architecture plays an important role in regulating the phenotype induced by nanotopography.

Tension-driven myofibril self-assembly

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Headline (399/max 400 characters including spaces)

Self-assembly of myofibrils in muscle cells requires mechanical tension, yet the physical mechanisms establishing their periodic sarcomeric patterns are not understood. Based on extensive data on myofibrillogenesis in the fruit fly, we put forward a new theory of sarcomere assembly based driven by reciprocal interactions between catch-bond actin crosslinkers and force-generating myosin filaments.

Abstract (max 2 pages for the whole document)

Voluntary motions and heartbeat in animals is driven by contractions of myofibrils, millimeter-long acto-myosin bundles with characteristic periodic patterns of micrometer-sized sarcomeres. It was shown that the self-assembly of these "cytoskeletal crystals" requires mechanical tension [1]. Yet, the underlying physical mechanisms driving this self-assembly are not understood to date.

Here, we report data on early myofibrillogenesis in *Drosophila* flight muscle demonstrating that myosin molecular motors and actin-crosslinking Z-disc proteins form sarcomeric patterns first, while actin becomes polarity-sorted only hours later [2]. This data informs a new mathematical model of sarcomere self-assembly. We postulate a feedback loop between myosin filaments binding to an actin bundle and actin-crosslinking proteins, which relies on the catch-bond behaviour of Z-disc proteins such as α-actinin in response to active myosin forces. This model is robust to small-number fluctuations for a wide parameter range in agent-based simulations, providing a plausible mechanism of early sarcomere self-assembly.

Next, even after the establishment of sarcomeric patterns, new sarcomeres are added to myofibrils, although the myofibrils are under constant mechanical tension. We report a new, unpublished mechanism of controlled "self-rupture" of sarcomeres in which a mother sarcomere divides into two daughter sarcomeres by splitting its myosin stack, and establishing a new Z-disc in between.

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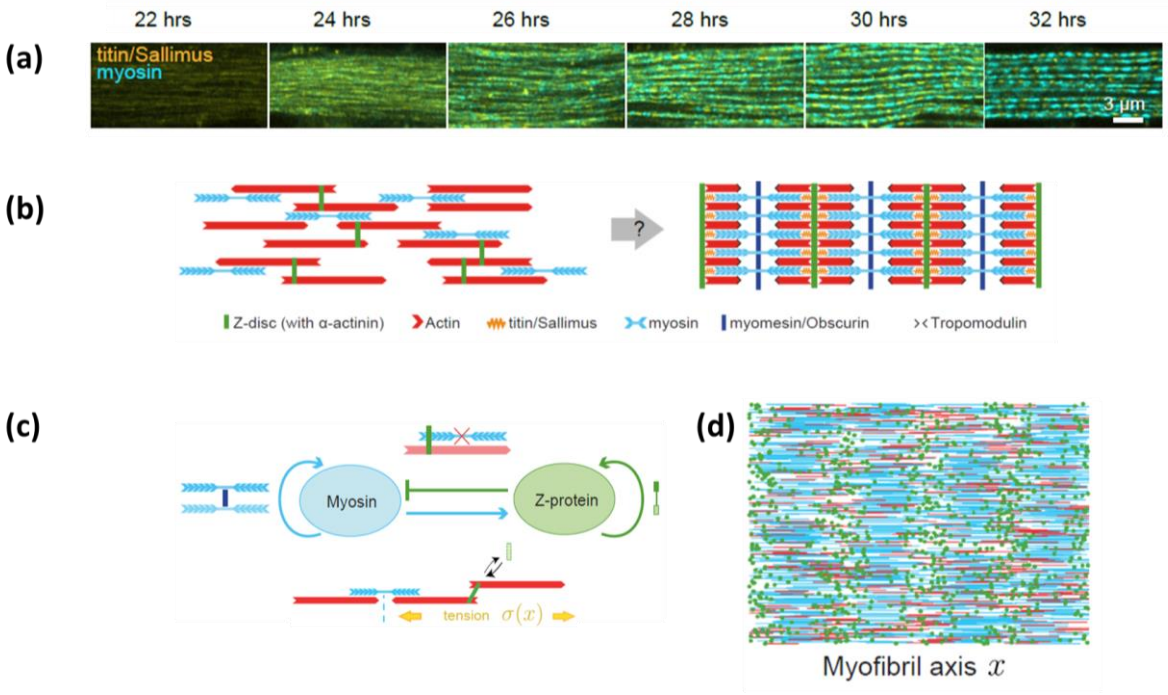

Figure 1. (a) Formation of periodic sarcomeric patterns in developing myofibrils in fruit fly flight muscle. The tagged N-terminus of titin/Sallimus serves as proxy for nascent Z-discs, which alternate with myosin filament stacks. (b) Problem statement: how do unstriated acto-myosin bundles remodel into myofibrils with periodic pattern of sarcomeres? (c) Proposed feedback between myosin filaments binding to actin bundle and cross-linking Z-disc proteins: active myosin forces stabilize aggregates of Z-disc proteins such as α-actinin as result of their catch-bond behaviour. Together with previously reported autocatalytic attachment and steric interactions, this defines a feedback loop capable of spontaneous pattern formation. (d) Agent-based simulation of the model depicted in (c), demonstrating the robust self-assembly of sarcomeric patterns.

Mechanical memory of confined migration

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Headline

Do cells migrating in confined microenvironments retain memory of their previous confined experiences?

Abstract

The migration of epithelial cells through dense tissues and tight spaces[1] is a crucial process in tissue development, homeostasis, and diseases such as cancer. However, how spatial confinement affects cell migration dynamics is still not well understood. We investigated the transient migration events of epithelial cells on adhesive dumbbell-shaped micropatterns[2] that lead to repeated back and forth migration events. By tuning the dimensions of the central narrow bridge that connect two squared-shape adhesive sites, we show that the spatial confinement imposed by the bridge geometry influences the migration velocity. Our findings show that imposing narrower bridges increases the cell migration speed through large cellular extensions. Interestingly, extending the length of the narrower bridges significantly increases the success rate of crossing up to 85%. We show that the crossing rate and the dynamics of transient migration are both controlled by a morphological switch imposed by the bridge aspect ratio. Indeed, epithelial cells on longer bridges switch from an extended and slow morphology to a fast and compacted phenotype with a steady polarization state, raising the question of the existence of a polarization memory in confined cells. To address this question, we the mechanism of mechanical memory maintenance through the reorganization of cytoskeletal components and explore how it is possible to erase this mechanical memory.

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Single objective light sheet allows volumetric super-resolution imaging of thick samples

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Headline (max 400 characters including spaces)

We show how to implement highly confined single-objective light-sheet and its application to achieve volumetric PALM in thick samples. This approach is used to map the spatial distribution of efflux pumps in bacterial biofilms, complex communities where microbes gain enhanced resistance to biocides. Visualizing these pumps at subcellular resolution provides crucial insights into the molecular strategies that enhance biofilm resistance.

Abstract

Introduced by Tokunaga et al. in 2008 [1], HILO microscopy employs a single-objective inclined light-sheet illumination strategy that significantly reduces unwanted background fluorescence from out-of-focus planes by exploiting the refraction of the excitation beam at the glass/water interface. This method illuminates a confined region of the sample volume with a thin sheet of light, enhancing image quality and allowing for detailed observation of biological samples at the sub-cellular level.

We have developed a theoretical model describing the propagation of the inclined beam, validated through comprehensive far-field and near-field experiments [2]. This model enables precise prediction of the beam's geometrical features at the sample level, facilitating a straightforward alignment and beam shaping procedure that improves image quality for both conventional fluorescence microscopy and localization-based super-resolution microscopy. Moreover, thanks to the simple introduction of a linear slit to shape the excitation beam we could reduce the thickness of the inclined beam to less than 3 micrometers while maintaining a field-of-view suitable for cell imaging, and we have more than doubled the number of single-molecule localizations in super-resolution PALM/STORM applications. This enhancement not only extends resolution but also reduces the time required for acquisition, thus representing a significant leap forward in microscopy techniques.

We further extended the application of highly confined inclined beam by demonstrating volumetric PALM in thick samples through scanning of the light-sheet along the optical axis of the objective. In fact, we performed volumetric PALM to reveal Efflux Pumps (EPs) spatial distribution in bacterial biofilms [paper under revision].

Biofilms are complex community of bacteria organized in a three-dimensional mesh structure, of some hundreds microns thickness, exhibiting enhanced resistance to biocides. This resistance, often mediated by efflux pump (EP) transmembrane complexes like the AcrAB-TolC protein complex, challenges the

treatment of infections caused by biofilms. The understanding of the molecular mechanisms behind such an increased resistance would be of great importance, however it poses technical challenges to reach subcellular resolution in biofilms. Our work leverages the capabilities of HILO microscopy combined with super-resolution techniques to visualize and quantify the expression levels of the AcrAB-TolC pump within biofilms at an unprecedented resolution.

Importantly, by utilizing a combination of PALM with single-objective light-sheet excitation and precision genome editing, we have achieved single-molecule localization of endogenous efflux pumps (EP) with 20 nm resolution. This methodology allowed to explore the spatial distribution and density of EPs under their native regulation for the first time over the biofilm volume, revealing a decrease in pump density with increasing depth inside the biofilm and over time during its maturation. Such findings suggest a stratification of defense mechanisms within the biofilm, with bacteria at the periphery exhibiting higher levels of efflux pump expression, potentially as a direct response to environmental threats.

Our study provides the first single-molecule map of protein distribution within a biofilm, employing HILO microscopy to offer new insights into the molecular mechanisms underpinning biofilm resistance. This breakthrough not only enhances our understanding of biofilm biology but also opens new avenues for the development of targeted therapies to combat biofilm-related infections. Furthermore, the methodologies that we developed have broad applications, extending beyond biofilms to the study of other thick biological samples, thereby setting a new standard for imaging techniques in the biological sciences.

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Mechanical interplay between cells and the microenvironment, associated with cancer invasion and immunotherapy

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Headline

Mechanical and chemical interactions between cells and the surrounding extracellular matrix, trigger multiple signaling events that regulate cell behavior, and fate. We would like to present here specific mechanosensitive features of the "cancer microenvironment", including description of a process whereby invasive cancer cells penetrate the nearby matrix by up-regulating protrusive integrin adhesions, termed "invadopodia", and a novel "synthetic biology" strategy whereby cancer-specific cytotoxic T cells are stimulated ex-vivo by a "synthetic immune niche", consisting of immobilized chemokine and adhesion molecule, increasing their capacity to both proliferate and kill target cancer cells.

Abstract

Living cells maintain a dynamic mechanical cross-talk with their cellular and non-cellular microenvironment. These interactions are mediated by diverse membrane receptors that mediate either adhesive processes or activate transmembrane signaling pathways that, conc ertedly trigger changes in cell shape, fate and behavior, including cell proliferation, differentiation, gene expression, signaling, morphogenesis, migration and survival. Cellular sensing was shown in recent years to respond to multiple features of the pericellular environment, including its chemical properties, molecular composition (commonly sensed via specific membrane receptors, that activate specific signaling pathways) and its physical properties, like rigidity, viscosity, roughness and the like (often sensed via multiple "mechanosensory", cytoskeleton-associated adhesion complexes). In my talk I will address two such systems, one related to cancer invasion and the other to cancer immunotherapy. The first story will be focused on **invadopodia**, their structure, mechanical properties, their capacity to use the nucleus for amplifying their penetration force and the regulation of their formation by the HIPPO signaling pathway. We will highlight the molecular mechanism involved in the generation of protrusive forces that drive cancer invasion, and discuss the capacity of YAP and TAZ to regulate the levels of TKS5 and MMP14, key components of invadopodia. The second story, will address a novel approach for enhancing cancer immunotherapy, using a **Synthetic Immune Niche** (SIN), mimicking the pericellular microenvironment in the lymph-node. Multiple studies carried out in recent years demonstrated that cell-based cancer immunotherapies, using tumor infiltrating lymphocytes (TILs) or CAR-T-cell therapy, often fail, when the effector T cells become exhausted, following massive proliferation. We demonstrated that exposure of the immune cells to immobilized chemokine (CCL21) and adhesion molecule (ICAM1) can enhance both the proliferation of cancer-specific cytotoxic T-cells and, at the same time, retain or even enhance the cytotoxic potency of these cells, thereby increasing their cancer-eradicating capacity.

Control strategies for fast scanning in dynamic mode AFM

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Headline

In AFM imaging scanning speed and topography reconstruction are connected, and improving one leads inevitably to a worsening of the other. Many are the limitations we are trying to overcome, first the parachuting that arises at high speeds and causes artifacts in the reconstructed topography. In this work, an easy solution for removing parachuting is suggested.

Abstract

AFMs have been extensively used as powerful tools for imaging applications. Although there exist more different working modalities, the dynamic mode is the one most suitable for this purpose since it allows weaker interaction forces between the tip and the sample. In this modality the cantilever is excited close to its resonance frequency by an actuator, with a free vibration amplitude A_0. However, due to the interaction forces, the oscillation amplitude $A(t)$ varies proportionally with the separation distance between the tip and the substrate. Therefore, a PI controller is exploited to keep the amplitude close to a set point value A_R by translating vertically the cantilever support so that the error signal $e(t) = A_R - A(t)$ is kept to zero. From this regulation process, the sample topography can be reconstructed. However, this tracking method is not satisfactory whenever there are deep valleys in the sample surface that lead to the *parachuting phenomenon*. In fact, the presence of deep valleys causes the tip to disengage from the sample and the error signal saturates to the constant value $A_R - A_0$ which is small since A_R is typically chosen to be closed to A_0. In return, the PI controller reacts slowly to this error signal causing artifacts in the topography. Additionally, those artifacts are more evident at fast scanning speed, hence the parachuting limits also the maximum achievable velocity [1]. It is evident that a compromise between scanning speed and the minimization of the error in the topography should be found in order to improve the performances.

In [2] the authors propose an effective strategy for limiting the parachuting in the so-called auto-tapping mode, i.e., the cantilever is self-excited by using a nonlinear feedback loop, while ensuring high speeds. Their idea is to adapt the free vibration amplitude to the variations in the sample profile. Following this approach, we propose a technique that can be applied to any commercial AFM microscope working in dynamic mode. Our algorithm aims to increase A_0 whenever the parachuting is detected. This can be easily implemented with a switching logic, where the excitation force, exerted by the actuator on the cantilever, is amplified just during the parachuting. Allowing A_0 to increase in this phase, permits a quicker response of the PI controller and hence it reduces the presence of artifacts in the reconstructed topography even at fast scanning speeds.
The algorithm has been implemented on a Moku:Pro, a flexible FPGA-based real time architecture [4]. With expanded application-programming interface support in different computer languages such as MATLAB and Python, Moku:Pro allows users to implement custom algorithms in an intuitive way. An additional benefit is that it allows users to change custom parameters' values in real time.
For the experiment we used the DriveAFM from Nanosurf [5], equipped with a breakout box which allows the users to manipulate the input signals of the AFM, a NCSTAu cantilever (Nanosurf) and a calibration grating sample with square holes pattern. In Figure 1 a comparison between the classical scanning technique (a) and the one corrected using our proposed algorithm (b) is reported. Both images

assume a scanning direction from left to right and a scanning speed of 50 μm/s. In Figure 1.(a) the effect of parachuting is evident, (blurred area), where a wrong interpretation of the real height is given. On the contrary, in Figure 1.(b) the parachuting is completely vanished: we can notice sharp profiles where before we saw blurred valleys. Such result confirms the goodness of the proposed algorithm.

In the future, as first step, we would like to extend the proposed algorithm to the auto-tapping mode, [2]. This modality is particularly beneficial when working in fluid environments and hence with biological samples, since it simplifies the identification of the resonance frequency and improves the signal to noise ratio. Then, we would like to proceed modifying the control law, bypassing the traditional PI controller, in order to have better overall performances.

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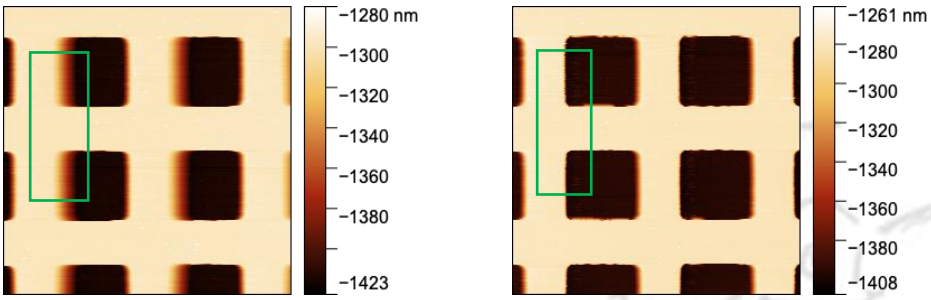

Figure 1. Sample topography using classical dynamic mode (a) and with the proposed algorithm (b).

A novel in silico – in vitro approach for material design in viscoelastic mechanotransduction studies

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Headline

An in silico-in vitro approach was defined to investigate viscoelastic mechanotransduction on agarose-dextran gels. In silico models were used to predict the optimal polymer combination to achieve gel time responses matching cellular timescales. Tests with adipose derived stem cells confirmed the computational results allowing the identification of substrates for controlling cell fate.

Abstract

The understanding of mechanisms governing mechanotransduction has become a key goal of scientific research in recent decades for designing more physiologically relevant in vitro models and understanding the role of the extracellular matrix (ECM) viscoelastic properties in the evolution of pathological conditions [1]. Hydrogel manufacturing techniques have been extensively researched in this context to fabricate cell culture substrates with variable mechanical properties, even though the majority cannot describe the role of the sole viscous properties of the hydrogel. In this context, predicting hydrogel viscoelastic properties is critical for accurately designing viscoelastic mechanotransduction studies. Even though different computational models have been proposed to simulate hydrogel mechanical behaviour, most of them can involve high computational costs and the a priori knowledge of input parameters that are difficult to achieve [1-3].

Here, we present a novel in silico - in vitro approach (Fig.1) for tailoring and predicting agarose hydrogel viscoelastic behaviour. In particular, the time-dependent properties of the gels were modulated by tuning liquid phase viscosity thanks to different dextran concentrations. Optimal polymer combination was defined using an in-silico model able to predict and select gels with the relaxation time (τ_{rel}) within cell focal adhesion lifetime (τ_l) and the binding time (τ_b).
The in silico model performed on Matlab 2022a describes the agarose and dextran hydrogel viscoelastic behaviour by analysing the interaction between liquid phase diffusive properties - described through the reaction-diffusion equations (eq.1) - and viscoelastic mechanical response - described by the lumped-parameters schematization (eq.2) - as a function of the liquid phase dextran concentration. An apparent diffusion coefficient (eq. 3) was introduced starting from the Einstein-Stokes coefficient and including additional coefficients for considering the porous matrix hindered diffusion (eq.4) and the connection between diffusive and mechanical properties via the average mesh size (eq.5-6) [3-4]. An adapted generalised Maxwell Standard Linear Solid (eq.2, Fig.1) lumped parameter model reflecting dextran interaction with the viscous phase was used to predict the mechanical behaviour [1,5].

$$\frac{\partial c}{\partial t} = D_{app} \cdot \nabla^2 c - \bar{v} \cdot \nabla c - R_{dex} \quad \text{(eq. 1);} \qquad \sigma(t) = \dot{\varepsilon}\,(E_0 t + (\eta_1 + \varphi \cdot \eta_2)(1 - e^{-\frac{E_1}{(\eta_1+\varphi\cdot\eta_2)}t})) \quad \text{(eq.2)}$$

$$D_{app} = \beta(R_h, \kappa) \cdot \delta(\xi_{avg}) \cdot \frac{k_b T}{6\pi\eta R_H} \quad \text{(eq.3);} \qquad \beta(R_h, \kappa) = \left\{1 + \left(\frac{R_H}{\kappa}\right)^{\frac{1}{2}} + \frac{1}{3}\left(\frac{R_H}{\kappa}\right)\right\}^{-1} \quad \text{(eq.4)}$$

$$\delta(\xi_{avg}) = e^{-\frac{\pi}{4}\left(\frac{R_f + R_h}{R_f + 0.5\cdot\xi_{avg}(t)}\right)^2} \quad \text{(eq.5);} \qquad \xi_{\text{avg}}(t) = (18 k_B T/\pi\alpha^{\frac{2}{3}} E(t))^{\frac{1}{3}} \quad \text{(eq.6)}$$

The model predictive power was assessed and validated by comparing the computational predictions to the experimental characterization of 5 mg/mL agarose hydrogel and 0, 20, 30 and 40 mg/mL dextran

using the epsilon dot method [5]. Indeed, despite an increase in dextran content and therefore liquid phase viscosity, the mesh size rises, resulting in a decrease in the relaxation time (Fig.2B), whereas the equilibrium elastic modulus remains constant (Fig.2A).

The in silico tool was used to select the substrate compositions for performing adipose-derived mesenchymal stem cells (ADSC) viscotransduction study for 7 days: a) 5 mg/mL agarose + 20 mg/mL dextran that provides a τ_{rel} within $[\tau_b,\tau_l]$; b) 5 mg/mL agarose + 40 mg/mL dextran that has $\tau_{rel} = \tau_b$; c) Tissue culture plastic (TCP) substrate used as control and can be considered with $\tau_{rel} \rightarrow \infty$. The morphological, immunological (e.g., YAP distribution), and histological (adipogenic, osteogenic differentiation or stemness) features were analysed for describing the cell response to the different viscoelastic stimuli through immunostaining and histological staining techniques and confocal images analysis (ImageJ). Cell testing revealed the model's capacity to optimise the viscoelastic mechanotransduction study more quickly and easily. Indeed, morphological, immunological, and histological analysis demonstrated that when τ_{rel} was equal to the ADSC τ_b (40 mg/ml dextran gels), the mechanical tension provided by the agarose substrate was enhanced by the viscous properties, resulting in YAP nuclear transmigration (Fig.2C) and thus activation of the adipose differential pathway (Fig.2E). The differentiation pathway was also followed on the TCP controls, even though toward an osteogenic lineage (Fig.2D) due to the highest elastic modulus of the substrate (≈GPa). In the case of 20 mg/mL agarose, the viscoelastic dynamic can preserve the ADSC stemness, since the YAP remained located in the cytoplasm (Fig.2C), thus the viscoelastic dynamic occurred outside the temporal window of cell mechanical response.

The presented in silico-in vitro framework proved to be a valuable and useful tool for the design of cell viscoelastic mechanotransduction studies, and more broadly for the design of hydrogels for regenerative medicine applications and advanced in-vitro models.

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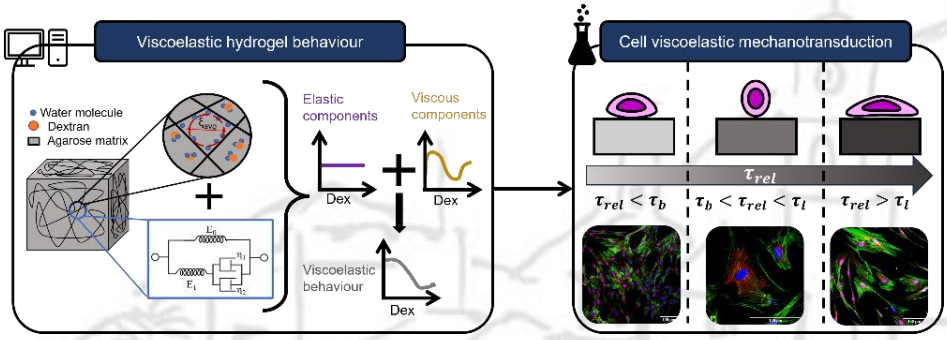

Figure 1. Graphical schematization of the in silico – in vitro approach framework

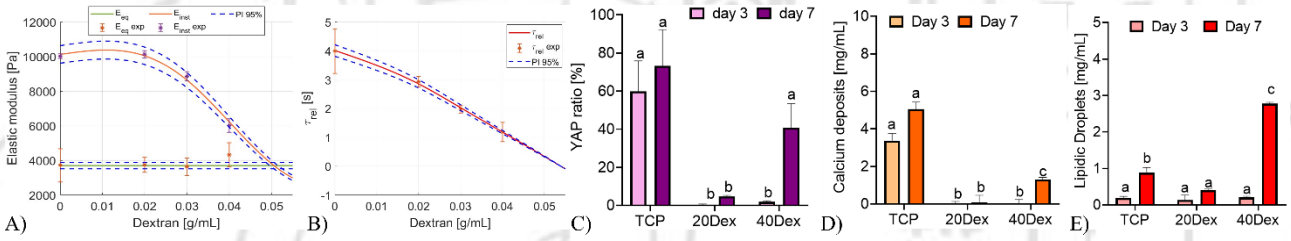

Figure 2. (A) Equilibrium, instantaneous elastic moduli and (B) relaxation time of agarose hydrogels as a function of the dextran concentration; (C) ADSC YAP nuclear distribution, (D) calcium concentration (Alizarin red) and (E) lipidic droplets concentration (Oil Red O).

Native extracellular matrix probes to target patient and tissue-specific cell–microenvironment interactions by force spectroscopy

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Headline

Atomic Force Microscopy (AFM) is successfully used for the quantitative investigation of the cellular mechanosensing of the microenvironment. To this purpose, force spectroscopy approaches aim at measuring the adhesive forces between cells and their microenvironment typically exploiting functionalised tips mimicking the ECM. Our work focuses on the creation of native, patient-specific ECM probes.

Abstract

Aiming at reproducing the native cell–ECM interface within a typical AFM-based force spectroscopy experiment, we developed native ECM probes and demonstrated that they can be reliably used to scrutinise integrin-related adhesive interactions between cells and their microenvironment. While the current approach to mimic ECM interactions relies on probes functionalized with single ECM components, our AFM probes faithfully reproduce the entire structural and biochemical complexity of the extracellular matrix.

This was achieved by attaching to an AFM cantilever a micrometric slice of native decellularised ECM, which was cut by laser microdissection. We demonstrate that these probes preserve the morphological, mechanical, and chemical heterogeneity of the ECM. As proof-of-principle, we tested a rat bladder ECM probe against the AY-27 rat bladder cancer cell line. On the one hand, we obtained reproducible results using different probes derived from the same ECM regions; on the other hand, we detected differences in the adhesion patterns of distinct bladder ECM regions (submucosa, detrusor, and adventitia), in line with the disparities in composition and biophysical properties of these ECM regions.

Our results demonstrate that native ECM probes, produced from patient-specific regions of organs and tissues, can be used to investigate cell–microenvironment interactions and early mechanotransductive processes by force spectroscopy. This novel adhesion force spectroscopy approach is therefore characterised by a high potential versatility, which could be a key element in the nanotechnological toolkit of nascent personalized medicine.

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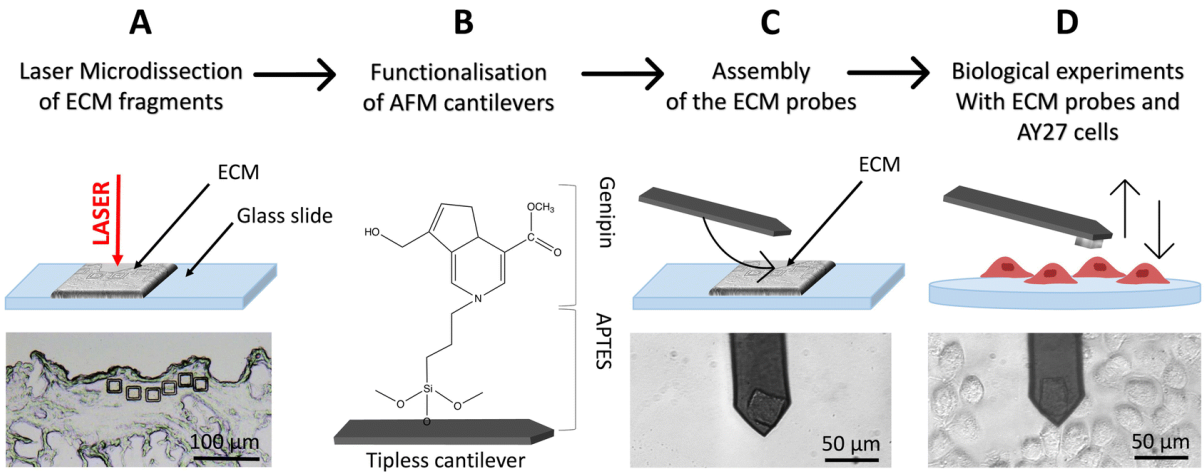

Figure 1. Schematics of the ECM probe production. (A) Optimized use of the LMD system to produce native ECM probes: mounting the decellularized tissue directly on a standard microscope glass slide, allows the UV laser to physically separate ECM fragments from the surrounding matrix, without detaching them from the glass support. (B) Tipless cantilevers are functionalised with APTES and genipin (covalently bonded), and then used for the attachment of the ECM probes (C), exploiting the XY micro-translation stage of the optical microscope that includes the AFM (stand-alone stages can be used equivalently). (D) The resulting ECM probes are used for biological experiments, such as force spectroscopy measurements on top of living cells.

Adherens junction dynamics and YAP localisation are modulated by cell confinement on micropatterns.

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Headline (max 400 characters including spaces)

Mis-regulation of adherens junctions can lead to loss of cell-cell adhesion and contribute to metastasis. We use micropatterning to impose stable, reproducible forces, and dissect the role of intrinsic forces in the maintenance of a stable epithelium. We dissect the interplay between biophysical properties of confined cells and correlate this to junction composition, YAP localisation and motility.

Abstract (max 2 pages for the whole document)

Adherens junctions initiate cell-cell adhesion and play important roles in tissue development and homeostasis. Mis-regulation of adherens junction dynamics can lead to loss of cell-cell adhesion, epithelial to mesenchymal transition and contribute to tumour invasion. At present, there are no markers of cell-cell contact maturation. Using a Junction Unit model, the Braga lab demonstrated that the biophysical properties of a cell can provide insight into the junction maturation status. Adherens junction morphology and junctional protein distribution is altered in response to intrinsic cell rheology. (1)

Cell pairs confined to triangular micropatterns have elevated intrinsic stiffness, short, straight junctions with reduced density of proteins present at the junction. In contrast, cell pairs grown on circular micropatterns are more compliant and have longer, sigmoidal junctions with increased receptor density at junctions. (Figure 1) This data led us to infer that cells grown on less tensile environments have junctions associated with an immature, unstable phenotype, akin to tumour cells.

Here, we decipher the mechanisms by which junction maturation can be mechanically regulated. We build up complexity of the model by increasing the number of cells and the area available to spread to understand how intrinsic forces play a role in the maintenance of a stable epithelium or promote a tumour like phenotype. We dissect the interplay between biophysical properties of confined cells and correlate this to junction composition. We dissect the key signalling pathways associated with junction maturity and those driving tumour cell motility, enabling us to devise novel anti-metastatic strategies to inhibit tumour cell invasion.

YAP is a transcriptional coactivator that shuttles between the cytoplasm and the nucleus to regulate the transcription of genes involved in proliferation, differentiation, motility etc. YAP activity can be modulated by the hippo pathway, signalling receptors and mechanical cues. We demonstrate that YAP localisation is modulated by cell confinement on micropatterns. Interestingly, the localisation of YAP on micropatterns deviates to that shown on epithelial monolayers as YAP is heterogeneously distributed among cells sharing a micropattern. We dissect the key biophysical properties driving YAP heterogeneity in response to geometry, increasing adhesive area and number of neighbours. The mechanism by which YAP is translocated into nucleus to cytoplasm is unknown. Evidence suggests that adherens junction complexes may play a role in sequestering YAP in the cytoplasm. We correlate

the composition of adherens junctions with YAP nuclear intensity and identify cellular responses that correlate with increased YAP nuclear intensity under confinement.

The model developed will allow us to dissect the key contribution of individual signalling pathways that co-ordinately maintain stable, mature junctions.

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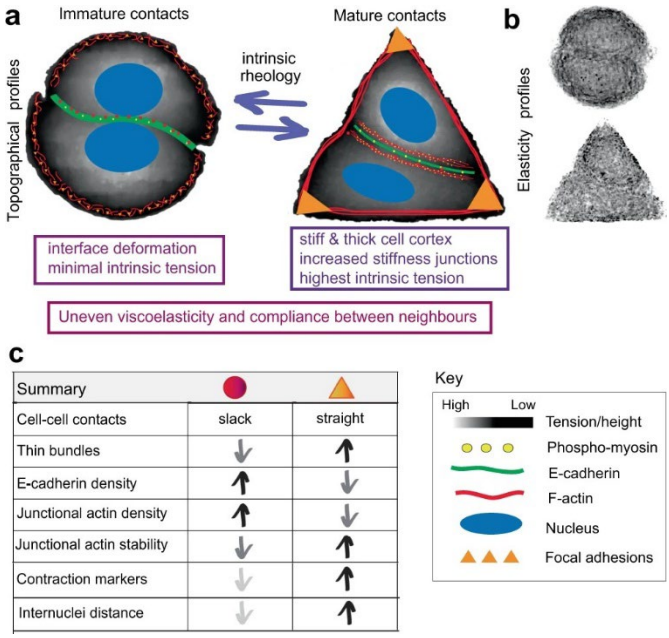

Figure 1. (a) Intrinsic cell rheology drives junction maturation—summary (b) Diagram depicting the topographical maps of circular and triangular cell pairs in grey scale, representing height as shorter (dark grey) versus higher length values (white) of cell pairs. Representation of junctions under different tensile stresses are shown as the shape of cell-cell contacts (E-cadherin, green), organization of actin filaments (red) at junctions and periphery of micropatterns as: lamellae (circles) or stress fibres (triangles), focal adhesions (orange colour) and labelling of PMLC (yellow dots). b Elasticity profiles of circular and triangular cell pairs highlight the presence of stiffer spots on regions/subcellular structures and junctions (white colour, hight stiffness) and areas of lower mechanical stress (dark grey). a, c Text boxes below diagrams (a) and summary table (c) show the identified rheological and molecular properties, respectively. In the table in c, micropattern geometries are represented as red circles or orange triangles.(1)

Progressive alteration of murine bladder elasticity in actinic cystitis detected by Brillouin microscopy

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Headline

We use Brillouin Microscopy as a novel imaging technique to retrieve biomechanical changes of murine bladders in healthy and fibrotic conditions, thus being able to distinguish between progressive stages of actinic cystitis.

Abstract

The biological consequences of altered tissue mechanics greatly affect organs homeostasis and functions. In vivo, the local microenvironment within tissues continuously exerts mechanical forces (i.e., shear, compressive, extensional forces) on cells, triggering the activation of a cascade of biomechanical pathways critical for morphogenesis, homeostasis and development [1].

Bladder mechanical properties are critical for proper organ function and tissue homeostasis: alteration of its elasticity is thus linked to disease onset and progression [2]. This study aims to characterize the tissue elasticity of the murine bladder wall in healthy conditions and in actinic cystitis, a state characterized by tissue fibrosis that is a frequent complication of radiation therapy. Here, we exploit Brillouin microscopy, an emerging technique in the mechanobiology field that allows mapping tissue mechanics at the microscale, in non-contact and label-free mode [3].

We show that Brillouin imaging of murine bladder tissues is able to recognize different anatomical components of the bladder wall, confirmed by histopathological analysis, showing different tissue mechanical properties of the physiological bladder (Figure 1), as well as a significant alteration in the presence of tissue fibrosis. Our results point out the potential use of Brillouin imaging on clinically relevant samples as a complementary technique to histopathological analysis, deciphering complex mechanical alteration of each tissue layer of an organ that strongly relies on mechanical properties to perform its function [4].

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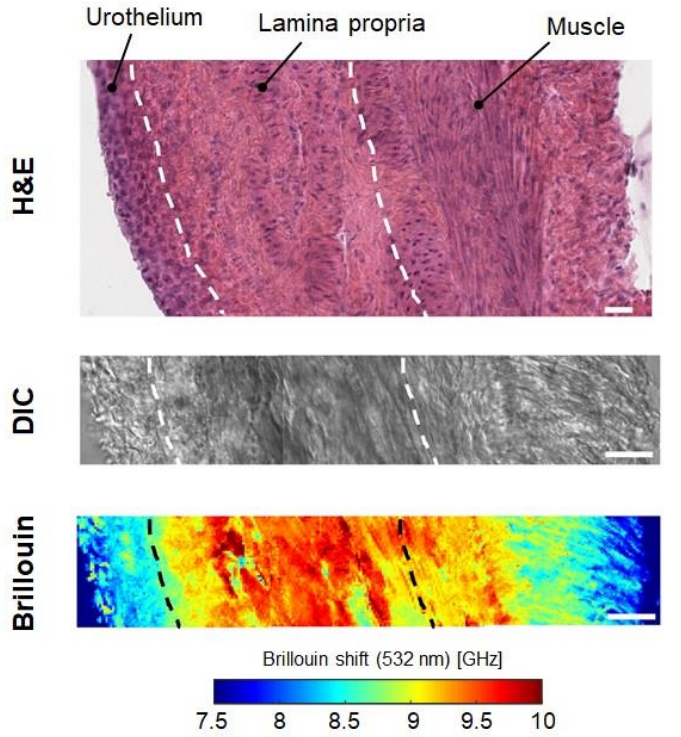

Figure 1. Healthy rat bladder wall imaging by different microscope techniques. From top to bottom: Hematoxylin eosin (H&E) staining of the bladder wall, showing the different anatomical tissue layers (Urothelium, Lamina propria, Muscle); Differential interference contrast (DIC) image; corresponding Brillouin map of healthy bladder walls from urothelium (left) to muscle (right), imaged with our custom-buil Brillouin Miscroscope. Scale bars = 20 µm.

Harmony in Motion: Unveiling Single Cell Dynamics with Simultaneous Mass and Stiffness Measurements

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Headline (max 400 characters including spaces)

Our enhanced AFM technology introduces simultaneous cytoplasmic stiffness and mass measurements of single live cells through Q-factor and resonance frequency analysis, promising insights into cell growth-stiffness correlations across states and diseases. When applied to integrin-knockout fibroblasts, it revealed diverse mechanical properties.

Abstract (max 2 pages for the whole document)

Investigating the interplay between regulatory processes such as cell growth and stiffening is essential to understand the development of multicellular organisms. However, how cells use different membrane proteins to regulate cellular stiffness and mass by sensing extracellular information remains unclear. Recently, an AFM technology based on photothermally actuated microcantilevers was established to monitor the total mass of single adherent living cells in culture conditions over the course of several hours, while imaging them via light microscopy [1-2]. In this approach, the attachment of a cell to the free end of the microcantilever leads to a shift in its resonance frequency, which is used to calculated the attached mass. In order to expand this technology, we introduced a way to extract the quality (Q-) factor of the coupled microcantilever-cell system alongside its resonance frequency. The Q factor represents a measure of the damping of the overall system and it depends on its spring constant. Therefore, by simultaneously tracking both the resonance frequency and the Q-factor of the microcantilever over continuous frequency sweeps, we aim to simultaneously measure the mass and stiffness changes of living cells. To better understand how integrins regulate cell growth and stiffness, we applied the above-described method to characterize the mass and Q-factor changes three different engineered integrin-knockout fibroblast cell lines which exclusively express αv-, β1- or both αv/β1-class integrins and of their parental wild-type cell line. Interestingly, we observed different Q-factor behaviors for the engineered fibroblasts lines expressing specific integrins, indicating that these cell lines might possess different mechanical properties. Thus, we have developed a theoretical model to extract the stiffness properties of the cells from the changes in Q-factor that we observe upon cell attachment to the microcantilever. To validate our theoretical model of the Q-factor, we characterized the mechanical behavior of the aforementioned fibroblast lines by distinguishing between the two fundamental components that govern cell mechanics: the cytoskeleton and the cytoplasm. We utilized AFM indentation to measure cytoskeletal stiffness and conducted active microrheology with optical tweezers to assess cytoplasmic mechanical properties. Notably, the measurements obtained with optical tweezers correlated well with the stiffness data derived from the Q-factor results. Thus, we propose a novel functionality of the picobalance technology, enabling simultaneous measurements of cytoplasmic stiffness and mass changes. This advancement holds significant promise as it facilitates a comprehensive understanding of how cell growth correlates with stiffness variations in diverse cellular states and under diseased conditions.

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Epithelial to Mesenchymal Transition modulation in breast cancer cells: a frontier for new investigation approaches

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Headline

Over 90% of cancer deaths are due to metastases, however the effectiveness of therapy in reducing them has reached a plateau. To increase our arsenal for one more effective treatment, we need new targets and therefore our knowledge and the investigation approaches. Here, we analyse the ability of a 3D photopolymerized substrate to investigate breast cancer mechanotransduction in EMT.

Abstract

INTRODUCTION

Breast cancer is the most frequently diagnosed cancer, and the leading cause of death, among women. Its onset is associated with phenotype transition from epithelial to mesenchymal (EMT), a process generating cancer stem cells (CSCs) [1, 2]. Markers of EMT are loss of cellular contacts, β-catenin nuclear localization, cytoskeleton reorganisation, vimentin expression and increased motility [3]. There are several factors of the tumour microenvironment known to influence EMT, such as hypoxia, immune response and stiffening of the extracellular matrix [4]. We developed a culture substrate, called Niche, which is a custom-made, microporous, optically accessible 3D scaffold fabricated by 2-photon laser polymerization [5], in which cells adhere and grow in a spatial configuration different from the spheroid standard culture condition. Here, we analyse the ability of this substrate to sustain the EMT phenotype in CSCs.

METHODS

Malignant breast cancer cell lines (MCF7 and MDA-MB-232) were grown in different cell culture conditions: on 2D flat glass coverslips, on 2D soft collagen based-hydrogels (with Young's modulus of 10Pa), in the 3D Niche, and in conventional3D spheroids. We used a pharmacological control to destroy actin cytoskeleton and reduce intracellular tension. Immunofluorescence assays were used to characterize the organization and localization of EMT-key markers: actin, vimentin, and β-catenin. The role of the various substrates in modulating cell migration was investigated by time-lapse microscopy.

RESULTS

Results show that the Niche and the pharmacological control limit the cell capability to migrate. The actin fibers number, related to intracellular tension, is highest on flat, and reduced in the Niche and spheroids. Vimentin is less expressed in the Niche than 2D glass and protein localization in the Niche is similar to spheroid samples. Only the Niche and the spheroids influence nuclear β-catenin translocation.

DISCUSSION & CONCLUSIONS

Our results suggest that the Niche, influencing cellular adhesion properties, can modify key parameters related to cancer phenotype, primarily cell motility. This substrate also guarantees optical access to the samples to monitor in real-time the phenomenon evolution. Therefore, the Niche is a valuable tool for 3D-cancer-models and pharmacological research in vitro. We are now using this substrate to evaluate how mechanobiology can improve knowledge and research approaches related to cancer biology.

ACKNOWLEDGEMENTS: ERC-project BEACONSANDEGG G.A.10105312

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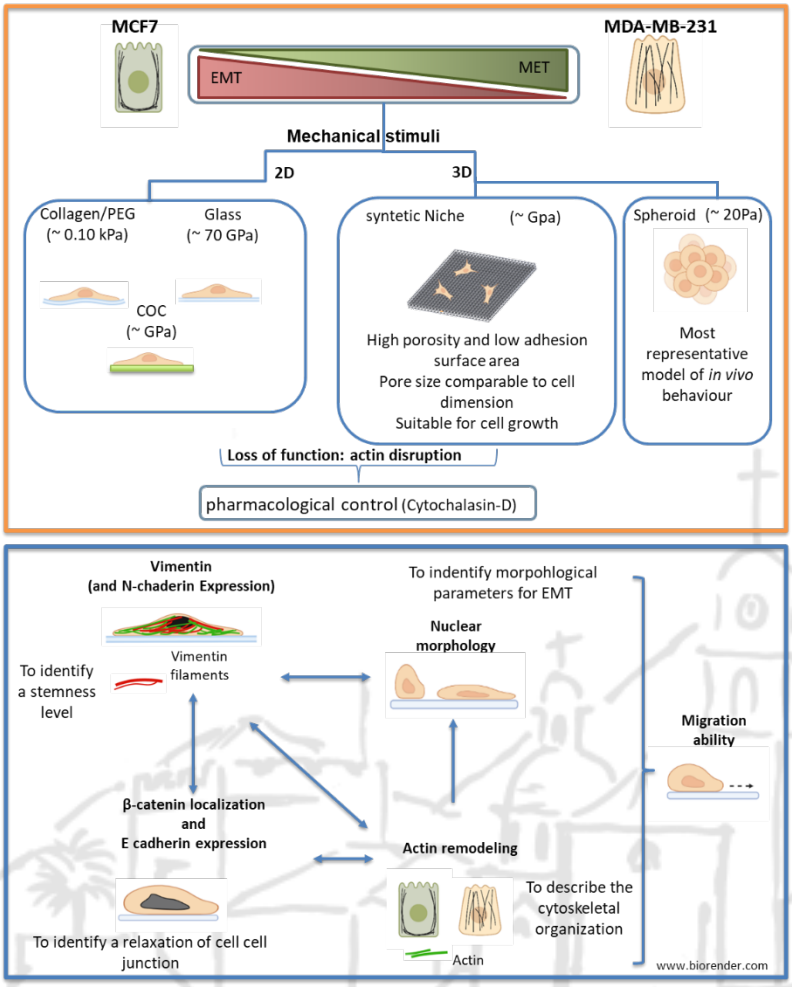

Figure 1. Mechanotransduction investigation of EMT: concept of the work

Morpho-mechanics of human early brain organoid development

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Headline

Long-term, live light sheet imaging captures developmental morphodynamics at single-cell resolution using sparse and multi-mosaic fluorescent human brain organoids, revealing quantitative insights into neuroepithelial morphogenesis and patterning in the presence and absence of extrinsic extracellular matrix.

Abstract

Brain organoids enable mechanistic study of human brain development and morphogenesis and provide opportunities to explore self-organization in unconstrained developmental systems[1–3]. We have established long-term light sheet microscopy on unguided multi-mosaic neural organoids generated from fluorescently labelled human iPSCs, which enables tracking of tissue morphology, cell behaviours, and subcellular features over weeks of organoid development. We developed an analysis pipeline to demultiplex labels and segment cells in multi-mosaic brain organoids using deep learning to provide quantitative measurements of tissue and cellular dynamics, using Actin, Tubulin, plasma membrane, nuclei, and Lamin labels. Based on live imaging and single-cell transcriptome modalities, we find that lumenal expansion and cell morphotype composition within the developing neuroepithelium are associated with modulation of gene expression programs involving extracellular matrix (ECM) pathway regulators and mechanosensing. We show that an extrinsically provided matrix enhances neuroepithelium alignment, polarization, and lumen expansion as well as telencephalon formation. Unguided organoids grown in the absence of an extrinsic matrix have altered tissue and lumen morphologies with increased neural crest and casualized tissue identity. Finally, we show ECM induced morphological changes and patterning guidance is linked to modulations via the interactions between the HIPPO and the WNT signalling pathway, including spatially restricted induction of WNT pathway genes that mark the earliest distinction between telencephalic and non-telencephalic lineages. Altogether, our work provides a new inroad into studying human brain morphodynamics and supports a view that mechanosensing dynamics have a central role in constraining brain morphodynamics and regionalization[4].

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Stronger adhesion to the substrate contributes to increased fragility of senescent endothelial monolayers

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Headline

We analysed the mechanical resilience of young and aged endothelial cells exposed to different stretch conditions. While young cells were resilient to acute uniaxial and cyclic biaxial loading, their senescent counterparts showed impaired monolayer integrity. Based on the results of a new computational model, stronger adhesion to the substrate has been identified as a cause of increased fragility.

Abstract

Endothelial cells are constantly exposed to mechanical stimuli, of which mechanical stretch has been shown to have various beneficial or deleterious effects depending on whether loads are within physiological or pathological levels, respectively. Vascular properties change with age, and on a cell-scale, senescence elicits changes in the cell's mechanical properties that together can impair monolayer response to stretch.

In recent experiments [1], we investigated the behaviour of endothelial monolayers when exposed to stretch of their substrate. The monolayers were comprised of either young (HUVECs) or senescent (derived from aged patients or young HUVECs exposed to an in vitro senescence-inducing treatment) cells. We applied acute uniaxial stretch up to supraphysiological levels, as well as cyclic equibiaxial stretch within a physiological regime. In case of supraphysiological uniaxial stretch, young monolayers were able to withstand strains of up to 40%, while their senescent counterparts showed severe cell death and detachment, and thus, compromised monolayer integrity at such levels of strain. Upon closer inspection, we found that young monolayers exhibited intercellular holes immediately after application of uniaxial stretch that healed after 30 minutes. The senescent monolayers also exhibited holes within the cellular domain, which we call intracellular holes. Stretch induced damage in cyclically stretched monolayers was evident for senescent cells even for physiological strain regimes of 10%, while, again, young monolayers preserved their integrity under strains reaching up to 15%.

Due to morphological and structural differences between cell phenotypes, we asked if cytoskeletal mechanical properties could contribute to the observed damage behaviour. To answer this question, we developed a computational model of endothelial cells representative of their mechanical behaviour as single cells and in a monolayer.

To this end, we created a discrete network model of the actin cytoskeleton, linked with elements representative of nucleus, cellular membrane, focal adhesions, and cell-cell junctions. Model parameters were determined based on traction force microscopy data we previously generated [2]. We showed that our model is able to accurately represent the endothelial cell's contractile behaviour on the unstretched substrate as well as its mechanical response to equibiaxial stretch [3].

We then extended the model to represent a monolayer. We created monolayers representative of morphological and structural characteristics of young and senescent cells and performed simulations of the acute stretching event.

Among several hypotheses considered, we speculated that increased stability of senescent cells' connection to their underlying substrate [4] might lead to the observed intracellular damage. In our simulations, a higher number of focal adhesions caused senescent cells to follow the deformation of the substrate more tightly, leading to increased deformation energy within the cells. Indeed, by conducting a new set of experiments in which we biochemically decreased the adhesion to the substrate of senescent endothelial monolayers and stretched them uniaxially, we showed that levels of cellular detachment after 30 minutes relative to levels immediately after stretch were significantly dampened in senescent monolayers with decreased adhesion when compared to the senescent monolayers of the previous experiments [1].

To understand intra- and intercellular damage processes, we then introduced cytoskeletal fracture in the computational model and determined stretch dependent fracture criteria of the individual bonds. The results of endothelial damage computations for young and senescent monolayers were compared with distributions of intra- and intercellular holes that were quantified with the help of machine learning algorithms. The presentation will discuss model parameters influencing the formation of inter- and intracellular holes and their link with cell phenotype.

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Figures

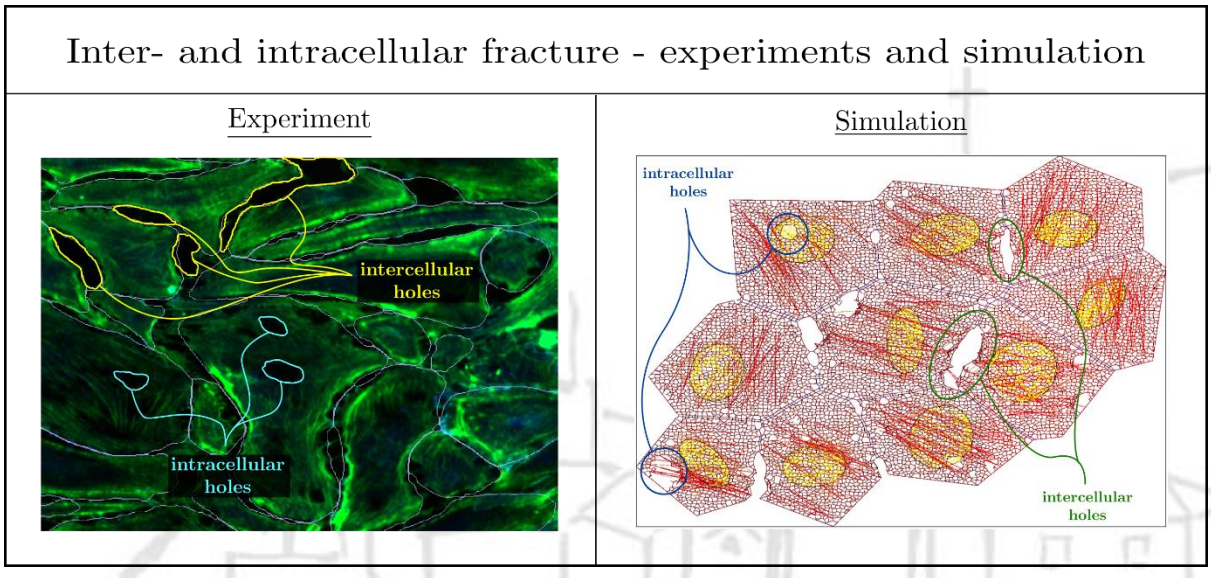

Figure 1. Endothelial monolayer fracture upon acute uniaxial stretch. On the left: experimental results of fracture in senescent endothelial cells exhibiting both inter- and intracellular holes. On the right: simulation of the senescent monolayer model leading to inter- and intracellular holes**.**

Unveiling the relationship between mechanical, morphological and nuclear changes in cells subject to nanovibrational stimulation

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Headline

Here, atomic force microscopy, gene expression and morphology analysis, expand our understanding of cellular vibration response. Within three hours, the cell stiffens, correlating with increased actin formation and nuclear area, before relaxing. Actin plays a crucial role in cell response, whilst vibration induces changes in nuclear envelope gene and protein expression.

Abstract

Cells are mechanosensitive to their surrounding environment and have been shown to respond to applied mechanical stimulation [1]. The application of forces, strains, or stresses have all been shown to result in responses from cells, such as migration, differentiation and morphological changes [1]. The cytoskeleton plays a role in numerous cell processes including cell adhesion, motility and the production of cytoskeletal tension [2]. Cytoskeletal contraction occurs due to the interplay between actin filaments and myosin. Actin-myosin complexes extend to the nucleus, forming a connection between focal adhesions on the cell surface and the nuclear envelope. The nucleus interacts with actin through binding proteins present on the surface of the nuclear membrane, collectively known as the linker of nucleus and cytoskeleton (LINC) complex. LINC is essential in force transmission to the nucleus, which leads to a biological response as it connects the nucleoskeleton, nuclear envelope and the cytoskeleton together [2]. Studies have also found that connections through LINC are essential for the remodelling of the actin cytoskeleton [3].

Mechanical stimulation in general has shown to increase cellular tension and deformation of the nucleus [4]. Applying nanoamplitude vibrations to cells grown *in vitro* has been found to result in changes in adhesion, ion channels and gene expression [5, 6]. Nanovibration has successfully been used to induce an osteogenic response in mesenchymal stem cells [5, 7]. However, despite the clear changes to cellular phenotype, the effects of nanovibrational stimulation on cell stiffness have not been investigated. Additionally, the relationship between mechanical and morphological changes has not been explored. This study used the fibroblast cell line NIH 3T3 and the osteosarcoma cell line MG63 to investigate morphological and mechanical changes following nanovibrational stimulation over periods of up to five days. Cells were vibrated at 1 kHz and 30 nm amplitude, with fluorescence microscopy being used to analyse the morphology of cells and the LINC protein sun1. Atomic force microscopy (AFM) was used to acquire mechanical measurements. qPCR was used to perform gene expression analysis related to LINC proteins and osteogenic markers.

The application of nanovibrational stimulation was found to increase the Young's moduli in both the nucleus and cytoplasm of NIH 3T3 cells. This response was highest within the first few hours of stimulation, before decreasing back to control levels during prolonged vibration up to 72 hours (Figure 1). These mechanical changes correlate with morphological changes in the nucleus and increased actin fibre formation. Fibroblast cells appeared to initially respond to vibrational stimulation through morphological changes and actin fibre reorganisation, leading to an increased tension and thus increased cell stiffness. To confirm the role of the cytoskeleton, actin polymerisation and contractility were separately inhibited, with cells no longer responding to vibration and instead maintaining Young's moduli matching non-vibrated controls.

Meanwhile, MG63 cells did not show as strong a change in mechanical properties as observed in fibroblasts. Whilst a stiffening was observed within the first few hours of stimulation, this response was not as strong as previously seen in NIH 3T3 cells and was limited to the cytoplasm. Upon gene expression analysis, changes were observed in nuclear envelope gene sun1 which was found to decrease in cells vibrated for 5 days as compared to non-vibrated controls. Immunofluorescence revealed an increased protein expression for sun1 at the same time point, suggesting an increased production in some nuclear proteins in response to vibration. This, coupled with an increased nuclear area suggests similar mechanisms are being stimulated within both cell types with cytoskeletal-LINC proteins playing a central role. Understanding mechanical responses to vibration may offer an alternative route to phenotypic assessment and allow us to maximise vibrational stimulation parameters.

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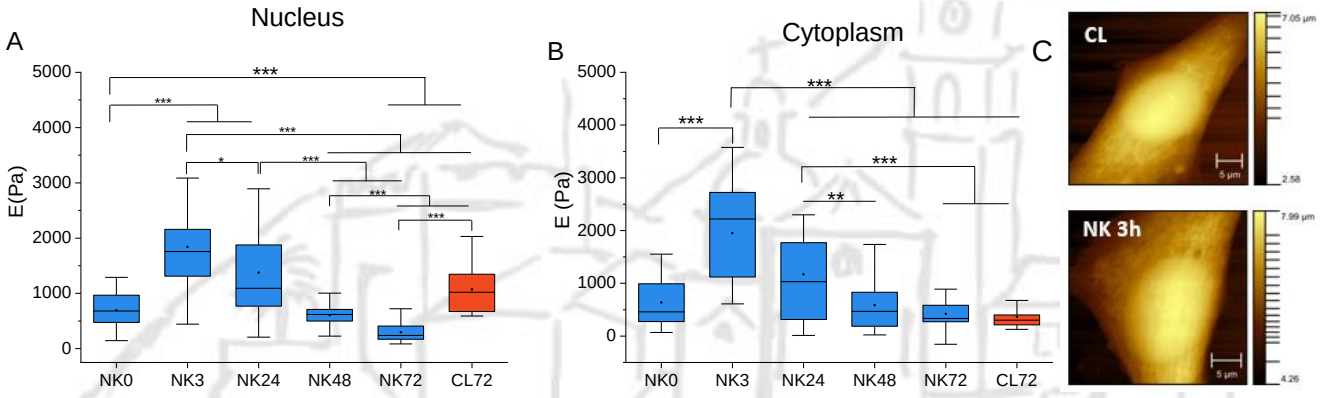

Figure 1. AFM measurements of NIH 3T3 cells at multiple timepoints during nanovibrational stimulation using an Asylum AFM. (a)Nuclear stiffness shown to increase significantly in the first 3 hours of stimulation before decreasing at later time points; (b) Cytoplasm stiffness shows a similar response, stiffening following 3 hours of stimulation and reducing at later time points; c) Topographical images of fixed cells, both control and after 3 hours of nanovibrational stimulation, obtained using a JPK AFM.

Combined optical manipulation with FRET-based molecular tension sensor for the investigation of living cell mechanotransduction.

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Headline (max 400 characters including spaces)

We present a setup for the investigation of mechanotransduction in living cells using a combination of optical tweezers and FRET-based molecular tension microscopy. The setup allows simultaneous force application and probing with a trapped particle and FRET signal recording using synchronised excitation lasers.

Abstract (max 2 pages for the whole document)

Mechanotransduction, the process by which cells sense and respond to mechanical stimuli, is a fundamental aspect of cellular physiology with implications for various biological processes. Here, we present a setup designed for investigating mechanotransduction dynamics within living cells. The setup utilizes optical tweezers to create 3D-controlled mechanical stimulation with a trapped microbead and molecular tension microscopy based on the fluorescence resonance energy transfer (FRET) mechanism to capture the response of the cell. Simultaneous fluorescence and brightfield imaging with synchronized donor and acceptor excitation pulses allow precise FRET measurement while applying the force on the cell. The sample is placed inside a microscope incubator with controlled temperature, humidity, and CO2 levels for extended acquisitions (several days).

Additionally, we provide mechanical stabilization of the microscope using a corrective feedback loop that utilizes fiducial markers like micro- or nano-particles. The algorithms based on local gradient [1] calculations enable precise three-dimensional localization of single particles, thus enhancing image resolution. This method demonstrates superior performance in noisy conditions, achieving sub-millisecond calculation times and sub-nanometer stabilization. The mechanical stabilization plays a crucial role in long-lasting measurements to counteract thermal focus drift and other mechanical noises.

The developed setup allows precise control of the mechanical stimulation and allows synchronised detection of the live cell response with optical force measurements, FRET-based detectors and visual analysis of the brightfield images.

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Lymphocyte mechano-regulation for immunotherapies

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Revolutionizing Biomedical Engineering

Unveiling the Nexus of Mechanical Forces and Immunotherapy. Our research exposes the intricate ties between mechanical forces and immune cell activation, transcending boundaries between biology and immunotherapy. This research, focused on T and B cell pathways, explores the interplay of external mechanical cues and immune cell activation, introducing fresh perspectives with biomedical concepts. The presentation zooms in on the links between mechanical cues, gene expression, and spatial organization within immune cells, utilizing cutting-edge techniques like super-resolution imaging. The integration of these insights could revolutionize quality control in CAR-T cell therapy, offering promising applications in clinical contexts.

Abstract

In the dynamic field of biomedical engineering and immunotherapy, my recent research unveils the intricate relationship between mechanical forces and immune cell activation, bridging the realms of biology and immunotherapy. This study aims to unearth innovative pathways for adoptive immunotherapies, emphasizing the role of biomedical and mechanobiological insights in broadening our understanding.

The core focus lies in decoding the mechanobiological pathways governing T cell and B cell activation. It explores the interplay between external mechanical cues and the immune cell activation process [1-4], with a nod to biomedical concepts for fresh perspectives. In my presentation I will focus on links between mechanical cues, gene expression profiles, and the spatial organization of genomic loci and transcripts within immune cells [1]. Combining optical super-resolution imaging, traction force [2] and molecular force microscopy [4] with functional read-outs – such as single cell transcriptomics and mitochondrial activity - opens possibilities for quality control in chimeric antigen receptor T (CAR-T) cell therapy. The research shows a potential for translation into clinical applications, here our most recent research harvests the effect of nanoporous substrats to boost lymphocyte activation without TCR-ligand stimulation (see Figure 1).

This interdisciplinary approach holds the potential to revolutionize adoptive immunotherapies, incorporating insights from biomedical and life sciences.

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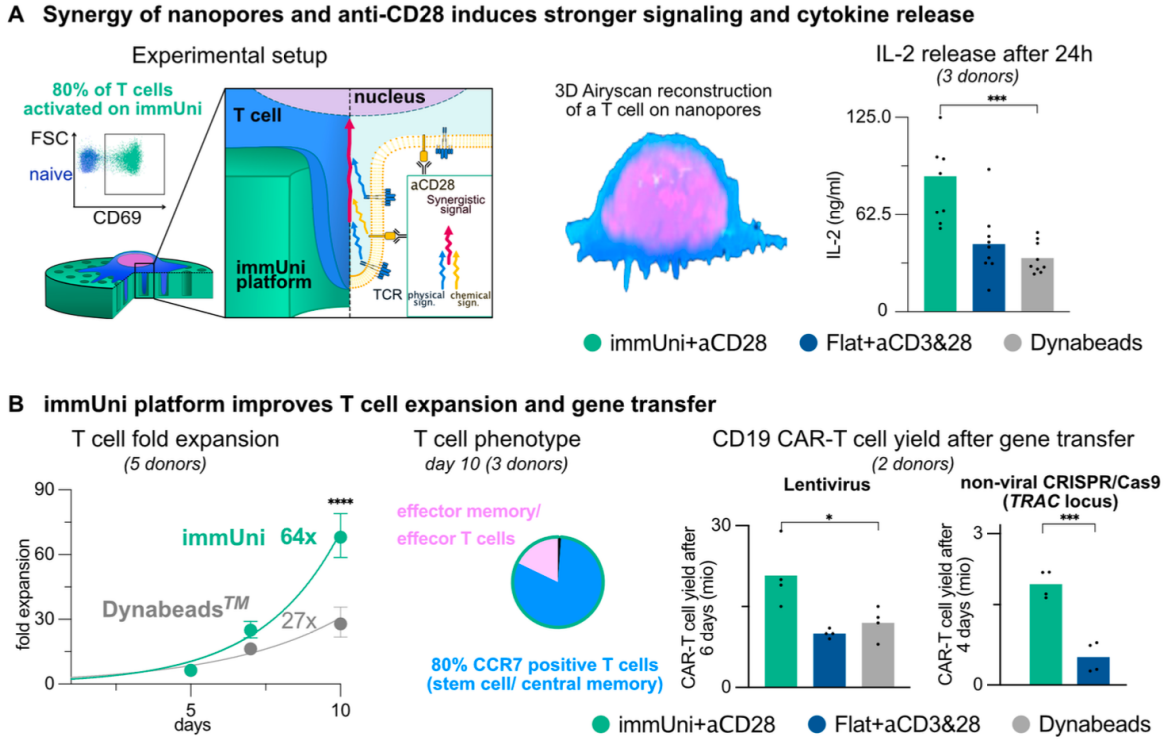

Figure 1. Nanoporous activation of T cells for adoptive immune therapy independent of TCR-ligand.

A Magnetic Artificial Cilia Platform for Studying Dynamic Mechanotransduction

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Headline (max 400 characters including spaces)

Mechanotransduction is crucial for cell function. We have realized a platform using magnetically actuated artificial cilia to apply sub-cellular mechanical stimulation to cells in a highly controlled, dynamic and versatile manner. Here, we show the fabrication and function of the platform, demonstrating artificial cilia actuation, cell viability under magnetic actuation and cell-cilia interaction.

Abstract (max 2 pages for the whole document)

Mechanotransduction is the process through which cells pick up on and respond to mechanical stimuli, and it plays an important role in the development and function of many tissues[1]. These stimuli can have different origins, like reaction forces from the extracellular matrix depending on the matrix' stiffness and topography[2,3], or from dynamically applied extracellular forces[4]. This dynamic mechanotransduction is very significant as dynamic stimuli are abundantly present in biology. However, the exact role of dynamic mechanotransduction in cellular development is not fully understood yet, partly due to the absence of a platform that would enable systematic in-vitro studies and analyses of the process. Submicron magnetic artificial cilia (nanoMACs) are well suited for locally stimulating individual cells, however their actuation in a water-based cell culture medium has not been addressed yet. The nanoMACs, made from a hydrophobic elastomer, show a very high response in polar solvents like ethanol[6], but actuating them in water requires a surface treatment using surfactants like Triton-X or soap molecules[7] which hamper their use in cell studies. In this work, we present a method for fabricating nanoMAC from a hydrophobic elastomer, standing on a hydrophilic surface, which enables the actuation of cilia with maximum possible responsiveness.

The nanoMAC fabrication process is shown schematically in figure 1. We use a polycarbonate track-etched (PCTE) membrane as sacrificial template, of which we can use different design parameters to alter the nanoMAC dimensions (length, diameter, areal density). The nanoMAC are made of a cured ferrofluid of magnetite particles in aminopropylmethylsiloxane-dimethylsiloxane (AMS) copolymer. The base material is a hydrophilic polymer on which the cilia are grown (fig.1). We use the MG-63 osteoblast-like cell line for this work, because of its known mechanosensitivity and robust consistent phenotype. We demonstrate the ease of culturing cells on AMS-made devices in fig.2A, illustrating that no protein coating is required for good cell adhesion, in contrast to PDMS surfaces. NanoMACs are especially suited for our research because of their small size, because the forces the nanoMACs exert are in a realistic order of magnitude to mimic the forces present in a biological context[5] (fig.2B), and because we can precisely control their actuation magnitude and frequency using an external magnetic field (fig.2C). The cells maintain their viability under magnetic actuation (Fig. 2D), and they assume different morphologies depending on the nanoMAC areal density (fig.2E,F).

Taken together, our magnetic artificial cilia platform is a promising tool for systematic dynamic mechanotransduction studies by applying controlled sub-cellular mechanical stimulation to cells.

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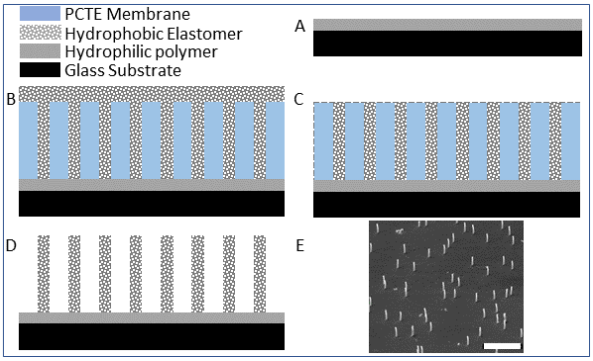

Figure 1. *A-D:* Schematic overview of the nanoMAC fabrication process. E: SEM image of a nanoMAC device with cilia length of 23um, diameter of 2um and areal density of 1E5cm-2. Scale bar = 50um.

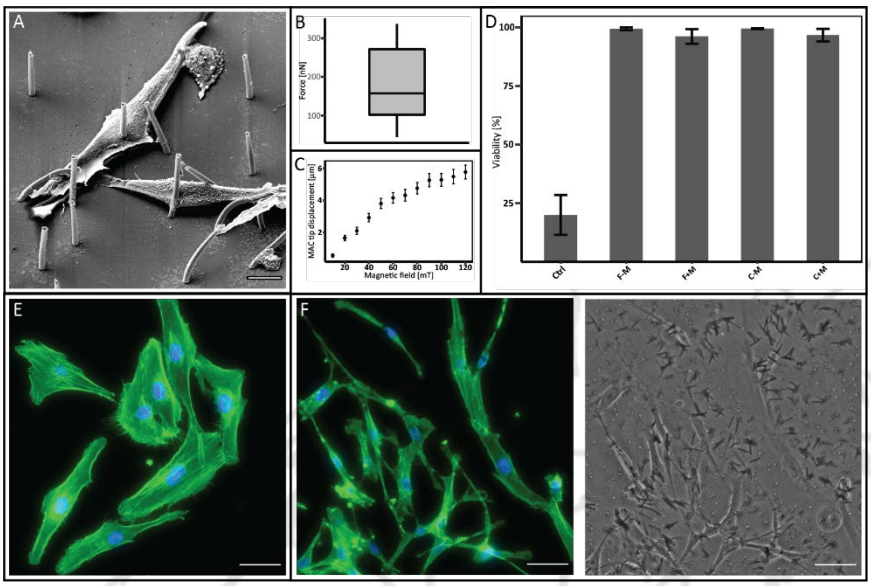

Figure 2. A) Scanning electron micrograph of MG-63 cells interacting with nanoMAC. Scale bar: 10um. B) Estimated force exerted by MG-63 cells onto MAC. C) MAC tip deflection as function of magnetic flux [mT]. D) MG-63 viability after 2 hours of magnetic actuation. E, F) Fluorescent micrographs illustrating MG-63 cell morphology on a flat surface (E) and MACs (F). Scale bars: 50um.

Reengineering a mechanosensitive feedforward circuit with photons as synaptic transmitters

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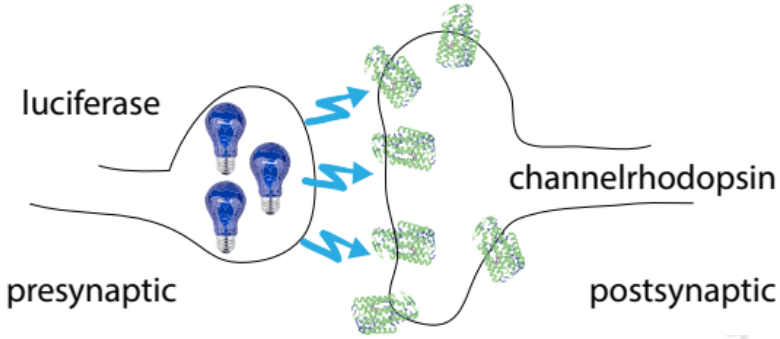
Headline (max 400 characters including spaces)

The tailored design of a whole connectome towards an individual need is the holy grail of synaptic engineering [1]. To this end, we leverage color-matched luciferases and channelrhodopsins expressed in two neighboring neurons of a mechanosensitive avoidance circuit to generate an all-optogenetic neurotransmitter system based on photons instead of chemicals.



Abstract (max 2 pages for the whole document)

Cell-cell communication is fundamental for the development of multicellular living organisms as well as for their correct homeostasis. Defects in intercellular signalling are known to cause many diseases and can even lead to organismal death. On the other hand, precise spatiotemporal control over intercellular signalling cascades would enable researchers to cure currently intractable diseases, but also allow to engineer organisms and neuronal networks bottom-up [1]. In this work, we describe a powerful and versatile cell communication system based on light, which we have named PhAST for Photon-Assisted Synaptic Transmission [2]. PhAST relies on the expression of a calcium-sensitive luciferases, e.g. a cyan NanoLantern, whose light emission is triggered by endogenous calcium dynamics. Importantly, the emitted photons carry information about cellular state and activity across space and can conveniently be imaged on a camera sensor [3]. More importantly, these photons, given they spoectrally overlap, may activate co-expressed photosensors. If installed in the same or neighboring circuit of a neuronal network, the functional coupling of the luciferase-emitted photons to a postsynaptic light sensitive ion channel, e.g. channelrhodopsin, facilitates signal transmission between a pairs of neurons. In a set of proof-of-principle experiment, we demonstrated the performance of this system driving a mechanosensitive behavior in *C. elegans* by replacing the flavor of a chemical transmitter with the color of a photon. We further show that PhAST sensitizes a sexually dimorphic avoidance circuit to noxious mechanical stimuli and, by coupling a green NL to the spectrally matched inhibitory anion chanelrhodopsin (ACR1) suppresses the pain response by

inhibiting signal transmission from the sensory to the interneuron. The engineered system is versatile and non-invasive and builds on genetically-encoded sensors and actuators which can be used in any organism and cell type, independent of the species [4]. Together, our approach enables fast signalling between cells or neurons in particular, and can readily be applied to complex multicellular systems, including organoids and developing embyos.

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The Response of Human Dermal Fibroblasts to Stretch and its Mechanocoupled Stimuli

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Headline

The coupling of stretch and the dermal mechanome was described in five parameters. This allowed to quantify the corresponding variations of matrix stiffness, interstitial fluid flow velocity, hydrostatic pressure, osmotic pressure, and electric field. Live cell calcium imaging showed that dermal fibroblasts feel these variations, and respond by activation of AKT/YAP, as well as gene expression.

Abstract

The influence of skin deformation, particularly through stretching, has a significant impact on skin physiology and pathology. This is evidenced by its crucial role in skin growth during pregnancy, as well as its impact on wound healing or scarring. In vitro, it has been shown, that dermal fibroblasts cultured in a 3D hydrogel under cyclic stretch respond with a significant increase in cell proliferation [1]. But little is known about the stimuli that are unavoidably mechanocoupled to skin stretch in vivo, making up the skin mechanome. Here we show, that stretch leads to a variety of biophysical stimuli acting on the cell environment.

With a set of multiaxial, time dependent mechanical experiments on human dermis, we identified the parameters of a model describing the coupling between stretch and the dermal mechanome. When stretch is applied to skin, it inherently leads to water efflux from the tissue, which leads to a variation in matrix stiffness, interstitial fluid flow, hydrostatic pressure, osmotic pressure and the electric field (Fig. 1). To quantify the corresponding variations of these parameters, a set of experiments was undertaken using different bioreactor setups for mechanobiological studies of cells in 2D and 3D collagen hydrogel culture. Live cell calcium imaging experiments demonstrated that primary human dermal fibroblasts feel the variation of the biophysical variables associated with interstitial fluid. The calcium signaling by the cells was particularly strong for changes of flow velocity, hydrostatic pressure and the electric field (Fig. 2). For these stimuli, dermal fibroblasts also responded by activating AKT and YAP signaling, and by modulating gene expression. Further study plans towards the analysis of fibroblast response to tension-release are presented.

Current studies on mechanobiology focus on the interlink between stretch and stiffness as the primary biophysical variables activating intracellular pathways. Our results indicate that the stretching of skin induces a broader array of stimuli, which should be considered when studying the mechanotransduction of human dermal fibroblasts. Particularly in the examination of mechanical forces' impact on fibrosis, growth, and remodelling, it is essential to take into account the entire mechanome of the human skin.

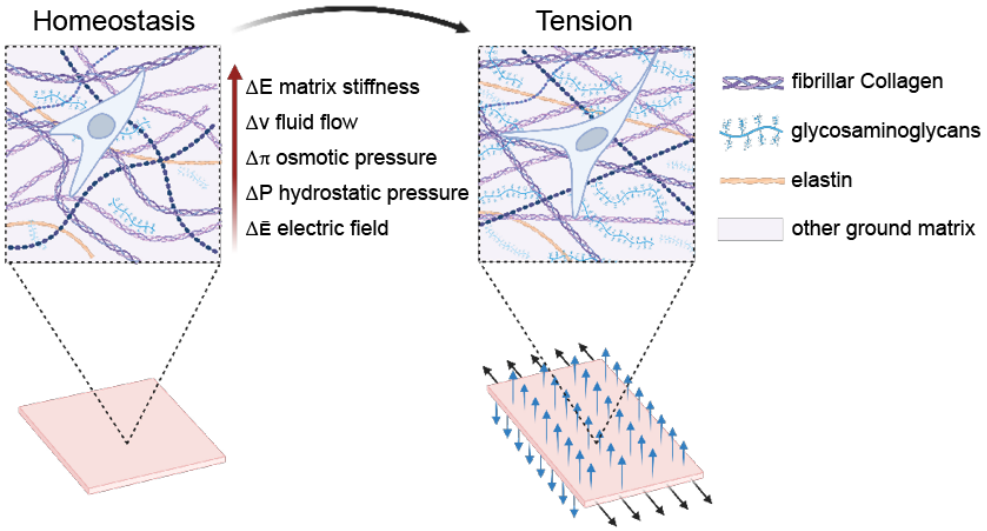

Figure 1. Skin stretch and its mechanocoupled stimuli acting on the fibroblast cell environment.

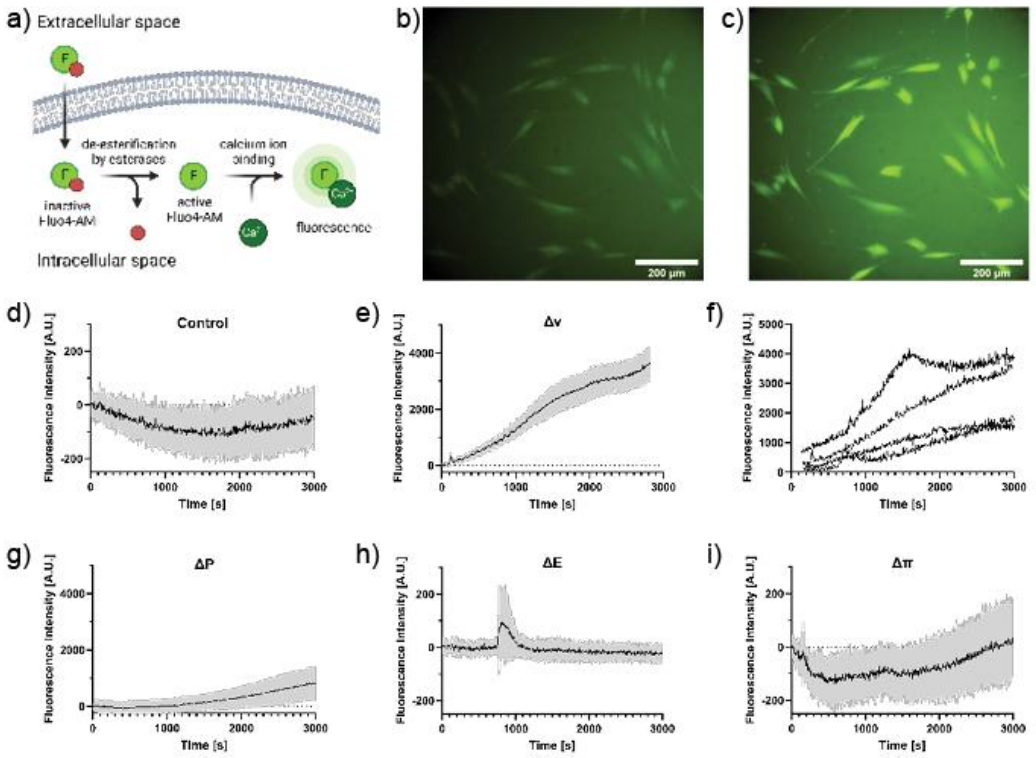

Figure 2. Calcium response of adult human dermal fibroblasts to mechanocoupled stimuli. The intensity of the calcium signal was measured for cells under no stress (d), fluid flow of Δv=10-20um/s resulting in 0.356mPa shear stress (e-f), hydrostatic pressure of ΔP=20kPa (g), electric field of 20mV/mm (h), and osmotic pressure by addition of 10mM sorbitol (i). The strongest response was measured for a variation of the fluid flow, followed by hydrostatic pressure and the electric field.

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Scaffold-induced fibroblast phenotypic variability

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Headline (max 400 characters including spaces)

Fibroblast can be a heterogeneous population, and this matters for the functional outcome of fibroblast-laden engineered skin substitutes. We investigate the time-dependent phenotypic variability and mechanical memory induced by passaging fibroblast in/on different hydrogel scaffolds (plastic, collagen, PEG) and compare to the phenotype of fibroblasts in their native tissue.

Abstract (max 2 pages for the whole document)

Tissue engineering holds the promise of growing functional tissue replacement in the lab, such as skin patches for treatment of large wounds can be grown in the lab [1]. Such approaches require both a large number of cells, usually expanded from an initial small amount, and a three-dimensional (3D) architecture that mimics the native tissue. Skin cell expansion is typically done on tissue culture polystyrene (TCPS) prior to integrating the cells in a 3D scaffold. However, TCPS is known to artificially induce a contractile myofibroblast phenotype, through mechanical memory effects [2]. An alternative could be to expand and assemble cells directly in a 3D scaffold, even if this method would be slower. We chose to test the use of 3D scaffolds for maintenance and passaging of fibroblasts. We used scaffolds made from Collagen I, or synthetic polymers such as poly(ethylene glycol) (PEG) functionalized with adhesion motifs. We asked whtether these 3D scaffolds would lead to attenuated mechanical memory compared to TCPS, or to other forms of phenotypic drift or population bias. To understand how 3D scaffolds affects the functional capacity of the cell population, we decided to systematically compare parallel cell populations passaged either in TCPS conditions or in 3D scaffolds. Our aim was to identify the traits that vary during passaging and cell expansion, and determine if this variability is scaffold-induced or scaffold-independent.

To remove any TCPS-induced bias, we decided to first isolate primary human fibroblasts from donated skin tissue, and to either encapsulate them directly into a 3D hydrogel, or to seed them on TCPS, and passage the cells up to 3 times. We hypothesized that cells passaged in 3D scaffolds would remain phenotypically and transcriptomically closer to the native tissue, as has been shown for cardiac valve interstitial cells on 2D soft gels [3].

Methods. Surplus human skin from surgeries were obtained from the University of Zurich Hospital. The basement membrane was digested and the epidermis removed. The dermis was digested by collagenase and the dermal cells collected by centrifugation. The cells (mostly fibroblasts) were either seeded on TCPS or mixed in a gel precursor solution. Collagen solution was polymerized at 37°C and 5% CO2 . PEG precursor solutions contained cell-degradable cross-linkers and adhesive binding motifs CRGDS. The gels were photopolymerized (λ = 405 nm, I = 14.5 mW/cm^2) by a radical-initiated reaction. TCPS

samples were passaged every 4–5 days, hydrogel sample every 10–14 days. After 3 passages, samples were collected for protein and gene expression assays, or fixed and stained.

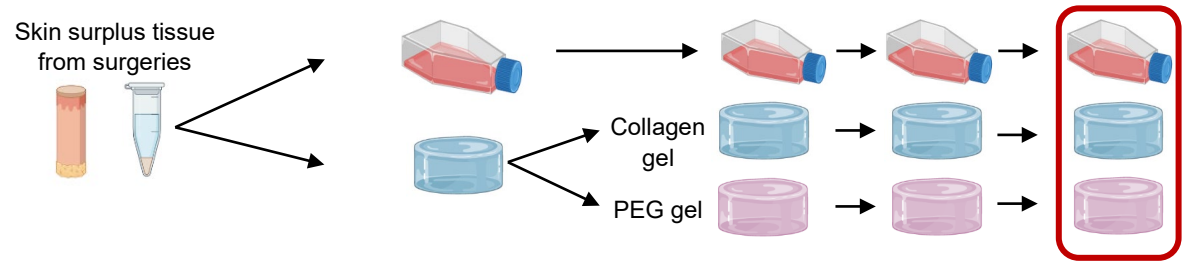

Figure 1: Passaging scheme for fibroblasts isolated from skin tissue in TCPS, Collagen gels, or PEG gels

Results. We first found differences in proliferation between cells cultured in 3D hydrogels compared to TCPS, with cells cultured in the PEG-based hydrogels being least proliferative. We then compared the gene expression of fibroblast and myofibroblast markers of the cells in/on the different scaffolds at passage 3 by qPCR. We found that 3D scaffolds induce upregulation of matrix metallo-proteinases and integrin subunits, but no reproducible changes in fibrotic markers.

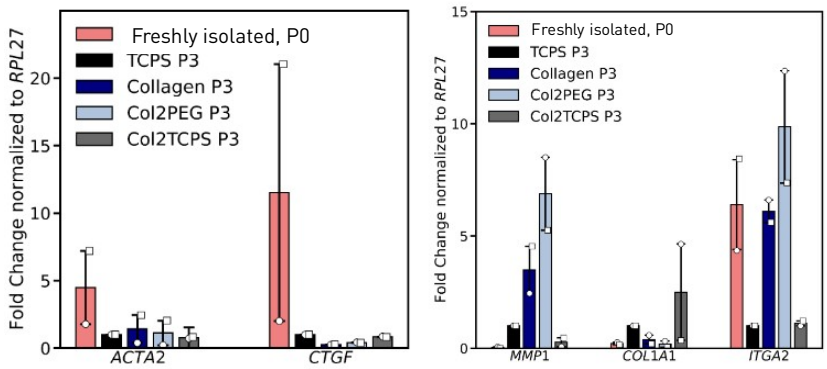

Figure 2: Gene expression for 1 donor, comparing cells in different passaging conditions. Expression is normalized to RPL27. Bars show mean +/- std on 2 samples.

Conclusion. We observed functional differences in cell proliferation and differences in gene expression between the 2D TCPS and 3D scaffolds. To date, it is not known how these accumulate over multiple passages. We plan to further explore if there are more functional divergences between the cell populations, and which scaffold-type maintains the cells closest to the native tissue phenotype. We will in the future probe genome-wide differences with RNAseq as well as interrogate the heterogeneities of the populations using immunostainings for proteins coded for by differentially expressed genes.

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Correlation between extracellular matrix stiffness distribution and peritoneal metastasis development

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Headline (max 400 characters including spaces)

The research shows application of atomic force microscopy in the clinics. Mechanical properties of normal and neoplastic extracellular matrix of patients with peritoneal metastases from colorectal cancer were studied and the results showed correlation between mechanical properties of ECM and progression of peritoneal metastasis.

Abstract

Peritoneal metastases (PM) are one of the most common routes of dissemination for colorectal cancer (CRC) and remain a lethal disease with a poor prognosis[1,2]. The compositional, mechanical and structural properties of the ECM play an important role in cancer development[3]; studying how these properties change during the progression of the disease is crucial to understand CRC-PM development.

The elastic properties of ECMs derived from human samples of normal and neoplastic PM in different pathological conditions were studied by atomic force microscopy (AFM); results were correlated to patients' clinical data and to the expression of ECM components related to metastatic spread, like collagen and αSMA – signature of cancer associated fibroblasts (CAF) activity[4–7].

Our results show that PM progression is accompanied by stiffening of ECM as a common feature; spatially resolved mechanical analysis highlighted significant spatial heterogeneity of the elastic properties of both normal and neoplastic ECMs, which show significant overlap in the two conditions (Figure 1). On the micrometre scale, ECMs that are considered normal according to the pathological classification possess stiffer spatial domains, which are typically associated with cancer associated fibroblasts (CAF) activity and tumour development in neoplastic matrices; on the other hand, softer regions are found in neoplastic ECMs on the same scales. Our results support the hypothesis that local changes (stiffening) in the normal ECM can create the ground for growth and spread from the tumour of invading metastatic cells.

Mechanical changes correlate well with the presence of CAF and an increase in collagen deposition, which are well known markers of cancer progression. Furthermore, we have found correlations between the mechanical properties of the ECM and patients' clinical data like age, sex, presence of mutations in *BRAF* and *KRAS* genes and tumour grade.

Overall, our findings suggest that the mechanical phenotyping of the PM-ECM has the potential for predicting tumour development.

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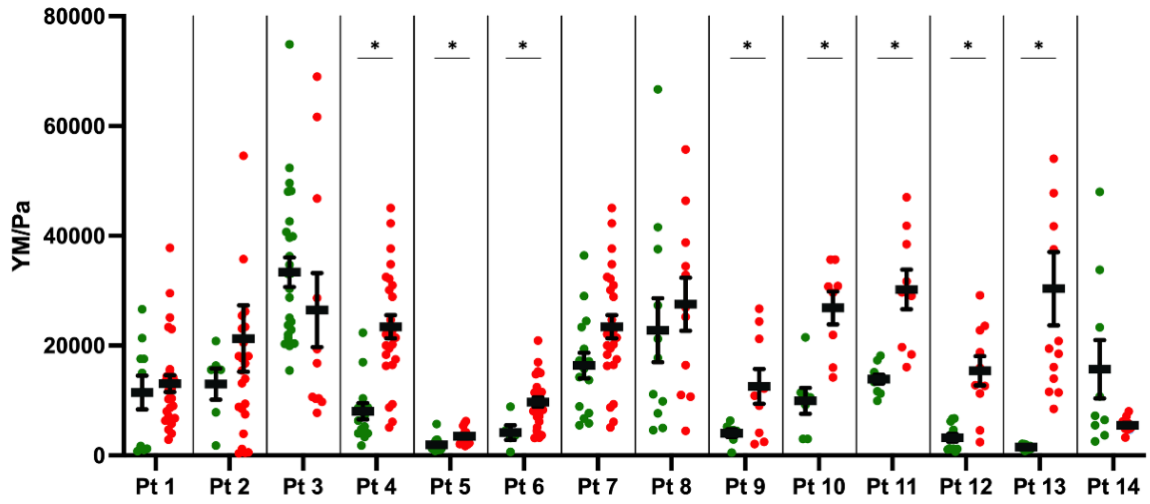

Figure 1. YM distributions for the normal (green) and neoplastic (red) conditions from peritoneal ECMs for the 14 patients considered in the study. Plots showing the distribution of median YM values measured from all FVs collected in different ROIs for each specific condition. Black dots and bars represent the mean median values and the corresponding standard deviation of the mean, respectively. The asterisk indicates statistical significance of the difference ($p<0.05$).

IRSp53 controls tissue fluidification during breast carcinoma progression

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Headline
We identify the I-BAR containing protein IRSp53 as novel determinant of tissue fluidification in breast cancer, by controlling the architecture of cell-cell adhesions and regulating supra-cellular tension in epithelial tissues. IRSp53 ablation profoundly impacts on cell mechanics, ultimately affecting collective cell migration and fluid-to-solid transition in confluent monolayers.

Abstract
Jamming-to-unjamming transition has been suggested as a key event in tumor progression and in the metastatic process in which cancer cells leave the primary tumor and infiltrate other parts of the body. Under which conditions do normal solid-like tissues evolve in cancer liquid-like ones? An increasing number of determinants have been shown to be lost or perturbed during this process, and to correlate with aggressive disease: cell–cell and cell–ECM adhesion, generation of traction forces and tissue tension, and cell shape transitions are the mechanisms at play.

Our hypothesis is that proteins that physically link the plasma membranes with the underlying cytoskeleton in epithelial tissues may be key regulators in the generation and transmission of such forces. The family of proteins that contain the membrane-bending and -binding I-BAR proteins appears to have all of the key structural features to perform this function. Among them, IRSp53 regulates the dynamic interplay between the plasma membrane and the actin cytoskeleton during directional migration and invasion of cells by sensing and promoting membrane curvature through its I-BAR domain. Moreover, IRSp53 works as direct effector of Rho-GTPases, enabling the recruitment of various actin regulatory proteins. IRSp53 has also a role in cell–cell and cell–ECM adhesions and is required for the polarized architectural organization and morphogenesis of epithelial tissues.

We recently focused our attention on the role of IRSp53 in the context of collective locomotion, employing in vitro 2D and 3D assays of MCF10DCIS.com cells. We collected a number of

evidences suggesting that IRSp53 removal affects the capacity of cells to “feel” each other in collective mode of migration by affecting the architecture of cell-cell junctions. This further promotes wetting of 3D spheroids, mimicking reduction in collective cell tension. These deficiencies translate in the loss of unjamming-to-jamming transition, allowing cells in confined condition to escape the physical constraint imposed by cell crowding. IRSp53 is therefore emerging as a crucial factor in the solid-to-fluid mechanisms linked to tumor progression.

Time-sharing optical tweezer microrheology reveals the compartmentalized subcellular mechanics during development and age

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Headline

Recording the mechanical response of biological samples, the cell's interior and complex fluids in general, would enable deeper understanding of cellular differentiation, ageing and drug discovery. Here, we present a time-shared optical tweezer microrheology (TimSOM) pipeline to determine the frequency- and age-dependent viscoelastic properties of biological materials.

Abstract

Optical tweezers-based active microrheology enables the characterization of the viscoelastic properties in the interior of cells and tissues non-invasively [1]. Commonly based on the application of sinusoidal perturbations onto an optically-trapped microbead inside the cell, it allows to obtain the frequency-dependent *shear* or *G* modulus which informs, among others, about the deformability or stress dissipation in the biological material under test. The measurement of the shear modulus requires accurate detection of the optical force and the subnanometer displacements of the microbead during the oscillation. To do so, two lasers –for trapping and detection- are typically used, which increases the complexity of the optical trapping set-up. Here, we introduce time-shared optical tweezer microrheology (TimSOM), consists in splitting a single laser beam into two near-instantaneous time-shared optical traps to carry out simultaneous force and displacement measurements with sub-nanometer and sub-picoNewton accuracy during sinusoidal perturbations to obtain the rheological spectrum [2].

We apply the so-called time-shared optical tweezer microrheology (TimSOM) pipeline in three relevant examples for state-of-the-art mechanobiology. First, we explore the viscoelastic maturation of condensates of the MEC-2/stomatin protein, which copartitions with UNC-89/titin and bears mechanosensation in *Caenorhabditis elegans* through mechanoelectrical transduction channels [3]. Second, we assess the compartmentalized mechanics of highly motile zebrafish stem cells. Here, we find that the nuclear elasticity, necessary for cellular proprioception through nuclear mechanotransduction, is facilitated by the nuclear envelope and nucleoskeletal proteins, while the interior nucleoplasm is characterized by a more fluid-like and soft microrheology [2].

Finally, we address the mechanical ageing in living worms and study the effect of different mutations that are commonly used to model different types of nuclear envelopathies, such as progeria and Emery-Dreifuss muscular dystrophy [2]. Here, we apply our microrheology pipeline directly onto endogenous

lipid droplets in the C. elegans intestine. Their greater refractive index compared to the cytoplasm allows to exert optical forces on the order of 100 pN and indent cell nuclei. As a signature for the ageing process, we find that the worm intestinal tissue undergoes cytoplasmic softening, which is accelerated by *lmn-1/lamin A*, *emr-1/Emerin* and *lem-2/LEMD2* genetic alterations. Together, our advances afford rapid phenotyping of material properties of cell compartments and protein blends, opening avenues for biomedical and drug screening applications.

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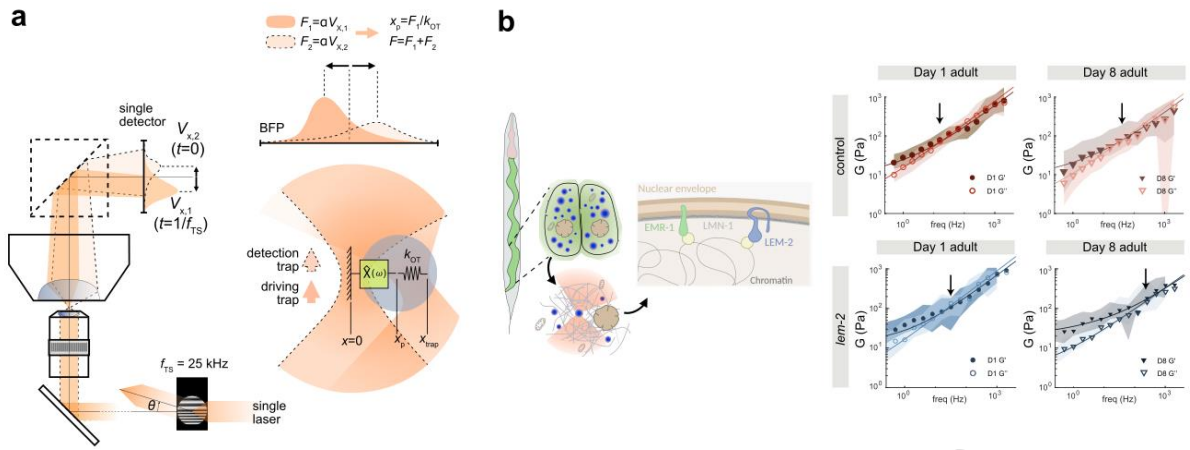

Figure 1. **a**, Schematic of time-shared optical microrheology with direct light momentum sensing of optical forces. A single 1064-nm laser beam is time-shared at 25 kHz between a *driving* and a *detection* trap. The driving trap (orange) oscillates around the trapped particle, while the detection trap (light orange, dashed line) monitors the particle position as x_p. The optical force acting onto the probe particle corresponds to the addition of forces exerted by the two traps ($F_1 + F_2$), which are obtained as $F_{1,2} = \alpha V_{1,2}$, where *alpha* is here the volt-to-picoNewton conversion factor of a single, light-momentum direct force sensor. **b**, *C. elegans* sketch with the intestinal tissue highlighted in green and the pharynx in red. The close-up sketch shows a pair of posterior intestinal cells with lipid droplets in blue, schematic of cell interior and traps (down) and (right) details of the distribution of nuclear envelope-forming proteins studied in this work. Curves represent frequency dependent shear modulus for two different ages of wildtype and age-matched *lem-2* mutants. The median and ±25%quantiles are represented by lines and shadows, respectively.

Investigating Time Scales of Mechanotransduction of Dermal Fibroblasts and Macrophages during Wound Healing using a New Material for *In Situ* Stiffening in 3D

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Headline

We aimed to design a biomaterial suitable for 3D, *in situ* stiffening to mimic changes to the dermis during fibrosis and wound healing. By adapting Methacrylated Hyaluronic Acid (MeHA), used for 2D *in situ* stiffening studies, to create a 3D macroporous gel comprised of fibrous microgels, we hypothesize we will be able to dynamically increase matrix stiffness without increasing cell confinement, allowing us to investigate timescales of stiffness mediated fibroblast and macrophage activation.

Abstract

Mechanical cues, such as extracellular matrix (ECM) stiffness, regulate fibroblast [1] and macrophage phenotype [2] and coordinate macrophage–fibroblast interactions [3]. Furthermore, the time scales over which matrix mechanics change are relevant, as cells are exposed to dynamic changes rather than static conditions during wound healing [4–9]. In this context, biomaterials capable of stiffening *in situ* and in the presence of cells have been used to identify new disease-relevant mechanotransduction mechanisms [4–9]. However, these studies often employ 2D culture and lack the dimensionality of native tissue. While 3D culture methods are improving, 3D mechanobiology studies are often unable to reproduce 2D findings. One challenge when studying the effects of matrix stiffness in 3D is that stiffness and cell confinement are often coupled [10]. Therefore, we aimed to design a biomaterial suitable for 3D, *in situ* stiffening to mimic changes to the dermis during fibrosis and wound healing. Importantly, our design allows for stiffness tuning independent of confinement and macrophage incorporation for co-culture to study inflammation. We adapted Methacrylated Hyaluronic Acid (MeHA) **(Figure 1)** a material compatible with 2D *in situ* stiffening studies via photo-initiated polymerization [5,6], to create a 3D macroporous gel, comprised of fibrous microgels. We hypothesize the macroporous nature of the gel will allow fibroblast spreading, matrix deposition, and macrophage migration. We hypothesize that our platform will allow us to dynamically increase matrix stiffness over different time scales, without increasing cell confinement, using photopolymerization and therefore allow us to identify new mechanotransduction pathways contributing to dermal inflammation, macrophage polarization, myofibroblast activation, and macrophage–fibroblast crosstalk in 3D.

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Figure 1

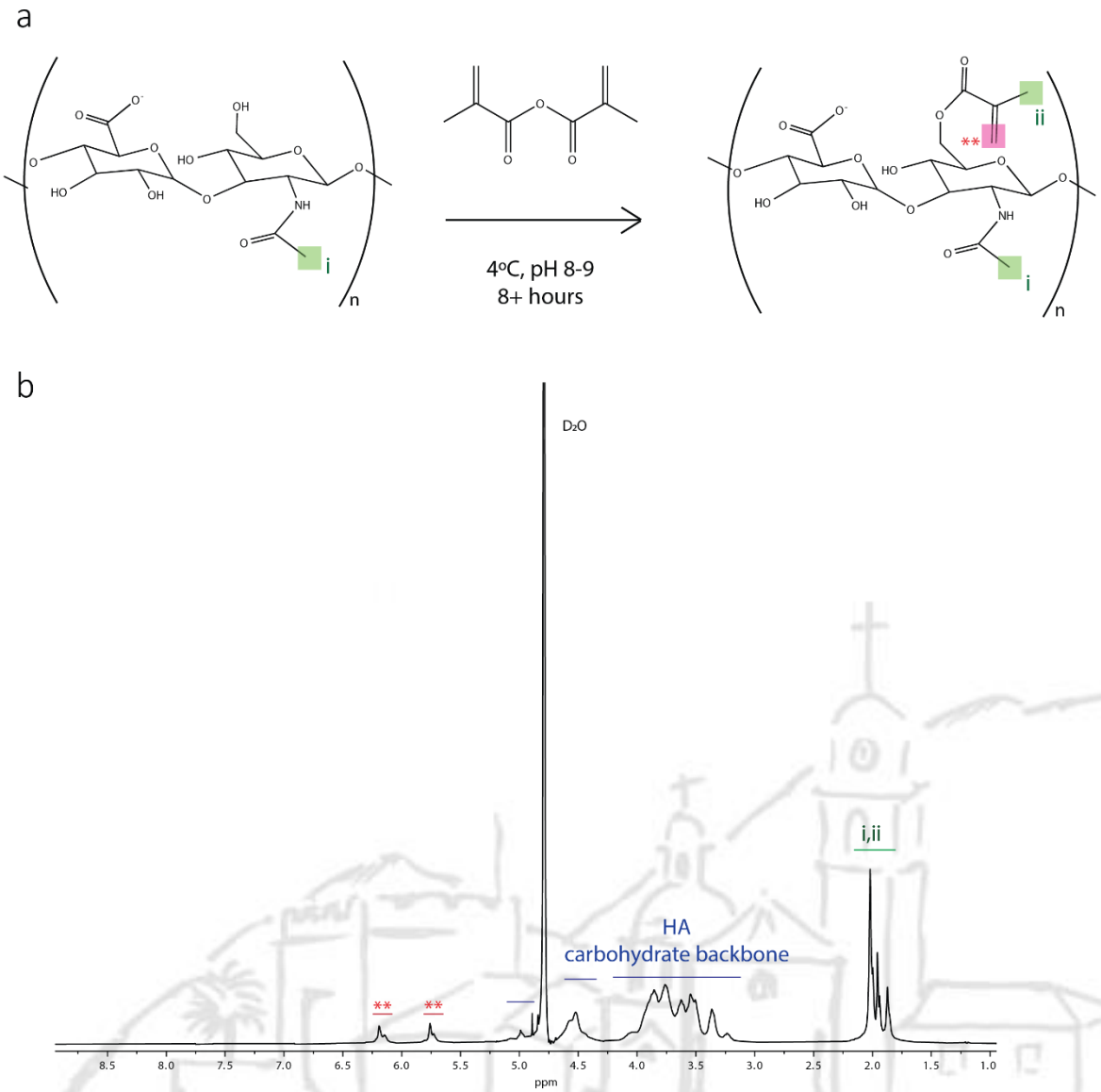

Figure 1. (a) 60kDa Sodium Hyaluronate was reacted with methacrylate anhydride at pH 8-9 for 8 hours on ice, then left overnight at room temp to synthesize Methacrylated Hyaluronic Acid (MeHA); (b) H NMR of synthesized MeHA with peaks corresponding to the corresponding groups labelled in (a) confirms ~40% of monomeric repeating units are functionalized with methacrylate.

A 3D Eukaryotic Cell model for flow simulations with sub-cellular components

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Headline (max 400 characters including spaces)

In this work we propose a model for the eukaryotic cell that explicitly implements the membrane, the nucleus and the cytoskeleton which are assumed to be the principal actors that affect the mechanical properties of the cell. We calibrate and validate the model using micropipette aspiration and microfluidics experiment data for the breast epithelial cells (MCF-10A).

Abstract (max 2 pages for the whole document)

We propose a model for the eukaryotic cell in flows that explicitly implements the membrane, the nucleus and the cytoskeleton which are assumed to be the principal actors that affect the mechanical properties of the cell [1]. The model, shown in Figure 1, is a simplified version of the one introduced by Lykov et al. [2]. We achieved this simplification by replacing the gel-like structure of the cytoskeleton with viscoelastic bonds that connect the cell membrane to the nucleus membrane according to a well-defined topology which main role is to ensure the uniform distribution of stress throughout the cell. To explicitly model the nucleus, we treat it as a viscoelastic material, employing the same approach as for the cytoskeleton with minor adjustments. The model is immersed and fully coupled with the fluid modelled using particle-based flow solver.

We calibrate and validate the model using micropipette aspiration and microfluidics experiment data for the breast epithelial cells (MCF-10A).

We believe that the main advantages of the proposed here simplified model are significant decrease in computational complexity and the reduction of the parameters to be estimated. Despite these simplifications, the model is still able to capture the viscoelastic behaviour of the cell in microfluidic experiment.

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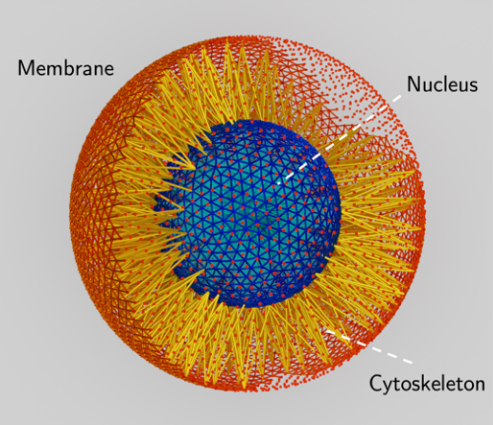

Figure 1. Visualization of the model with sub-cellular components

Microglia at the Crossroads: Mechanobiological Insights into Neuroinflammation

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Headline (max 400 characters including spaces)

Microglial cells are responsible for the initial immune response in the central nervous system (CNS). Like all other cells, microglia are mechanosensitive and links between their function and mechanotransduction events have been recently established. Here, we employ for the first time a 3D tissue engineered platform to further dissect and explore these processes.

Abstract (max 2 pages for the whole document)

Neurodegenerative disorders of the CNS pose a tremendous socioeconomic impact, which is expected to escalate with the increasing life expectancy. These are a heterogeneous group of pathologies characterized by degeneration of nervous tissue including Alzheimer's and Parkinson's disease, multiple sclerosis, among others. Although some of these pathologies have been described more than 100 years ago, they remain incurable. There are several known aspects that play a role in the pathological mechanisms including genetic, environmental, and endogenous. Among these, neuroinflammation is a common hallmark while its causes can be diverse: presence of toxic protein aggregates/metabolites, infection, traumatic injury or autoimmunity. The blood-brain barrier separates the CNS from the systemic immune system, making microglia the primary effector in this region. When activated, microglia become highly mobile, have phagocytic capabilities, and produce a plethora of cytokines and chemokines [1].

Our main hypothesis is that microglia activation status is highly dependent on the mechanical properties of their surrounding environment and that this interaction is mediated by post-translational modifications of mechanosensing pathways.

In this study we propose to assess, for the first time, the impact of mechanical stimuli on microglia function in a 3D environment, that more closely mimics the glial tissue.

Along with glia activation, one can observe in the lesioned tissue extensive extracellular matrix (ECM) remodelling and de novo deposition, all in all contributing to the formation of a glial scar [2]. Hence, microgliosis and astrogliosis strongly contribute to the outcome of the glial scar, which is seen as a hostile terrain impeding neuroregeneration. Besides presenting a characteristic biochemical composition, the glial scar has been proven to have different mechanical properties of the healthy CNS tissue, both regarding stiffness and topography [3]. In fact, a link between mechanical properties and microglia response has been shown both in vitro [4] and in vivo [5]. However, the link between microglia function and mechanosensing is poorly understood with key questions remaining to be answered.

Here we present a 3D microglial tissue engineered model that recreates key features of neuroinflammation. Primary rat microglia were embedded within alginate hydrogels containing the cell adhesion (RGD) and matrix metalloproteinase-sensitive (PVGLIG) peptides to recreate the ECM environment. These modifications to the alginate backbone allowed microglia to extend cellular processes and acquire a 3D morphology. By varying the final concentration (1 and 2% w/v) of alginate and the degree of oxidation we were able to produce matrices with different mechanical properties, regarding both stress-relaxation and stiffness. We found that microglia respond differently to a pro-inflammatory challenge (10 ng/mL of lipopolysaccharide for 24h) depending on the surrounding matrix mechanical properties, with cells in matrices with higher stress-relaxation and stiffness displaying a less pronounced pro-inflammatory-like phenotype. This was assessed by quantification of the production of nitrites and the expression of genes of interest through qPCR (*IL-1β, IL-6, NOS2, CD14*). Notably, we found a decrease in *TLR4* expression in matrices with higher stress-relaxation and stiffness, suggesting a possible regulation mechanism.

This work will contribute to the discovery of new therapeutic targets to tackle neuroinflammation and ultimately neurodegenerative diseases.

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Figures

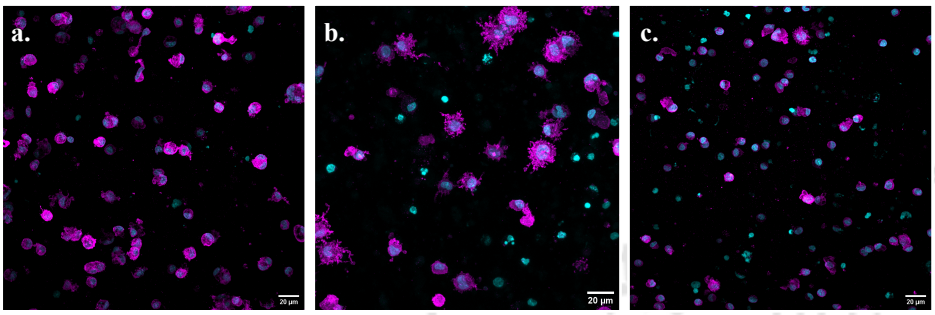

Figure 1. Microglia cells cultured in alginate matrices with different mechanical properties present different cellular morphologies. (a) 1% (w/v) with 60:40 oxidized:non-oxidized alginate; (b) 2% (w/v) with 60:40 oxidized:non-oxidized alginate (c) 2% (w/v) with 30:70 oxidized:non-oxidized alginate. Confocal microscopy images (Z-stack). Blue – DAPI; Magenta – IBA1.

Acknowledgments

The authors acknowledge the funding from the Air Force Defense Research Sciences Program, USA (Award FA9550-23-1-0599). M. R. G. Morais and E. D. Carvalho acknowledge FCT for their Ph.D. fellowships (grants 2022.13353.BD and SFRH/BD/140363/2018, respectively). G. Athanasopoulou acknowledges ASTROTECH Project for her Ph.D. fellowship (Grant Agreement number: 956325 — ASTROTECH — H2020-MSCA-ITN-2020). M. R. G. Morais and G. Athanasopoulou acknowledge the support of the Biomedical Sciences and BiotechHealth doctoral programmes from ICBAS, University of Porto, respectively.

Ultrashort self-assembling peptide hydrogels: AFM microscopy and Raman spectroscopy perspective on their mechanical and adhesive properties

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Headline

Synthetic ultrashort peptides can be rationally design to self-assemble into nanofibrous structures resembling the extracellular matrix. Their properties can be tuned at will by changing the aminoacid sequence. The physicochemical characteristics retrieved by AFM microscopy and Raman spectroscopy can shed light on different important aspects of their properties.

Abstract

Ultrashort self-assembling peptides are a class of short peptides (3-7 aminoacids) that can self-assemble in physiological condition to form nanofibrous hydrogels due to their amphiphilic nature [1]. Up to 99.9% of water can be retained in their soft-solid structure, which fully resembles the extracellular matrix. Moreover, the peptides self-assemble based on physical cross-linking, without the need of damaging UV initiator. In addition, they are fully biocompatible and non-immunogenic. Their synthetic nature allows a fine tuning of their sequence according to desired properties or applications. For instance, their sequence can be tweaked to accommodate different needs of 3D cell culture, organoid growth, 3D bioprinting, tissue engineering, and underwater adhesives [2-9]. Compared to other benchmark hydrogel matrices, such as Matrigel, the composition of USPs can be fully controlled.

By modifying the sequence of USPs we were able to generate hydrogels with different elastic moduli[7], enhanced underwater adhesion for coral restoration [10], and to effectively provide to the matrix, cell specific ECM derived adhesion cues [4].

In this context, AFM microscopy and Raman spectroscopy can provide a surprisingly high number of detailed physicochemical information from the single cell down to the single molecule level.

We will show how with these two techniques we were able to identify the most adhesive peptide down to the single molecule level from a set of Dopa modified peptides [10], the effect on cell metabolic activity with USPs of different stiffness [2], and the response to different peptide cues (laminin, fibronectin) expressed in terms of work of adhesion of living fibroblasts cells in physiological environment.

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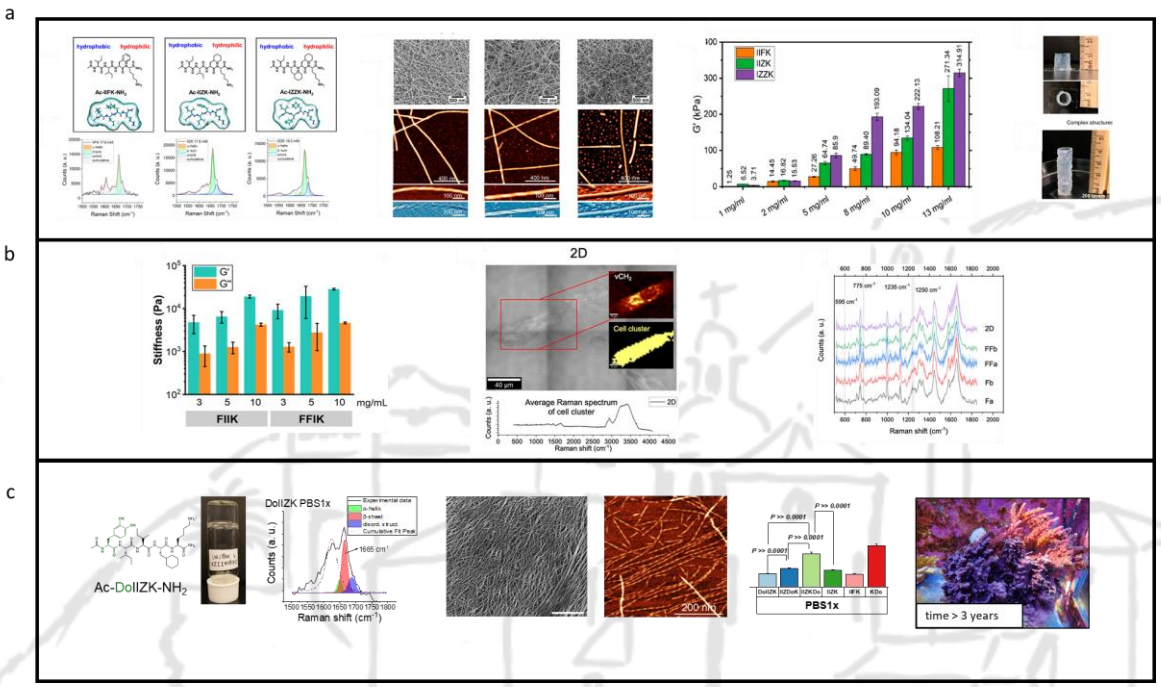

Figure 1. Various sequences and physicochemical properties of USPs and 3D cell cultures in USPs. (a) adapted from [7] (b) adapted from [2] (c) adapted from [10].

Effects of cell senescence to endothelial response to wall shear stress

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Headline

Exploring Cellular Senescence in Vascular Health: A Detailed Microfluidic Study on the Differential Responses of Senescent and Healthy Human Umbilical Vein Endothelial Cells (HUVECs) to Varied Wall Shear Stress Levels, Offering Novel Insights into Endothelial Mechanotransduction and Implications for Vascular Pathology

Abstract

This study presents a comprehensive analysis of the response of Human Umbilical Vein Endothelial Cells (HUVECs) to different wall shear stress (WSS) conditions in a microfluidic environment, focusing on the distinct behaviors of healthy versus TNF-alpha-induced senescent cells. The role of endothelial cells in vascular health is critical, and their dysfunction is linked to various vascular diseases, such as atherosclerosis, hypertension, and thrombosis. Cellular senescence in endothelial cells, particularly under mechanical stress like WSS, plays a pivotal role in vascular aging and disease progression, but its impact remains insufficiently explored.

Methods: Two distinct groups of HUVECs were prepared: healthy cells and those subjected to senescence induction via TNF-alpha treatment, a method recognized for simulating an inflammatory, senescent state [1]. Both groups were seeded in a custom-designed Polydimethylsiloxane (PDMS) microfluidic device, which provided precise control over the cellular environment and facilitated detailed observation of cellular behavior. The device, measuring 200x800 µm, allowed for effective endothelialization of its channels. Post-seeding, cells were cultured in non-supplemented Endothelial Cell Growth Medium (ECGM) with 1% penicillin/streptomycin, reaching confluency and ensuring full endothelialization. A bioreactor system with a peristaltic pump was then employed to simulate different flow conditions, exposing cells to WSS levels of 1.4 Pa (lower physiological limit), 2 Pa (upper physiological limit), and 6 Pa (supraphysiological level) over an 18-hour period. A static control group was also maintained. Subsequently, cells were fixed using paraformaldehyde and stained for nuclear visualization (DAPI), cell-cell junction assessment (beta-catenin), and senescence detection (beta-galactosidase for TNF-alpha-treated cells).

Results: The analysis, conducted using a custom-developed MATLAB algorithm, revealed notable differences in cellular orientation in response to varying WSS. At 1.4 Pa, healthy HUVECs began aligning parallel to the flow, while senescent cells showed a more pronounced parallel orientation, indicating an altered or heightened sensitivity to WSS. At the upper physiological limit of 2 Pa, healthy cells continued to align parallel to the flow, but senescent cells exhibited a surprising perpendicular orientation, suggesting a distinct response

mechanism in senescent cells. At the high stress level of 6 Pa, both cell types showed a uniform perpendicular orientation, possibly as a protective mechanism against excessive mechanical stress.

Conclusion: The study demonstrates that senescent HUVECs actively respond to WSS variations, exhibiting distinct orientation behaviors compared to healthy cells. This suggests enhanced sensitivity or altered mechanotransduction in senescent cells, particularly at physiological WSS levels. These findings provide novel insights into endothelial cell dynamics under shear stress, highlighting the potential impact of senescent cells in vascular health and disease. The results pave the way for further research into targeted treatments for vascular pathologies involving endothelial dysfunction and senescence.

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Role of a mechanosensitive activation mechanism of ADAM proteases through Piezo1 and TRPV4 in endothelial homeostasis and function

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Headline

Mechanical excitation has a huge impact on endothelial cell behavior. Our findings provide new evidence of a direct link between the mechanical stimulus and certain cellular responses, including permeability or tissue repair processes, via the activation of ADAM17/10 by the mechanosensitive ion channels Piezo1 and TRPV4.

Abstract

In the organism, endothelial cells are constantly exposed to mechanical forces from blood. Changes of flow mechanics are sensed by various mechanosensors including the ion channels Piezo1 and TRPV4. They rapidly convert mechanical stimuli into an intracellular signal by the influx of calcium ions, leading to a variety of signaling pathways that may be relevant for adaptation of cells to the altered flow conditions, influencing cellular functionality. Surface expressed metalloproteases can be important mediators in this process by reacting immediately to the mechanotransduction with a functional response. The two most prominent members of the a disintegrin and metalloprotease (ADAM) family, ADAM17 and ADAM10, are well known to convert important cell surface expressed proteins such as growth factors (e.g. HBEGF), signaling molecules (e.g. Notch1), numerous adhesion molecules (JAM-A, PECAM1 or VE-cadherin) and cytokines and their receptors (TNFα and TNFR1) into soluble variants by proteolytic cleavage. Thereby, both ADAMs play a major role in cell proliferation, permeability regulation and repair processes. These responses are of great importance when disease-driven pathological remodeling takes place, leading to infection or wounding and changes in mechanical conditions. In this study we investigated if endothelial ADAMs can be mechanically activated via mechanosensitive ion channels and whether this pathway could be relevant for vascular homeostasis and function.

Here we show that chemical- and flow-induced activation of Piezo1 and TRPV4 causes enhanced activity of the metalloproteases ADAM17 and 10 in cultured primary endothelial cells. Therefore, we investigated protease-specific substrates via ELISA, immunofluorescence and western blotting. Additionally, we used selective protease inhibitors to show the contribution of ADAM17 or 10 to the cleavage of the tested substrate. The activation of Piezo1 and TRPV4 correlates with an enhanced shedding of the endogenously expressed ADAM17 substrates JAM-A (junctional adhesion molecule A) and TNFR1 (tumor necrosis factor receptor 1), as well as with an ADAM10-specific induction of the Notch1-responsive gene *HEY1*. Furthermore, we observed a Piezo1 activity-dependent reduction of adhesion molecules such as JAM-A, PECAM1 (platelet and endothelial cell adhesion molecule 1) and VE-cadherin at the cell borders, which is thought to be related to the induced ADAM activity and may influence the permeability of the endothelial cell layer. Importantly, chemical- and shear stress-induced substrate release is suppressed by *PIEZO1* knockdown or Piezo1/TRPV4 inhibition. Of note, the activation of Piezo1 and TRPV4 is not sensitive to PKC inhibition which contrasts with the well-known

activation mechanism of ADAM17 by PMA. In addition, the data indicates a relevance of Piezo1- and ADAM17/10-induced effect on endothelial proliferation and migration. *PIEZO1* knockdown and ADAM inhibition leads to a strong reduction of both functional processes, which is the other way around when stimulated with Yoda1. These findings may be important for the mechanical regulation of many physiological and pathophysiological processes in endothelial cells, such as JAM-A-/VE-cadherin-dependent permeability, neovascularization, vessel repair or inflammatory responses.

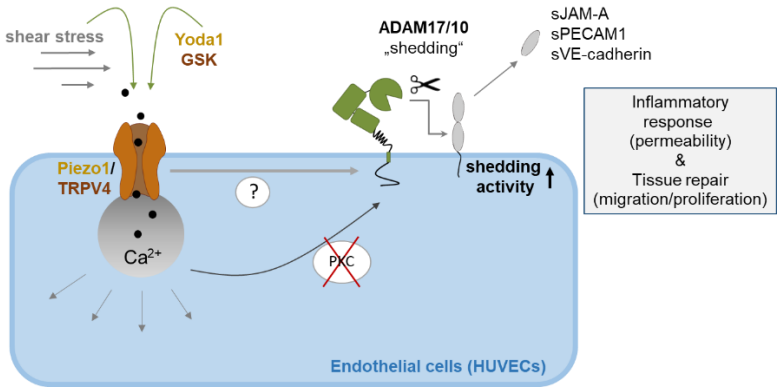

Figure 1. Piezo1 and TRPV4 activation by mechanical (shear stress) or chemical (Yoda1/GSK) stimuli induce an increased activity of ADAM17/10 through an unknown pathway which is independent of protein kinase C. The ADAM activation results in the proteolytic release of specific transmembrane proteins such as the adhesion molecules JAM-A, VE-cadherin and PECAM1 showing evidence for having an impact on functional responses like inflammation and repair processes.

The Biophysical Role of Desmoglein 3 in Pemphigus Vulgaris: Insights from Fluidic Force Microscopy and Total Internal Reflection Fluorescence Microscopy

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Headline

Exploring the role of Desmoglein 3 (Dsg3) in pemphigus vulgaris (PV) by measuring altered cell stiffness and cell-cell adhesion via Fluidic Force Microscopy (FluidFM) and by elucidating the mechanism of cell-substrate detachment process by combining the force monitoring of FluidFM with optical imaging of Total Internal Reflection Fluorescence Microscopy (TIRF).

Abstract

The existence of a transcellular communication network is implicit in the remarkable complexity of tissue morphogenesis and regeneration. Through this network, individual cells perceive the environment and coordinate their biological activity in time and space. Studying biophysical properties in combination with biochemical network analysis is required to understand the fascinating capacity of tissue self-organization. In this framework, the role of desmosomal cadherin-type adhesion molecules in integrating tension forces into biophysical and biochemical networks is emerging due to their suggested outside-in signaling activity and critical role in bearing mechanical tissue stress. One report linking a specific biochemical signaling component to altered tissue stiffness, a well-known mechanical read-out, highly supported such activity in cells treated with PV autoantibodies capable of disrupting Dsg3 transadhesion.[1] Pemphigus is a unique group of autoimmune diseases and Dsg3 is the major target in PV. [2] Since the role of Dsg3 in mechanosensing and signaling has yet to be clarified, we are using PV as a model to further investigate the Dsg3 biophysical network. We are applying the innovative Fluidic Force Microscopy (FluidFM),[3] a versatile tool in single-cell biology[4] that consists of a force-controlled pipette based on AFM standards with microchanneled cantilevers to measure altered biophysical parameters upon disruption of Dsg3 transadhesion in human primary epidermal keratinocytes (HPEK). Our investigation into the Dsg3 signaling network includes measuring cell stiffness via indentation experiments and assessing cell-cell adhesion by detaching individual cells from their monolayers in control and Dsg3 antibody-treated cells. During the cell detachment process of control cells, Force vs Distance curves obtained with FluidFM reveals a series of events: the cantilever first reaches a peak force, followed by detectable minor jumps known as tether-breaking events. We are therefore integrating FluidFM with Total Internal Reflection Fluorescence Microscopy (TIRF) to enhance our understanding of the cell detachment mechanism at the cell-substrate interface and to elucidate the dynamics of observed tether-breaking events.

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"Crafting robust patterns: the interplay of cell mechanical properties and 3D shapes in Drosophila wing disc development"

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Headline (max 400 characters including spaces)

This research investigates the interplay between mechanical forces and developmental patterning in the Drosophila wing discs. We combine assays to mechanically stress tissues *in vivo* and *ex-vivo* with mathematical modelling and 3D cell segmentation to study the relative contributions of cell size, 3D geometry, and mechanical properties on robust pattern formation and maintenance.

Abstract (max 2 pages for the whole document)

Animal tissues undergo development and take on their functional 3D shape while experiencing continuously changing mechanical forces. Despite decades of work into how genetic and biochemical programs guide morphogenesis, we do not yet understand how tissues buffer these constantly fluctuating forces to enable robust development. To understand how developmental patterning responds to mechanical stresses and uncover mechanisms that buffer these stresses, we combine *ex-vivo* and *in-vivo* approaches using the *Drosophila* wing disc as a model system.

Drosophila larvae are highly motile, and as a result their internal organs experience fluctuating mechanical forces which are transmitted through muscle fibers and attachments to the epidermis during crawling. Using rapid live imaging of whole larvae we are able to observe deformations of wing discs induced by the forces transmitted as the larvae crawl freely or inside microchannels with narrow constrictions, enabling us to characterize the mechanical strain experienced by organs *in vivo* (Figure 1A).

As a complementary approach, we employ a tissue stretcher[1] to deliver precisely-controlled mechanical stimuli to wing discs *ex-vivo* (Figure 1B). This allows us to apply different regimes of mechanical stress (e.g prolonged stretch vs cyclic stretch) while observing the response of live reporters of key signalling pathways that guide wing disc patterning. One of the processes we've been investigating is the development of sensory organ precursors (SOPs) at the wing margin, which are specified through Notch-Delta signalling during the 3rd instar larval stage and form two rows of cells on either side of the dorsoventral boundary (Figure 1C).

By stretching wing discs and quantifying the cells aspect ratios of SOPs and their neighboring cells we have observed a striking "stripe" pattern of cell elongation arise, which is due to the SOPs being very stiff and resisting stretching (Figure 2A). We hypothesized that these unique mechanical properties may

help confer robustness to the pattern and performed perturbation experiments where the SOPs are genetically softened with a Rok-RNAi.

In preliminary experiments we have observed that the wing discs with "softened" SOP have an altered pattern spacing (Figure 2B), as well as an increase in misaligned cells. This motivated us to further investigate the factors controlling the spacing between SOPs.

Predictions from a mathematical model of signalling in this system[2] show that the cells signalling range needs to exceed the first neighbours to obtain the experimentally observed spacing of roughly 3 cells apart (Figure 2C). We hypothesized that these additional contacts might arise through cell-cell intercalations occurring on the z axis, as wing disc cells are pseudo-stratified, extremely tall (50 um) and scutoid -shaped. By performing deep two-photon imaging of wing discs and machine-learning assisted 3D segmentation[3] we were able to show that SOPs and surrounding epithelial cells have a 2-fold increase in cell-cell contacts when the z dimension is considered compared to just the apical neighbours (Figure 2D). As packing of cells in the wing disc changes in different regions, we are now comparing the spacing of SOPs in regions where cells are more or less densely packed, as well as analyzing the effect of mechanical perturbation (like cell softening) on 3D cell shapes. Overall, this will allow us to understand the relative contribution of cell area, 3D geometry and mechanical properties with the effective inhibition that can be achieved through Notch-Delta signalling and final outcome in terms of patterning.

Combined with our *ex-vivo* and *in vivo* mechanical perturbation assays, this study will enable us to eventually understand how developmental patterns are (1) robustly achieved and (2) maintained in the presence of continuous mechanical perturbations.

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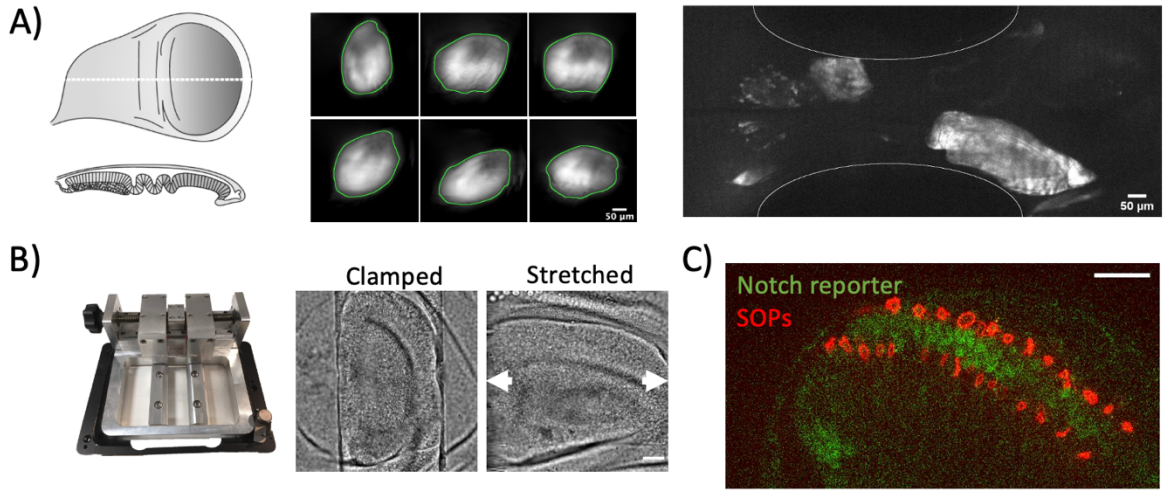

Figure 1. (A) Schematic wing disc (left), example of shape changes in a wing disc imaged inside a freely crawling larva (middle) and a larva crawling inside constricted PDMS channels (right) ; (B) tissue stretcher with example of a stretched disc; (C) the SOP pattern as seen using the neuralised marker for SOPs.

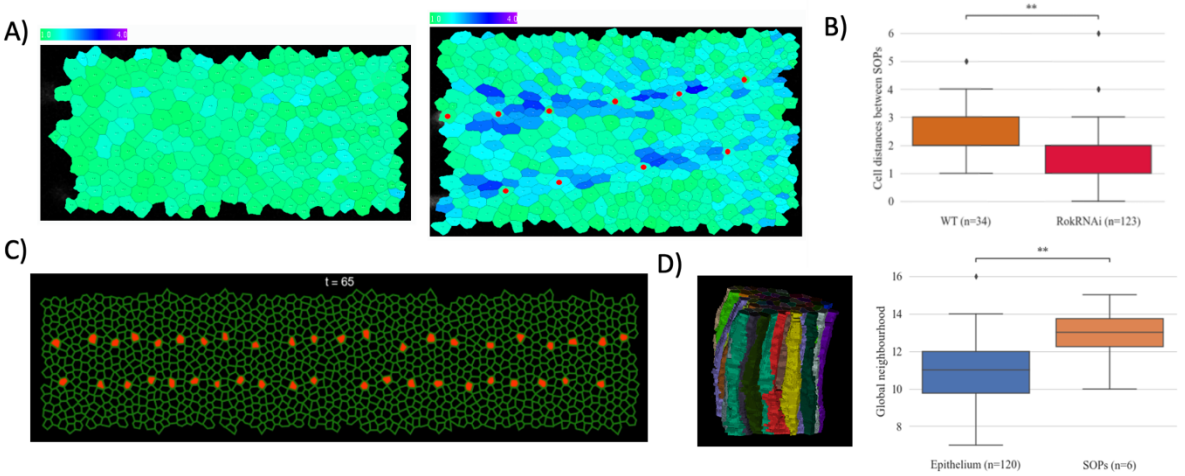

Figure 2. (A) Elongation ratio of cells before and after stretching, SOPs marked with red dots; (B) analysis of spacing between SOPs in control conditions and with “softened” SOPs; (C) example simulation of SOP patterning with a spacing consistent with experiments; (D) example 3D reconstruction of wing disc cells and quantification of total cell contacts across the whole volume.

Mechanobiology and Morphogenesis: New Tools for an Old Problem

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Headline (max 400 characters including spaces)

We introduce three tools to study cell-matrix interactions in high-throughput in the context of mechanobiology and morphogenesis. TEMPO is a cell line with advanced sensors, HYDRA is a technique to fabricate high-throughput hydrogels, and SEM2 is a new computational framework for cell and tissue mechanics.

Abstract (max 2 pages for the whole document)

Addressing Mechanobiology and Morphogenesis Challenges. Understanding mechanobiology and morphogenesis presents complex challenges, particularly in characterizing and modeling the myriad interactions between cells and the extracellular matrix (ECM). Our research introduces three novel tools - TEMPO, HYDRA, and SEM2 - each contributing uniquely to this field.

TEMPO: A Reference Cell Line with Advanced Fluorescent Sensors. TEMPO, a reference cell line, incorporates advanced fluorescent sensors, facilitating detailed phenotypic screening. We prototyped TEMPO in human epithelial cells (HaCaT) in various complex imaging experiments. Notably, the integration of structural and functional fluorescent sensors with a novel cell cycle indicator, FUCCIplex, allows for precise cell cycle stratification in imaging-based experiments, including tracking and smart microscopy. Then, we expressed the TEMPO sensors in human induced pluripotent stem cells (hiPSCs) and demonstrated its capabilities in stem cells, germ layer progenitors, and 3D organoids.

HYDRA: Automated Hydrogel Production for High-Throughput Drug Testing. HYDRA introduces an automated method for producing hydrogels in high throughput multiwell plates using liquid handling robots. We demonstrated this method by creating a 96-well plate featuring fish gelatin hydrogels cross-linked with microbial transglutaminase. The resulting hydrogels exhibited physiological stiffness and provided a more relevant substrate for cell culture studies compared to traditional materials. We then used TEMPO HaCaT cells to demonstrate the compatibility of these hydrogels with various imaging techniques, including digital holographic and fluorescent confocal imaging, which enhances their utility in imaging-based analyses.

SEM^2: A Computational Framework for Cell and Tissue Mechanics[1]. SEM^2 is an advancement in computational modeling that extends the subcellular element modeling (SEM) framework to incorporate analyses of particle-level stress and strain. This tool allows for the simulation of cell behaviours like division, migration, and proliferation in different culture environments. SEM^2 capacity to model multi-scale mechanics is crucial in studying stress and strain propagation, contributing to our understanding of tissue morphogenesis and cellular responses to mechanical stimuli. The application of SEM^2 extends to various experimental settings, including cell creep experiments and modeling of proliferation in constrained environments.

Conclusion. The development of TEMPO, HYDRA, and SEM2 addresses critical aspects of mechanobiology and morphogenesis in tissue engineering. TEMPO provides a platform for detailed phenotypic screening, HYDRA automates hydrogel production for high-throughput applications, and SEM^2 offers a computational framework for understanding cell and tissue mechanics. Collectively, these tools enhance our capacity to characterize and model cell-material interactions, offering valuable contributions to both the theoretical and practical aspects of tissue engineering research. All three of these tools are or will be made available to the academic community via open-science channels.

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New tools to understand the mechanics of the cell and its nucleus at large deformations

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Wedged Cantilever, Magnetic Pincher and Fluorescence Exclusion: quantitative tools for cell biologists who want to understand cell mechanics

Methods to access the mechanics of the cell nucleus, the mechanics of the cell cortex and to measure the cell volume, when cells undergo large deformations

Abstract (max 2 pages for the whole document)

We have been motivated to develop new tools or adapt existing ones, to assess cell mechanics, while there are already so many available, for two main reasons: 1) as cell biologists, we were often frustrated to not be able to interpret the cell mechanics measures provided by classical tools such as AFM or micropipettes, from the perspective of our knowledge of the cell complexity (e.g. what structure dominates the measure, is the volume constant, is the cell actively responding during the measure?), so we turned to methods that provide more direct measures that we hope to be able to interpret better; 2) we have investigated, from a cell biology point of view, contexts in which cell undergo large deformations, such as migration of immune and cancer cells through pores or dense tissues, circulation of cells in blood circulation, or deformation of cells due to proliferation in confined spaces. We found that mechanics associated to these large deformations involved taking into account phenomena such as volume loss, nuclear envelope unfolding, nuclear envelope ruptures, blebbing, that had not been taken into account in the usual small deformation regimes investigated in most studies of cell mechanics.

On this talk, I will present the results we obtained with three methods: a) the magnetic pincher, which allows a direct access to the thickness and mechanics of the cell cortex; b) a combination of wedged cantilever AFM and confocal imaging (a method developed by Daniel Müller's lab), applied to investigate the mechanics of the cell nucleus at large deformations; c) fluorescence exclusion (FxM), to measure the volume of the cell and the nucleus with a % precision, over short (ms) to long (days) timescales; showing that cells (and nuclei) loose a significant amount of volume when they are deformed.

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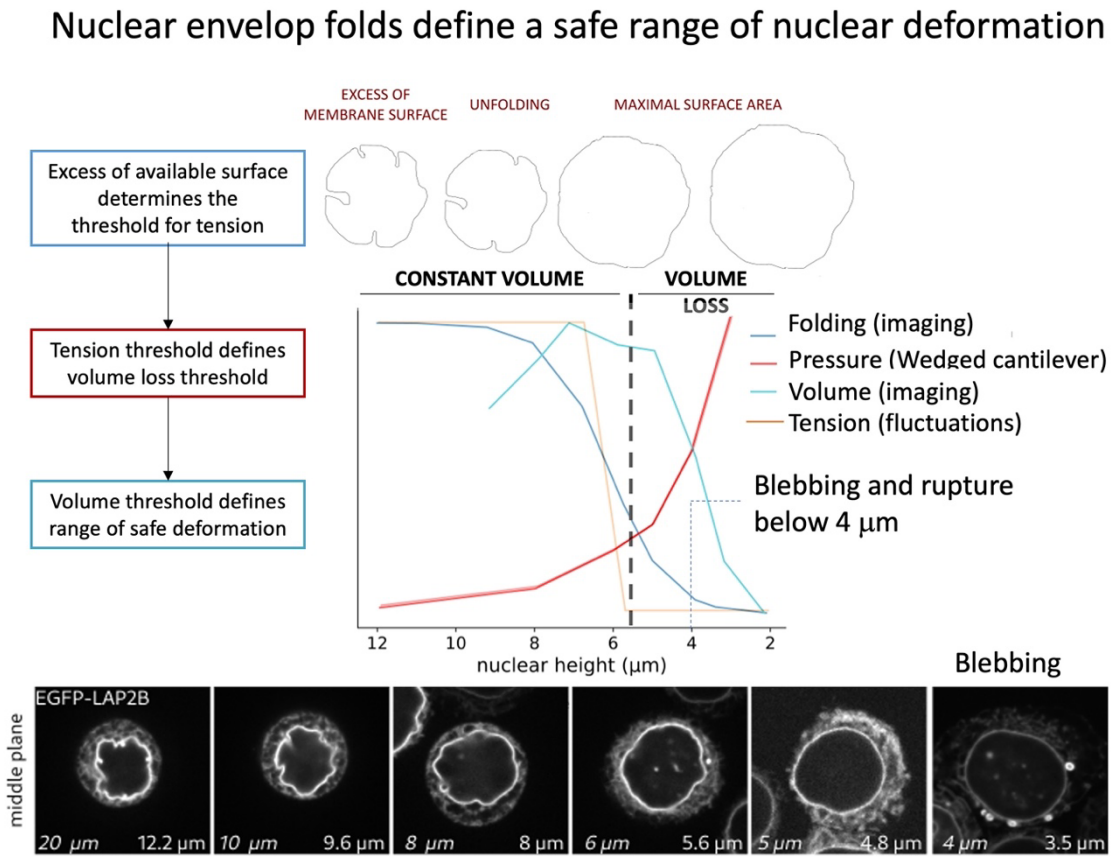

Figure 1. Mechanical states of the nucleus as it is progressively flattened. From a folded nuclear envelope, with a constant volume, to a tensed envelop with volume loss and eventually blebbing and rupture

Uncompensated apoptosis causes “mechanical wear” of the postmitotic retinal pigment epithelium

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Headline

Uncompensated apoptosis in the ageing postmitotic retinal pigment epithelium alters the monolayer structure and biomechanics, resembling material wear and tear. In our ageing-mimicking model, apoptosis induced age-associated histone modifications and changes in the expression of membrane-associated genes, suggesting both epigenetic and functional implications for the epithelium.

Abstract

Postmitotic cells are common in our body, especially in the heart, muscles and the central nervous system. The inability to proliferate makes their adaptation to mechanical challenges unique, causing fundamental mechanobiological processes to occur at a different temporal resolution. The retinal pigment epithelium (RPE), a postmitotic epithelium crucial for photoreceptor health, remodels during ageing due to uncompensated apoptosis. This remodelling includes accumulation of hypertrophic cells, changes in monolayer organisation, decrease in cell height and changes to the cellular cytoskeleton and apical membrane, all suggesting altered mechanical balance. Considering the importance of contractility for RPE function and RPE’s role in the pathogenesis of age-related macular degeneration, we examined how age-related cell loss impacts RPE biomechanics and function.

We hypothesize that local stretch due to apoptotic extrusion events may impose global mechanical burden on the epithelial monolayer during ageing, causing structural wear-and-tear and impacting crucial epithelial functions. To study this, we developed a unique, age-mimicking in vitro model of a postmitotic epithelium undergoing apoptosis. Mature, low-proliferative hiPSCs-derived RPE monolayers on soft hydrogels were subjected to apoptosis via drugs or on-demand caspase-8 activation. Our model reveals that age-mimicking cell loss alters RPE biomechanics, such as the tissue stiffens, its dampening capacity declines and traction forces increase. Furthermore, the monolayer structurally remodels – the cell height decreases, cells reinforce their adherens junctions and the apical microvilli become thinner. These findings demonstrate that uncompensated apoptosis in postmitotic epithelia may cause mechanical wear during ageing, like materials’ ageing.

Current work focuses on the impact of this material ageing of the postmitotic RPE on ageing-associated epigenetic and functional aspects. We found that monolayer-scale apoptosis causes increased number of double-stranded DNA breaks, as evidenced by an increased presence of the ageing-associated histone γH2AX. Functional enrichment of differentially expressed genes after bulk RNA sequencing revealed changes in pathways associated with membrane-associated processes implicating genes like ITGA3, PLS1 and EPHA2. These findings suggest that cell loss may have possible epigenetic implications, as

well as effect on membrane linked RPE functions like substance trafficking across the retina and photoreceptor outer segment phagocytosis.

Our work delivers novel insights into epithelial homeostasis from an unusual perspective and highlights the role of mechanics in preserving the health of the ageing retina. Future work will uncover the exact connection between the biomechanical and functional changes due to apoptosis in postmitotic tissues.

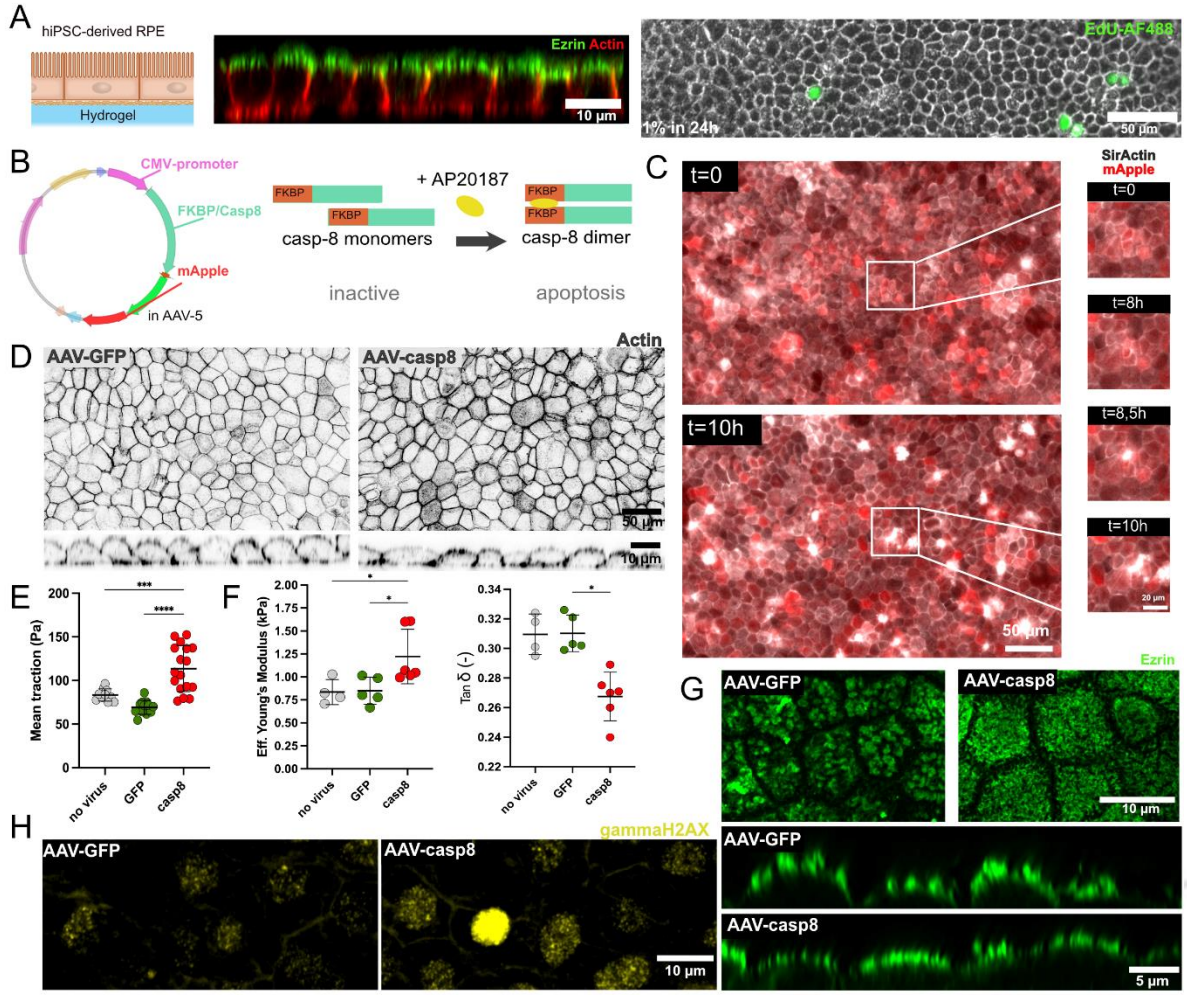

Figure 1. (A) In vitro model of a post-mitotic epithelium: hiPSCs-derived RPE cells on soft polyacrylamide hydrogels (left) polarize and express characteristic apical marker ezrin (middle) and show low level of proliferation (right); (B) Age-mimicking in vitro model based on on-demand apoptosis induction: AAV5 viruses are used to deliver a construct for the expression of a recombinant caspase-8, which can dimerize upon interaction with AP20187 and promote apoptosis; (C) Extrusion events during apoptosis induction are a large-scale monolayer occurrence that takes place approximately 10 h after AP20187 addition; (D) Apoptosis-based postmitotic ageing model alters cellular size and arrangement (top), as well as leads to decreased cell height (down); (E) Average monolayer traction force increases due to ageing-mimicking apoptosis; (F) Effective Young's modulus increases after ageing-mimicking apoptosis, while the dampening properties (ratio of loss to storage modulus determined by dynamic mechanical analysis) decrease; (G) Morphology and thickness of apical microvilli decreases in the ageing-mimicking model; (H) Double-stranded DNA damage-associated histone modification γH2AX is enhanced after ageing-mimicking apoptosis in the postmitotic hiPSCs-derived RPE model.

Photothermally driven AFM on biological samples

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Abstract

The atomic force microscope (AFM) is a multifunctional toolbox for characterization at the nanoscale. A critical aspect of AFM operation is control over the actuation of the cantilever. In this regard, photothermal excitation – an intensity modulated light source directed onto the cantilever – shows significant benefit over a dither piezo for clean and stable tip oscillation. Cantilever motion may be driven from DC to higher eigenmode frequencies, enabling new approaches for using the AFM.

In this presentation, we provide an overview of new developments and advances that take advantage of photothermal excitation for nanoscale characterization of materials with AFM. These include fast off-resonance imaging techniques, and mass and mechanical property measurements of cells and particles.

Extracellular vesicles, cells and mutual interactions: an AFM-based mechanobiology point of view

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Headline

AFM-based mechanical and topographical investigations of cells, models of cellular membranes and their interaction with Small Extracellular Vesicles produced by breast cancer cells.

Abstract

Atomic Force Microscopy (AFM) has gone through a fast development over the last decades mainly thanks to its versatility. Indeed, AFM has shown unprecedented potential in the field of scanning probe microscopy, being able to operate in diverse environments and specimens, as well as to map simultaneously the topography and various physico-chemical properties of the scanned sample on different spatio-temporal scales, from μm to nm and from minutes to ms/μs.

In parallel, the research interest on biophysical topics for theranostic purposes has been growing, along with the need for high resolution tools able to operate on biological systems in physiological conditions. Particular attention has been paid to the field of mechanobiology, given the demonstrated correlation between mechanical properties and the patho-physiological state of many biological specimens.

In this context, AFM has proved to be a valuable tool, allowing for the acquisition of simultaneous topographic and mechanical maps of biological systems, from the micro- to the nanoscale, such as cells [1], Extracellular Matrices (ECMs), biological macromolecules, and the investigation of their mutual interactions. In particular, keen attention has been recently dedicated to the analysis of cellular membranes models and their interaction with small Extracellular vesicles (s-EVs) [3], given the similarities of the latter to viral particles and their well-known capability to influence the fate and properties of recipient cells depending on their origin and the transferred cargo [2].

Here we will present our results on AFM-based mechanobiology on cells in the context of breast cancer, looking in detail how mechanical properties can be influenced by the cellular uptake of s-EVs produced by highly invasive metastatic cancer cells (see fig.1, adapted from reference [2]). In order to gain a more detailed understanding of the fusion mechanisms underlying the biomechanical and fate changes observed in cells after EVs interaction, we will then focus our attention on the topographical and compositional key features of cellular membranes model and their interaction with breast cancer derived-EVs (see fig.2, adapted from reference [3]) We will finally show our preliminary force-spectroscopy measurements on the cellular membrane models to quantify the mechanical and fluidity properties of these systems on the nanoscale and their changes induced by EVs. Altogether our results underline the importance of understanding fusion mechanisms of EVs in breast cancer framework since it represents the first step for potential applications in the theranostic field.

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Figures

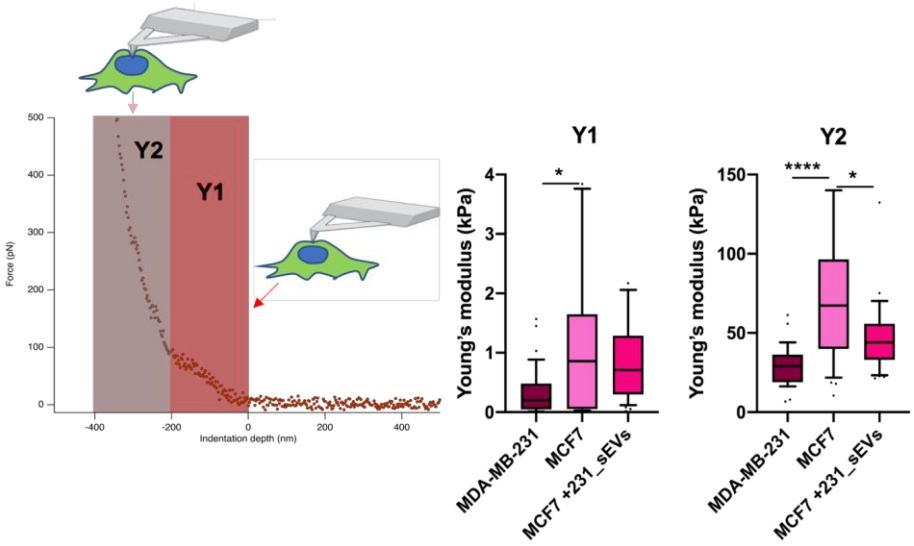

Figure 1. Young's moduli obtained through AFM force spectroscopy of MDA-MB-231, MCF7, and 231_sEV-treated MCF7 cells. (Left) Representative AFM force-indentation curve of cells by using a pyramidal tip (positioned over the nucleus) and by using the two-slope modified Hertz-Sneddon model to fit the force curves. (Right) Boxplots showing the elastic moduli Y1 (external layer) and Y2 (cell body) of the cells.

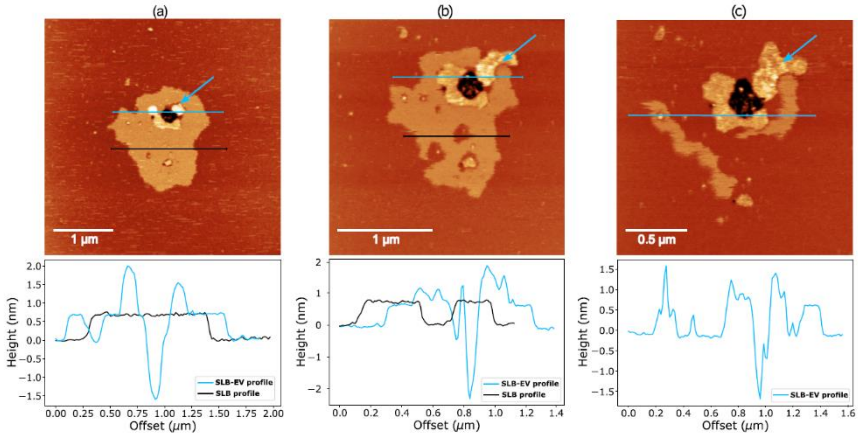

Figure 2. Time-resolved AFM topographic images of EVs (MDA-MB-231 cell line) interacting with DOPC/SM 2:1 (m/m) supported lipid bilayer with 17 mol% Chol and corresponding height profiles, acquired at 27 ◦C in Tris buffer 10 mM, with a time-lapse of 10 minutes (from a to c).

Quantification of stretch-stress generation and relaxation in model epithelium

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Headline

Throughout an organism's life, epithelial cells organize into a variety of tissue architectures, maintaining their structural integrity over time. In our study, we investigate the maintenance of homeostasis in an epithelial tissue subjected to stretch stress. We propose that the function of epithelial tissue in homeostasis is intricately related to its structure and morphology [1,2].

Abstract

Mechanical forces influence cells via a process known as mechanotransduction, in which cells within a tissue receive and respond to physical stimuli. Epithelia are constantly exposed to mechanical stimuli, originating from the environment, adjacent cells, and alterations in the extracellular Matrix (ECM). Various physiological processes like respiration, peristalsis, blood circulation in arteries, perturbation of heart involve cells consistently undergoing stretching, which may result in compromised tissue morphology and architecture [3,4]. Such alterations in tissue structure are often associated with a myriad of pathological conditions, including cancer. Understanding the impact of stretch deformation on tissue architecture and function is imperative for elucidating disease mechanisms [5]. In this study, we investigate the response of epithelial tissue by growing Madin-Darby canine kidney (MDCK) cells on a stiff PDMS substrate, which is later subjected to uniaxial stretch stress using a motorized stretch device (Figure1 (b)).

To examine the response of epithelia at diverse length and time scales, we apply static stress by subjecting homeostatic tissue to uniaxial stretch stress at a 10% stretch and a speed of 1 mm/s (Figure 1(a)). The study investigates the changes in the tissue due to the modulation of the mechanical properties of the environment in which the tissue is residing, the strength and frequency of driving the deformation, as well as characterizing the onset of tissue failure, correlating in with topological features in the tissue. This provides us with insight into tissue remodelling, growth, and death.

By applying a static stress, we demonstrate both immediate and long-term reactions of tissue to the stretch. We employ various image analysis techniques and statistical tools to quantitatively assess tissue remodeling, connectivity, and cell growth in response to tissue stretching. Our approach provides valuable insights into the mechanical feedback of tissue subjected to uniaxial stretch deformations, playing a crucial role in understanding the biophysical aspects of tissue response and disease progression.

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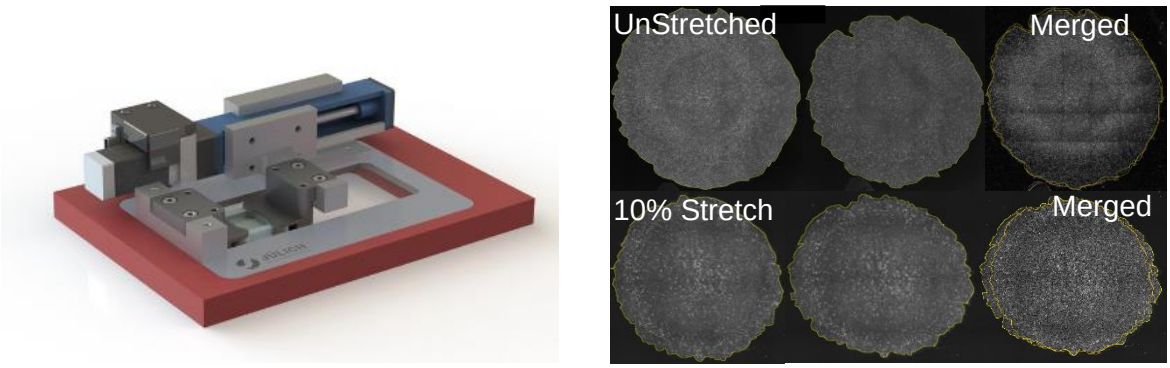

Figure 1. (a) A motorized stretcher system (b) MDCK tissue subjected to 0 and 10% stretch.

The Role of Viscoelasticity in the Bone Marrow Niche

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The bone marrow (BM) niche is the specialised environment in which hematopoietic stem cell (HSCs) reside. This environment provides a complex set of chemical and mechanical cues that regulate HSC self-renewal and differentiation (1). Its extracellular matrix (ECM) is not purely elastic but exhibits a time-dependent response to mechanical loading, a property known as viscoelasticity (2). However, efforts to understand the role of ECM viscoelasticity in regulating cell behaviour have only recently been made using 3D mimicking hydrogels (3). These have shown that viscoelasticity can affect cell behaviour independently from the hydrogel's stiffness (3), but little is known about its effects on cell mechanics. Here, we propose a novel approach to develop BM-mimicking isoelastic hydrogels pairs with varying stress relaxation rates. These hydrogels will then be used to investigate the effect of viscoelasticity on BM stromal cell morphology and mechanics.

Non-degradable polyethylene glycol-Maleimide (PEG-MAL) hydrogels were prepared using Michael-type addition, and functionalized using full-length Fibronectin (FN), a protein abundantly found in the BM ECM. Stiffness and viscoelasticity were varied by altering the percentage or molecular weight (MW) of the hydrogel components. Bulk mechanical properties including storage modulus (G') and stress relaxation were measured using a stress-controlled rotational rheometer. Furthermore, spheroids of human BM-derived mesenchymal stem cells (hMSCs) were encapsulated within hydrogels for a period of 5 days, after which spheroid morphology was investigated using confocal microscopy. hMSC spheroid mechanics were explored after 3 days using Brillouin spectroscopy.

We engineered three pairs of FN-functionalised PEG-based hydrogels, with BM-characteristic elastic moduli *E* of 1, 5 and 12kPa. Each pair comprises of a slow-relaxing, more elastic hydrogel and a fast-relaxing, more viscoelastic counterpart. When hMSC spheroids were encapsulated in the 5kPa hydrogel pair, we observed that spheroids in slow-relaxing matrices impaired spheroid outgrowth, with no significant changes in spheroid morphology by day 5. On the contrary, spheroids in fast-relaxing hydrogels demonstrated increased size, accompanied by the formation of finger-like projections that invaded the matrix. This morphological difference correlated with a change in spheroid mechanics, whereby spheroids in fast-relaxing hydrogels presented a significantly decreased Brillouin frequency shift compared to spheroids in slow-relaxing hydrogels. At higher stiffnesses (12kPa hydrogels) viscoelasticity did not affect spheroid morphology or mechanics, as spheroids in both slow- and fast-relaxing gels remained spherical and showed similar Brillouin frequency shifts. However, matrix stiffness significantly affected spheroid mechanics, with spheroids in rigid gels showcasing significantly higher Brillouin frequency shifts than those in the 5kPa hydrogel pair.

We engineered BM-mimetic FN functionalised PEG hydrogels with tuneable stress relaxation independently of their elastic modulus. Upon encapsulation of hMSC spheroids, fast-relaxing hydrogels of medium stiffness (5kPa) promoted cell invasion compared to slow-relaxing ones which instead restricted cell movement. Crucially, this change in cell behaviour could be detected via Brillouin spectroscopy as spheroid mechanics were affected by the stress relaxation of the surrounding matrix.

Finally, increased hydrogel stiffness (12kPa) significantly impacted spheroid mechanics, while viscoelasticity did not affect spheroid morphology or mechanics.

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Mitochondria as a unifying hub coordinating metabolism and nuclear gene expression in response to ECM mechanics

Patrizia Romani and Sirio Dupont

Extracellular matrix (ECM) mechanical cues have powerful effects on cellular processes influencing cell behavior. Recent findings in our laboratory indicate that ECM stiffness regulates mitochondrial fission dynamics via DRP1, which in turn triggers a transcriptional and metabolic antioxidant response. How general is such mitochondrial remodeling? How do forces and actomyosin tension regulate mitochondrial fission? We found that actomyosin tension regulates the phosphorylation of a mitochondrial fission factor to regulate fission, which in turn orchestrates the regulation of important transcription factors to control cell proliferation, differentiation, and metabolism in response to mechanical cues. The same mechanism also regulates key mitochondrial functions. Our findings point to a key role of mitochondria in the response of cells and tissues to forces.

Liquid-to-solid phase transition of MEC-2 condensates is required for mechanotransduction during touch

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Headline (max 400 characters including spaces)

MEC-2/Stomatin - a crucial protein for the sense of touch in *Caenorhabditis elegans* - forms viscoelastic biomolecular condensates that undergo a rigidity transition that switches transport along axons, into force-sensing, solid and immobile condensates to enable neuronal mechanotransduction.

Abstract (max 2 pages for the whole document)

Behind certain daily actions such as playing the violin, painting, or hugging our kids, there are ion channels that convert the physical stimuli into physiological signals that are interpreted by our nervous system to offer an adequate response, what is known as mechanotransduction. Even though in the last decades we have broadened our understanding of the ion channels and mechanoreceptor cells that govern this process, we barely understand how the mechanical stresses travel through the tissues and reach the molecular mechanosensors. While it is well established that many mechanosensitive ion channels respond to increased plasma membrane tension, emerging evidence points towards a physical tethering of these channels to the cytoskeleton and/or extracellular matrix within the sensory cells for an effective force transference[1].

Here, we focused on the mechanotransduction pathway of the nematode *Caenorhabditis elegans*' gentle touch, which relies on mechano-electrical transduction (MeT) platforms within the membrane of six touch receptor neurons. For more than a decade, it has been suggested that the pore-forming subunit of the MeT channel establishes a direct connection with the cytoskeleton[2] through a highly conserved and widely distributed protein, known as MEC-2, which shares structural similarities with Stomatin.

Membraneless cellular compartments experience liquid to solid-like phase transitions that exhibit different material properties over time, however, whether the naive and the mature states can have different biological functions remained elusive. Our study provides compelling evidence that MEC-2 assembles in liquid condensates that experience a shift in rigidity[3], transitioning from a fluid-like pool allowing transport along neurons, into solid-like states that are mechanoelectrically active (Fig. 1).

Using optical tweezers, we characterized MEC-2 maturation into gel-like platforms with a strong frequency dependent viscoelastic property, suggesting that it can transmit forces on short (>1Hz) but not on long timescales. We then directly delivered mechanical forces to animal's

body wall through a hybrid microfluidic-pneumatic chip and observed that only stiffened condensates, as opposed to fluid-like ones, are able to sustain mechanical forces. We showed that MEC-2 maturation depends on an unstructured proline-rich motif at its C-terminus with SH3-binding properties. By combination of neuronal calcium imaging and behavioural assays, we identified a role for the SH3 domain of UNC-89/Titin, which co-condensates with the mature pool of MEC-2 at mechanosensitive active sites and induces an elastic conversion *in vitro*.

This work introduces a new, physiologically relevant context in which biomolecular condensates tune their function upon maturation, facilitating neuronal mechanotransduction in response to tactile stimuli[3]. It also offers a new conceptual framework for understanding how animals, in a broader sense, perceive and respond to mechanical stresses.

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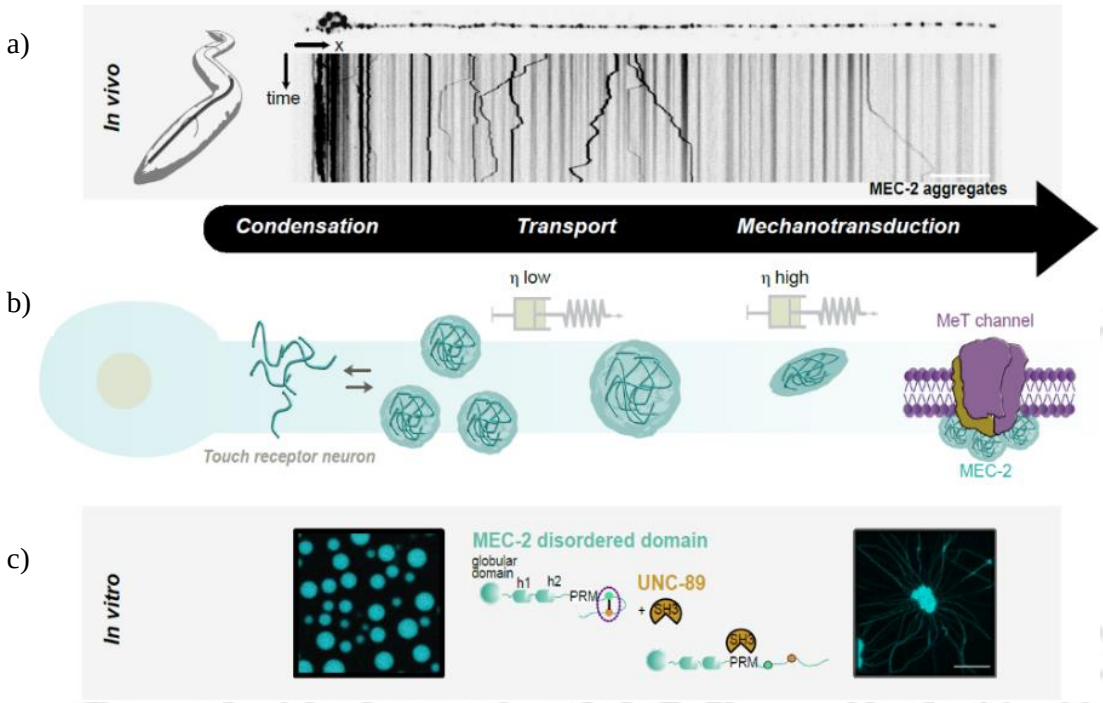

Figure 1. MEC-2 liquid-to-solid phase transition controls mechanotransduction. (a) MEC-2 aggregates mobility *in vivo*. Sketch of *C. elegans* touch receptor neurons (TRNs). Representative image and kymograph of MEC-2 along a TRN neuron from the cell body (left) towards the distal neurite (right). Scale bar = 20 µm. (b) MEC-2 forms biomolecular condensates with low viscosity that facilitate transport along neurites of varying diameter and thickness. Binding of the UNC-89 SH3 domain to MEC-2 disrupts the heterotypic buffering within the C-terminus and induces a rigidity transition of the MEC-2 condensates. The mature condensates form at sites of active mechanotransduction and can sustain stress transfer during body wall touch. (c) MEC-2 behavior *in vitro*. Confocal fluorescence microscopy image of MEC-2 C-terminus with UNC-89 SH3 domain at a molar ratio of 1:0.1 immediately, t=0 h (left image), or after 24 hours (right image) of sample incubation. Scale bar = 10 µm. In the middle, sketch of MEC-2 C-term with intramolecular interactions enhancing droplet formation. Upon UNC-89 SH3 addition, there is a transition into a solid-like state.

Role of spatiotemporal-controlled morpho-physical cues in the regulation of focal adhesion dynamics and cytoskeleton remodelling of adipose stem cells

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Headline

The dynamic interaction between cells and extracellular microenvironment can be in-vitro mimicked through the design and fabrication of new engineered materials. Particularly, micro-pneumatic platforms can be used to deeply investigate cellular behaviour. Here, a platform able to apply in a simultaneous manner different morpho-physical cues on stem cells has been developed.

Abstract

In biological systems, the geometric form and the biological functions are inherently correlated at multiple length scales. Particularly, at cell scale, there is increasing evidence about the role of morphological structure of the microenvironment on cell behaviour [1,2]. Cells, in fact, are constantly exposed to a variety of biophysical stimuli emanating from their microenvironment, among which morpho-physical cues like curvature and nano-topographies are starting to emerge as crucial regulators of different aspects of cellular behaviour such as cell proliferation, migration, and differentiation. Particularly, each specific cue like curvature or nano-topography [1,2] is able to induce a specific cellular response, but, in-vivo conditions, cells are modulated by a combination of signals. To apply different morpho-physical cues to cells, a specific platform has been designed in order to analyse in real-time the response of actin stress fibers and focal adhesions (FAs) of adipose derived mesenchymal stem cells (ASCs) induced by such signals.

The micro-pneumatic platform has been designed combining two different fabrication techniques: two-photon polymerization strategy and micro-milling method. Moreover, different materials were used: polymethylmethacrylate (PMMA) and polydimethylsiloxane (PDMS). The former technique allows the fabrication of a nanopatterned substrate used as master for obtaining the PDMS nanopatterned-membrane. The latter for PMMA layers production. As reported in Figure 1, the platform is composed by different layers irreversibly bonded by thermal solvent reactions. The curvature of the nanopatterned PDMS membrane was modified in a spatial-temporal controlled manner by using a pressure control system. ASCs transfected with pCMV–LifeAct®–TagGFP2 for actin stress fibers and pmKate2 for FAs were seeded on the PDMS membrane in order to study in real-time the dynamic behaviour of actin stress fibers and FAs.

The effects of curvature, nano-topography and their combinations were studied in static and dynamic conditions analysing both actin stress fibers and FAs response. Particularly, the curvature is applied along the longitudinal direction of the micro-channel whereas the nanopattern in the orthogonal one; this means that the two signals are opposite one to another. As shown in Figure 2A, after 24h of stimulation, in a static configuration both dose of curvature and nano-topography control the actin stress fibers and FAs re-organization with a cellular polarization along 45° with respect to such signals.

Conversely, in the dynamic cases (Figure 2B, C), the frequency of stimulation vehiculate the ASCs to polarize along the micro-channel (Figure 2B) or nano-pattern (Figure 2C) direction.

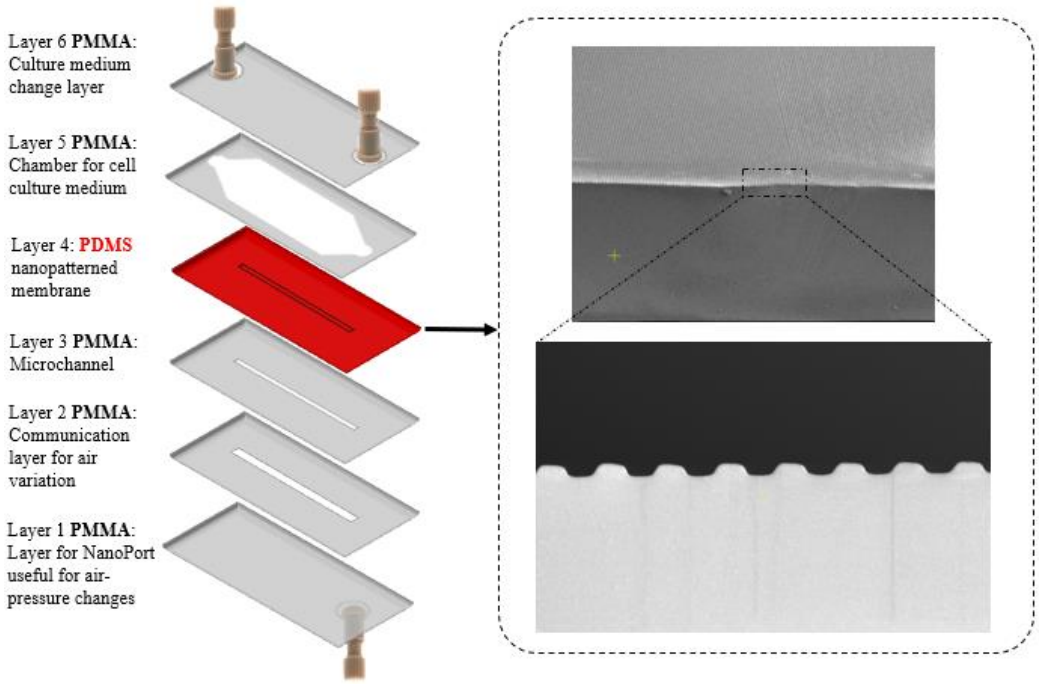

Figure 1: 3D view of the micro-pneumatic platform with scanning electron microscopy images of nano-patterned PDMS membrane

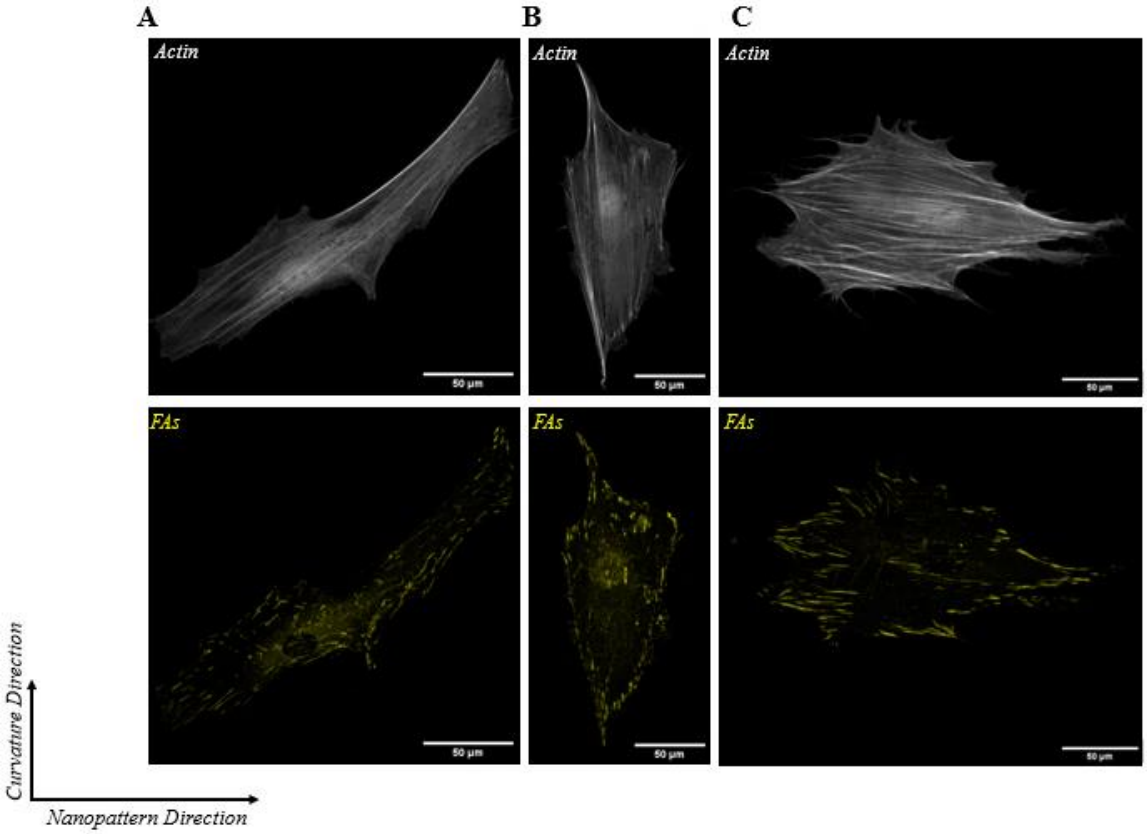

Figure 2: Representative images of actin stress fibers and FAs behaviour in static (A) and dynamic (B, C) configurations

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Investigation of mechanoradical formation in collagen through Electron Paramagnetic Resonance spectroscopy

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Headline

My project revolves around the investigation of mechanoradicals in collagen through EPR techniques. Mechanoradicals are the byproduct of covalent bond rupture, and by monitoring their formation in collagen samples we can obtain vital information on the mechanical properties of tendon tissues, and their behaviour in various age stages.

Abstract

Collagen is the most abundant protein in the human body by dry weight and is responsible for the structure and mechanical properties of bones, muscles, tendons and connective tissue in all bodily compartments. Although the structure of the collagen protein family has been investigated in detail through the years, a more in-depth understanding of the mechanisms by which collagen adapts to mechanical force is necessary. We are interested in elucidating the molecular basis for the formation of mechanoradicals in collagen, molecular species that form when covalent bonds in the protein break due to the application of mechanical forces. Recent studies have shown that the radicals arising from mechanical stress in tendons are of magnitude such that collagen itself may have evolved to protect itself from radical damage, mainly through post-translational modifications in key sites in the form of DOPA residues that act as radical scavengers [1, 2].

Furthermore, we wish to correlate the stress-strain behaviour of tendons to their age profile and level of collagen intramolecular crosslinks, as ageing has a negative impact on collagen structural integrity. The precise effects of ageing on collagen properties are not yet properly understood, and the stiffening that tissues such as tendons undergo has not to date been conclusively tied to a precise set of collagen modifications. Ageing connective tissue naturally develops crosslinks in the form of AGEs (Advanced Glycation End products) which could be responsible for the mechanical loss of function we observe; probing the rupture of bonds across age groups could yield valuable insights into these modifications.

We investigate the mechanical properties of rat (Rattus norvegicus) achilles, flexor and tail tendons through the application of vectorial force up to the failure point; the tendons are stretched in an extensometer device that allows for controlled stress application. At the same time, tendons are measured by EPR spectroscopy in the native state and in the stressed state, and the cw EPR signal readout of the stressed tendons is compared to the base signal. EPR spectroscopy detects the presence of unpaired electrons in a sample, and allows us to quantify the content of radicals in tendons after stretching as only radicals will give a relevant signal. The amount of mechanoradicals can be correlated

with the stiffness and crosslink level of the tendons; we compare the stretching response in three age groups (young, middle and old) to assess differences in tissue properties due to ageing.

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The impact of multiscale cell mechanics on the motility of epithelial cell collectives

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Headline (max 400 characters including spaces)

The mechanical properties of cells are emerging as crucial regulators of cell collectives behavior and during breast cancer progression and dissemination. Here, we investigate how molecular organization of junctional molecules impacts force transmission during the collective migration of epithelial monolayers, by using genetically encoded FRET force sensors and super-resolution microscopy.

Abstract (max 2 pages for the whole document)

Mechanical forces are emerging as pivotal regulator of cellular and tissue homeostasis and are also involved in pathological process, including breast carcinoma. In this context, we consider the evolution of ductal adenocarcinoma in situ (DCIS), breast cancer precursor, in invasive ductal carcinoma, in which metastasis are present. During this transition, breast cancer cells modify their mechanical proprieties: at the molecular level it entails critical rearrangements of cell-cell adhesions and force transmission through them. Additionally, at cellular level, it leads to the acquisition of cell motility, characterized by the transition from a solid to a fluid state. [1] Moreover, we found that the transition from a solid to a fluid state also exist within cell-cell junctional complexes. This transition corresponds to a biphasic behavior of the junctional protein alpha-catenin, which links the membrane protein E-cadherin to the actin cytoskeleton: while single alpha-catenin molecules bind to F-actin but do not actively resist force application ("liquid" slipping behavior), the concerted action of multiple molecules display cooperative binding to F-actin in response to force application ("solid" catch-bond behavior) [2]. Here, we investigate how junctional tension and molecular organization of junctional proteins are controlled and impact on tissue fluidification. Specifically, we aim to study the nanoclustering of cadherin-catenin complexes which is required to build up tension on adhaerens junction, in normal and tumorigenic epithelia, using single molecule and super-resolution imaging techniques [3-4-5]. Furthermore, we investigate how tissue fluidification impacts on transmission of intra and intercellular force, introducing genetically encoded fluorescence resonance energy transfer (FRET)-based force sensors [6] into crucial proteins of cell-cell junctions and of the actin cytoskeleton. This study will shed light on how force transmission occurs between cells in a tissue and within cells and how forces impact on the motility of cell collectives.

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Nanopatterned DNA Tension Probes Reveal Size-Dependent B Cell Activation and Mechanical Threshold at the Nanoscale

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Headline

B cells use mechanical forces to discriminate affinities towards antigens presented on antigen-presenting cells (APCs) in molecular clusters. Here, we employ nuclease-resistant DNA force sensors nanopatterned on 80 nm to 600 nm clusters and study the sub 10 pN B cell receptor-generated mechanical events and nanocluster size-dependent activation of naïve B cells on these APC mimicking surfaces.

Abstract

B cells, responsible for creating high-affinity antibodies against pathogens, use a proposed mechanism of mechanical forces to discriminate B cell receptor affinities towards the antigens. By presenting antigens in immune-complex clusters on their surfaces, a specific subset of APCs, called follicular dendritic cells, play an essential role in the affinity maturation process and selection of B cells with the highest affinities.

In this study, we employ nuclease-resistant DNA force sensors, measuring naïve B cell receptor generated sub 10 pN tension within well-defined pre-clustered nanopatterned regions of 80 nm to 600 nm in diameter. We observe a size-dependent activation threshold for B cell activation at the nanoscale and a differential mechanical behavior on these surfaces.

The nanopatterned surfaces are prepared over large areas via a sparse colloidal lithography. These assembled nanoparticles on the surface are a low-cost and rapid method for generating nanoscale masks. To create the protein nanopatterns, the surface with the particles is covered with a thin sacrificial metallic layer. Removal of the nanoparticles reveals nanoapertures to the non-coated substrate, which is used to direct the assembly of covalently bound biospecific PEG-Biotin brushes in place of the nanoparticles. By etching the sacrificial metallic layer and covering the remaining surface with an orthogonal biospecific PEG-Azide brush, a fully PEGylated ultra-low fouling surface decorated with 2 orthogonal tags of Biotin and Azide for click chemistry is prepared. [1]

These fully transparent, topography- and metal-free surfaces, with the same nanostructure global coverage across all sizes, are used to pattern small molecule hapten antigen-conjugated dynamic tension probes surrounded by ICAM-1, an adhesion molecule abundantly found on APCs, responsible for regulating the activation threshold of B cells. [2]

Live cell imaging of primary naïve B cells isolated from transgenic B1−8i$^{+/+}J\kappa^{-/-}$ mice with B cell receptors specific for the presented hapten antigens reveals that the cells generate more mechanical

events at around 5 pN upon interaction with B cells on the smallest 80 nm antigen nanoclusters compared to the larger structures. (Figure 1)

As a marker of early B cell activation, we used phosphorylation of SYK, a kinase responsible for initiating multiple B cell signaling pathways. Comparing the phosphorylation levels of SYK across different antigen nanopatterned surfaces and non-clustered homogenously covered surfaces with similar antigen coverage reveals that the B cells reach a higher activation state on clusters of 200-300 nm in diameter, and the lowest activation on the non-clustered antigens. Furthermore, these phosphorylated SYKs were colocalized with the site of the antigen nanopatterns. (Figure 2a)

Immunostaining of the phosphatase CD45, responsible for maintaining the hemostasis of the B cell activation, reveals clear exclusion of CD45 from the nanopatterned antigen surfaces (Figure 2b). Preliminary investigations using super-resolution DNA-PAINT microscopy reveal a larger overlap between CD45 and B cell receptors on the 80 nm clusters compared to 200 nm clusters, suggesting a potential geometrical mechanism of inhibitory phosphatase exclusion on the larger antigen clusters leading to a higher activation state in B cells.

These findings reveal new knowledge about the mechanism of B cell activation and its potential application in biomedical surfaces with tunable immune responses.

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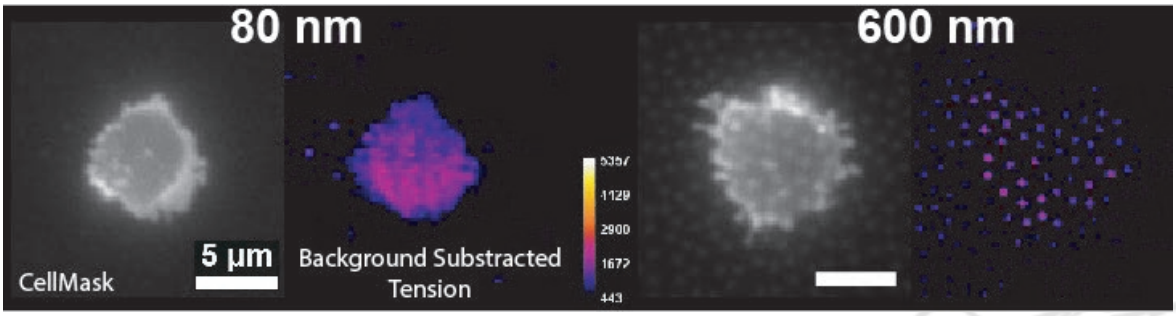

Figure 1. CellMask images and tension signal showing the cell-surface interaction regions and the tension fluorescence intensity as an indicator of the number of mechanical events. Naïve B cells generated mechanical events more abundantly on the 80 nm nanopatterns compared to the larger structures.

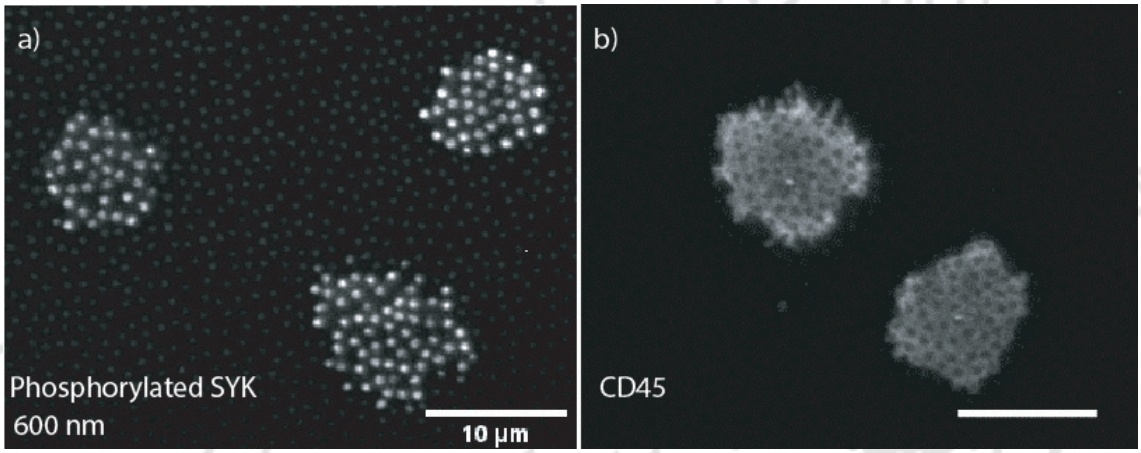

Figure 2. (a) TIRF immunostaining image of phosphorylated SYK on 600 nm antigen nanoclusters (b) exclusion of CD45 phosphatase from the antigen nanopattern sites.

The β-subunit dictates mechanoresponse for vitronectin-bound αV-class integrins

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Headline

Vitronectin (VN) is a matricellular protein that enables cell adhesion to various extracellular components like matrix proteins, cytokines, and chemokines. Here, we aim to understand the molecular and biophysical underpinnings of VN mechanosensing by fibroblasts that express VN-binding integrin receptors.

Abstract

αV-class integrins function across varied cellular paradigms ranging from fat and bone biogenesis, angiogenesis to tumour metastasis and age-related blindness. Given the ongoing research to develop αV-class targeting pharmacophores, drug-delivery strategies, and biomarker discovery, it becomes increasingly crucial to study and better elucidate how integrins respond to the varying magnitudes of extra- and intra-cellular forces during early steps of ligand recognition and binding. To this effect, we employ single cell force spectroscopy (SCFS) to quantify early stages (~120s) of adhesion strengthening, under varying mechanical load, and observe a non-monotonic response for αV-class integrins on vitronectin (VN) but not on fibronectin (FN). Interestingly, the non-monotonic response is distinct in a β-subunit-dependent manner. Moreover, this subunit dependence in the biophysical readout emerges from a differential involvement of cytoplasmic signalling molecules like - Arp2/3 complex, cSrc kinase and Phosphatidylinositol 3-kinase. Summarily, our work explores the effect of variable mechanical load on mechanotransduction during the early stages of vitronectin binding to the αV-class integrins at three levels – the ligand level, the receptor level and intracellular signalling level.

Studying the dynamics and interactions of the human PIEZO1 channel using molecular dynamics simulations.

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A computational approach to understanding PIEZO1 mechanosensation.

Mechanical forces are fundamental in a diverse range of biological contexts. The identification of PIEZO1 channels as sensors of mechanical force [1] presents them as key players in cellular force sensing. The membrane has been shown to play a key role in PIEZO1 force sensing. Using a computational approach to study PIEZO1 dynamics and interactions in model membranes offers new perspectives on PIEZO1 activity the molecular level, with a resolution inaccessible experimentally.

Abstract

PIEZO1 is a recently discovered mechanosensitive ion channel which has been identified as a bona fide force sensor [1]. PIEZO1 activation is fundamental for a plethora of physiological processes including erythrocyte volume homeostasis, innate immune response and vascular smooth muscle remodelling [2,3,4]. There is increasing evidence for the role of membrane lipids in PIEZO1 activation e.g., depletion of phosphoinositide lipids is associated with inhibition of PIEZO1 activation [5]. However, the mechanism by which these channels sense mechanical force and deliver proportionate responses to achieve the complex integration required for physiological force sensing remains poorly understood.

Here, we used a computational approach to model the full-length three-dimensional structure of the human PIEZO1 channel for which there is no solved experimental structure, based on the cryo-EM structure of mouse PIEZO1 [6]. We use multiscale molecular dynamics simulations at the coarse-grained and atomistic resolutions to simulate the dynamics and interactions of PIEZO1 channels with model endothelial membranes. Coarse-grained molecular dynamics simulations were employed to gain insight into how human PIEZO1 interacts with its local membrane environment, followed by conversion to atomistic resolution and simulation using all-atom molecular dynamics under applied tension.

These models reveal that human PIEZO1 alters the local membrane environment both by imposing a large membrane footprint on the local bilayer and via the specific interaction with lipids. We also gain insight into the conformational rearrangements which underpin human PIEZO1 activation in response to applied tension. Together, these data provide novel information about the dynamics, and interactions of human PIEZO1 in model membranes and provides insight into the molecular mechanisms by which mechanosensation is achieved.

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Figure 1. (a) figure caption; (b) figure caption (use Times New Roman 9)

High throughput deformability cytometry for the mechanical fingerprinting of adherent cells

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Headline (max 400 characters including spaces)

Osteosarcoma cell line, MG63, was utilised to study mechano-response to vibration with real-time deformability cytometry used to measure cellular mechanical properties (n>1000). Cells were vibrated at a frequency of 1 kHz and 30 nm amplitude for 72 hours. On average, vibrated cells were less deformable (stiffer) than control with the effect being mediated by cell-cell contact.

Abstract (max 2 pages for the whole document)

Osteoporosis is a condition that affects more than 500 million people worldwide – predominantly older demographics [1] and is defined as low bone mineral density. Since it is rooted in an imbalance of bone formation and resorption, current treatment options typically target osteoblast formation or osteoclast inhibition to re-establish homeostasis. New engineering approaches are emerging to provide a drug-free, avenue to osteoporosis treatment. Nanovibration for instance relies on mechanical stimulation, *i.e*. nano-amplitude vibration, to stimulate mesenchymal stem cell differentiation into osteoblasts. Results *in vitro* have already shown a positive correlation between 1 kHz, 30 nm amplitude vibration and osteogenic differentiation of mesenchymal stem cells [2], although the underlying mechanisms explaining this interaction remain unclear.

One key indicator to consider, is cytoskeleton rearrangement. Under stress, cells can undergo a positive feedback loop to create more actin stress fibres, and can even be considered as mechanosensors [3]. Cytoskeletal rearrangement has been seen in cells exposed to nanovibration, linking with a change towards an osteoblastic phenotype [4].

It has already been reported that adherent osteoblasts will be stiffer than their precursor cells when measured by atomic force microscopy (AFM) [5]. Real-Time Deformability Cytometry (RT-DC) is a different technique that classifies groups of cells according to their deformability under pre-determined stress. This technique allows us to measure cell stiffness under compression by forcing the cells through a microfluidic channel, while suspended in a fluid of known viscosity. This technique can be applied to measure thousands of cells within minutes, presenting population level mechanical data not achievable by AFM. In this study MG63, osteosarcoma cells were passed through a 30 μm RT-DC channel at flow rates up to 0.32 uL/s. Control cells were measured along with cells exposed to 72h nanovibration.

The results demonstrate that nanovibrated cells are significantly ($p<0.001$) less deformable than control cells after 72 hours of stimulation although the degree of cell-cell contact can introduce a confounding factor. Population level mechanical measurement highlights the mechanical heterogeneity within each sample, despite this, RT-DC is able to determine subtle changes between groups which may be missed by AFM or low throughput techniques. The data also highlights that control cells become more deformable with time, which correlates with cell density. We hypothesise that this is due to the inability to rearrange cytoskeletal filaments when under increasing confinement by surrounding cells. This new kind of cytometry can have a significant impact on research, even detecting mechanical changes of cells following detachment The potential for this methodology spans from diagnostics through to quality control for engineered cell therapies.

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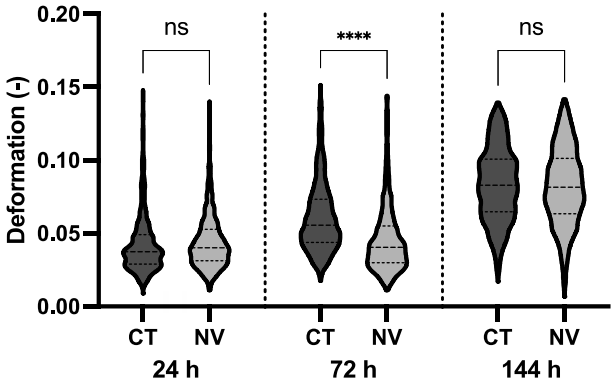

Figure I. Both at 24 and 144 h stimulation, there was no significant difference (p>0.05) between control and nano-vibrated groups. At 72 h, control exhibits significant difference (p<0.0001) between control and nano-vibrated group.

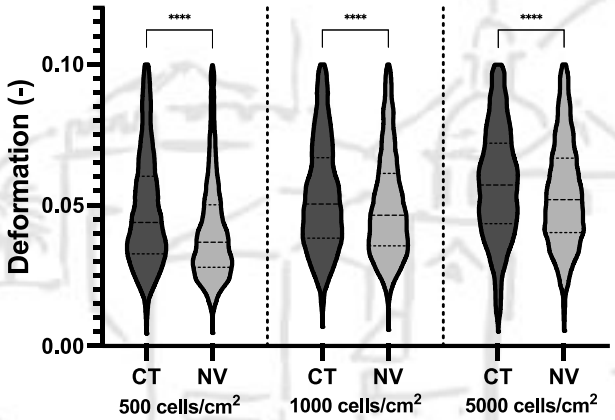

Figure II. Nano-vibrated groups were compared against their respective control groups. Deformability significantly increases (p<0.0001) as the seeding density also increases. When comparing CT versus NV, NV is significantly less deformable (p<0.0001) than CT in all three different seeding densities.

α5β1 integrins employ different adhesion strengthening modes to respond to the stiffness of fibronectin within seconds of adhesion initiation

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Headline

We here quantitatively investigate how fibroblasts employ fibronectin-binding α5β1 or αvβ3 integrins to sense and respond to stiffness of fibronectin during adhesion initiation. We show that both integrins distinct molecular mechanisms to initiate and strengthen adhesion within the first 360 s of contact and that α5β1 integrins sense and respond to fibronectin stiffness within seconds.

Abstract

In mammals, the dynamic adjustment of cell adhesion in response to the biochemical composition and the biophysical properties of the surrounding extracellular matrix (ECM) is essential for most physiological processes, including development, morphogenesis, homeostasis, and tissue regeneration [1]. ECM properties directly impact how cells interact with the ECM, which in turn triggers signaling cascades to regulate manifold cellular properties such as adhesion, mechanics, differentiation, and metabolism [2–4]. Hence, non-physiological ECM remodeling, which is swiftly sensed by cells, shows severe pathological consequences. In fact, the abnormal biochemical and biophysical properties of the ECM are the underlying mechanisms of many pertinent diseases, especially fibrotic and solid malignant tumors that assemble stiff and fibrotic ECMs [5, 6]. In recent years, heterodimeric integrins have emerged as sensors for the biomechanical properties of the environment [2]. Integrins and many integrin-related proteins are mechanosensitive, where force modulate their activity or expose cryptic binding sites. Hence, the intra- or extracellular forces that are applied to integrin-mediated adhesion sites have a major impact on the signaling output of the adhesion sites. However, how cells sense and respond to the stiffness of the environment the assembly of these adhesion sites remains elusive.

Here, we investigated by atomic force microscopy based single-cell force spectroscopy how actomyosin contractility-mediated mechanosensing regulates α5β1 or αvβ3 integrin-mediated adhesion initiation and strengthening of fibroblasts to fibronectin. While fibroblasts that adhere to fibronectin via α5β1 integrins switch from a slow adhesion strengthening to a fast adhesion strengthening mode within the first 360 s contact time on stiff fibronectin substrates (>5 kPa), they remain in the slow adhesion strengthening mode on soft fibronectin substrates (<5 kPa). This slow adhesion strengthening mode is independent of actomyosin contractility, paxillin, and focal adhesion kinase (FAK) signaling and hence depends mainly on the thermodynamics-driven binding of the integrin ligand and the maintenance of the ligand-bound state by talin and kindlin. The switch to the fast adhesion strengthening mode on stiff fibronectin substrates depends on myosinII-mediated contractility and a mechanosensitive signaling hub that includes the α5β1 integrin-fibronectin catch bond, paxillin, and FAK. Our results suggest that

the fast adhesion strengthening mode of α5β1 integrins involves force mediated integrin activation, recruitment, and clustering. Our results thus extend the function of paxillin and FAK in α5β1 integrin-mediated NA assembly to mechanosensitive signaling that activates and/or maintains the activity of α5β1 integrins Contrary, αvβ3 integrin-mediated adhesion initiation and strengthening does not respond to fibronectin stiffness and is myosinII-independent.

Our results extend a current model describing the talin unfolding-driven adhesion maturation during the first hours of attachment on fibronectin substrates stiffer than 5 kPa [7, 8], by showing that above a fibronectin substrate stiffness of 5 kPa α5β1 integrins employ a mechanosensitive signaling hub that activates and clusters additional integrins already at the onset of adhesion and thereby accelerates adhesion initiation and strengthening.

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Mechanical regulation of mevalonate pathway enzyme synthesis drives a malignant breast cancer cell phenotype

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Mechanical regulation of mevalonate pathway enzyme synthesis drives a malignant breast cancer cell phenotype

Abstract

Mechanical cues control cell fate in physiology and disease. In breast cancer, mechanotransduction from a stiffened extracellular matrix (ECM) drives proliferation and invasion. Here, quantitative mass spectrometry identified enrichment of ECM stiffness upregulated mevalonate pathway enzymes in breast cancer cells, suggesting sterol /isoprenoid metabolism reprogramming. Importantly, the rate-limiting mevalonate enzyme Hydroxymethylglutaryl-CoA Synthase (HMGCS1) protein was strongly upregulated in human breast cancer specimens and correlated with cross-linked ECM. ECM stiffness promoted HMGCS1 protein synthesis, not reflected at the mRNA level, and HMGCS1-RNAi blocked the stiffness-driven breast cancer proliferative and invasive phenotype. Mechanistically, we define mechanotransduction signaling through integrin beta1 and Rac1 to control HMGCS1 protein synthesis that drives the breast cancer malignant phenotype. Intriguingly, the Rac1-P29S cancer mutant promoted a malignant phenotype without stiff ECM in a mevalonate-dependent manner. In summary, we define a mechanotransduction pathway that regulates mevalonate pathway enzyme synthesis critical for breast cancer.

Glycerol-blended chitosan membranes with directional micro-grooves and reduced stiffness improve Schwann cell wound healing

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Headline

Our study aims to develop microstructured substrates for peripheral nerve regeneration, made of soft biodegradable materials able to be compliant with nerve cells mechanics.

Abstract

Regenerative medicine is continuously looking for new natural biocompatible and possibly biodegradable materials, but also mechanically compliant. Chitosan is emerging as a promising FDA-approved biopolymer for tissue engineering, however, its exploitation in regenerative devices is limited by its brittleness and stiffness. Scaffolds' material can be further improved, for example, by blending it with other materials and by tuning its superficial microstructure. We already demonstrated that nano/micro-gratings, ranging from ultra-small to micro topographies, are capable to direct neuronal and glial cell differentiation, polarization, and migration [1,2].

Here, we developed membranes made of chitosan and glycerol, by solvent casting, and micropatterned with directional geometries with different levels of axial symmetry. These membranes were characterized by light microscopy and atomic force microscopy (AFM), thermal, mechanical, and degradation assays, and then tested in vitro with glial Schwann cells (SCs) and co-cultures of SCs and neurons.

We show that soft microstructured glycerol-blended chitosan membranes can be produced through a simple and reproducible solvent casting procedure. The glycerol-blended chitosan (Gly-Chi) membranes are optimized in terms of mechanical properties, and present a physiological-grade Young's modulus (≈ 0.7 MPa) similar to the nerve tissue [3]. The superficial micropatterns are stable over time for several months, in simulated physiological conditions, in line with the nerves' regeneration timing (>3 months). The directional microtopographies still allow an optimal Schwann cell guidance in vitro, even if cells do not respond anymore to the substrate asymmetry. Importantly, Gly-Chi membranes improve significantly the collective migration performance of SCs, compared to pure chitosan membranes.

Finally, co-cultures of primary SCs and induced pluripotent stem cells (IPSCs)- derived nociceptors (sensory neurons) have been grown successfully over the Gly-Chi membranes. The microtopography is effective in directing not only the cytoskeletal organization of SCs but also the axonal elongation of the neurons.

Overall, we propose that a combination of a soft compliant biomaterial and topographical micropatterning can improve the integration of the scaffolds with Schwann and neuronal cells, which is a fundamental step in the peripheral nerve regeneration process.

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HYdrogel Dispensing with Robotic Automation For High Throughput Screening (HYDRA-HTS): a hydrogel-based cell culture platform for pharmaceutical Research & Development

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Headline

State-of-the-art drug development is routinely run on cost-effective stiff substrates. Softer substrates would better recapitulate the physiological microenvironment, but there is no scalable solution for their fabrication and use in high-throughput screening. To address this problem, we developed a scalable fabrication method to produce 2.5D flat gelatin hydrogels using a liquid handling robot.

Abstract

The development of new therapeutics has become increasingly long (> ten years), expensive (>1 B USD), and risky (<10% success rate). Up to 50% of potential therapeutics that succeed in the initial high-throughput pre-clinical assays ultimately fail in costly, ethically charged clinical trials. To overcome this issue, engineered cell culture systems - such as organoids and organs-on-chips - expose human cells to synthetic microenvironments that mimic native tissue and organ conditions. These complex systems often use hydrogels (water-swollen networks of polymers), such as gelatin hydrogels, to expose human cells to less stiff substrates than traditional culture ones, such as plastic and glass. However, generating engineered cell culture systems typically involves several manual fabrication steps, which have limited the platform and negatively impacted its adoption in the industry [1]–[3].

In this work, we demonstrated the fabrication of flat 2.5D fish gelatin hydrogels in a 96-well platform by Hydrogel Dispensing with Robotic Automation for High Throughput Screening (HYDRA). We reasoned that commercial high-throughput assays commonly use automated liquid handling robots to prepare plates with 96, 384, and 1536 plastic microwells for cell cultures. We selected gelatin from cold water fish skin (FG) as hydrogel protein to enable liquid handling of the hydrogel precursors. FG in phosphate-buffered saline (PBS) typically remains liquid at room temperature, facilitating dispensing by a liquid-handling robot. Mixing it with microbial transglutaminase (TG) results in a solution that can be cross-linked by incubation at 37 degrees Celsius. We identified hydrogel formulations with physiological stiffness compatible with the growth of human epidermal keratinocytes (HaCaT) cells. To produce flat 2.5D hydrogels compatible with most microscopy setups' working distance and depth of field, we developed a liquid handling protocol that produces thin films without touching well walls, avoiding the issues connected to meniscus formation [4]. We characterized our method by measuring the gels' thickness and flatness. Finally, we demonstrated how these hydrogel-based engineered cell culture systems can be implemented within a standard pipeline of a drug screening assay.

We conducted a rheological study to evaluate the hydrogel stiffnes for FG content (5, 10, 20% w/v), keeping the TG concentration constant at 2% w/v. By analyzing fish gelatin viscosity at room temperature, we found that zero shear rates increased ~2x when the fish gelatin concentration doubled: we measured from (0.67 ± 0.18) Pa·s to (2.11 ± 0.06) Pa·s. These findings suggest that fish gelatin solutions are suitable for dispensing at room temperature, even at higher solid concentrations, a feature not achievable with animal gelatin biomaterials [5]. The shear modulus G' of the hydrogels ranged from (500 ± 35) Pa at 5% FG and (3.1 ± 0.3) kPa at 20% FG (n = 3 for each FG concentration), comparable to the physiological range of soft tissues [6].

To fabricate gelatin hydrogel thin films in a scalable fashion, we used a robot (Integra Biosciences) that moves its 8-channel pipette along columns of the 96-well plate. We first dispense a small amount of liquid (12 µL) in the center of the well to avoid contacting the walls and consequent meniscus formation. Then, the tip approaches the bottom surface (≤ 100 µm tip-to-bottom distance) and aspirates the same liquid volume, leaving a thin gel layer behind. The whole fabrication process for a 96-well plate took 15 minutes, with a single dispensing&reaspiration step lasting less than 30 seconds.

We investigated the thickness and flatness of gels at 5%, 10%, and 20% w/v FG crosslinked with 2% w/v TG in dried and hydrated forms when seeded with cells. Specifically, we used an optical profilometer for the dried samples and confocal spinning disk imaging for the hydrated ones. Comparing pre- and post-rehydration gel heights revealed a concentration-dependent rise. Dried samples results in heights ranged from (1.8 ± 0.1) µm at 5% FG to (19.1 ± 1) µm at 20% FG (n=8 each). Post-swelling, heights increased from roughly (9 ± 1) µm to about (30 ± 5) µm across concentrations (n=5 each). The most notable change occurred at lower concentrations, consistent with known swelling ratios [7],[8].

To validate HYDRA fabricated gels for application in high throughput screening, we used HaCaT and evaluated the cell cycle inhibitory effect of Nocodazole and Paclitaxel drugs at different concentrations [9],[10]. Employing label-free digital holographic imaging for over 48 hours directly inside the incubator, we assessed the area covered by cells, i.e., *"% confluency"*, through time-lapse images. By comparing hydrogel and plastic groups, we determined drug IC50 values (Nocodazole: 11.01 ng/mL hydrogel vs. 11.32 ng/mL plastic; Paclitaxel: 2.53 ng/mL hydrogel vs. 2.47 ng/mL plastic). Significantly, we observed that the final confluence in the negative CTRL hydrogel group was ~2x compared to the plastic one ($P < 0.01$). At the lowest Nocodazole levels, cell growth halved on plastic compared to hydrogel, implying a stronger inhibition of cell proliferation on plastic ($P < 0.05$). This leads us to hypothesize that the surface type significantly affects drug screening outcomes.

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Phosphatidylinositol 4,5-bisphosphate modulates stability and dynamics of β-arrestin2–β_2 adrenergic receptor complexes

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Headline

Our atomic force microscopy-based single molecule force spectroscopy measurements and molecular dynamics simulations decipher how the allosteric modulator phosphatidylinositol 4,5-bisphosphate hand in hand with ligands and receptor phosphorylation tunes the stability and dynamics of the β-arrestin–β_2-adrenergic receptor complex.

Abstract

Even though eukaryotes express hundreds of different G protein-coupled receptors (GPCRs), a wide variety of signalling outcomes arises from only a handful of G proteins, a few GPCR kinases (GRKs) and two arrestins. Signals are further tweaked by allosteric modulators. In case of arrestins, ligands, receptor phosphorylation and membrane composition have been found to steer how arrestins engage with a GPCR and therefore modulate downstream signalling pathways. Specifically, phosphorylation of the β_2-adrenergic receptor (β_2AR) and the local membrane composition have been shown to cooperatively fine-tune GPCR-mediated signal transduction [1]. In addition, the recent discovery of phosphatidylinositol 4,5-bisphosphate (PIP_2) as a crucial factor in β-arrestins (βarr) binding to β_2AR further emphasizes the complexity of these interactions [2].

In this study, we employ atomic force microscopy (AFM)-based single molecule force spectroscopy (SMFS) to quantify how PIP_2, in conjunction with ligands and phosphorylation, modulates the thermodynamic and kinetic properties of βarr2–β_2AR interactions. We are able to delineate the contribution of the β_2AR C-terminal tail and core to the binding of βarr2 by probing their interaction forces. For this purpose, we study three configurations of βarr2–β_2AR complexes where i) β_2AR is phosphorylated and activated (tail-core complex); ii) β_2AR is phosphorylated and inactivated (tail complex); iii) β_2AR is not phosphorylated but activated (core complex). To shed light on the role of PIP_2, we explore these complexes in two distinct plasma membrane-mimicking environments, with and without PIP_2.

Our experimental data indicate that the presence of PIP_2 enhances the stability of the βarr2–β_2AR core complex. Furthermore, we observe that arrestin interacts with the tail of the receptor within milliseconds, while (additional) core engagement requires tens of milliseconds. Interestingly, in the absence of PIP_2, tail-core complexes are significantly more long-lived than the tail and core complexes. We complement our SMFS data with molecular dynamics simulations under comparable conditions. These simulations provide detailed insights into the role of the membrane and specific receptor entities, such as intracellular loops, in βarr2 binding. Notably, our simulations underscore the substantial impact of PIP_2 on the βarr2–β_2AR complexes probed with AFM.

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Complementary integration of AFM nanomechanics and micropipette micromanipulation for single-cell mechanobiology

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Headline (max 400 characters including spaces)

We present the combination of AFM-based nanomechanics and micropipette-based micromanipulation for 3D manipulations and subsequent nanomechanical measurements of single living cells. Experiments on living cells showed the significant changes in mechanics when cells were in different states.

Abstract (max 2 pages for the whole document)

The invention of atomic force microscopy (AFM) provides a powerful tool to measure the mechanical properties of single cells. In particular, providing a capability to manipulate the cells in a three-dimensional (3D) space allows additional applications of AFM measurements in cell biology to access more biological issues. In this work, we describe the complementary combination of AFM and micropipette manipulation, which maintains the full capabilities of AFM itself and at the same time allows 3D manipulations of single cells achieved using a micropipette, allowing controllable investigation of the mechanical properties of individual specific cells in different states (Fig. 1A) and offering novel possibilities for single-cell mechanobiology. Here, the micropipette with an inner diameter of 2–3 μm at its tip was used for single-cell manipulations (Fig. 1C). The system also had a small cell incubator which provided the environment (37 °C, 5% CO2) required for the growth of cells. The Petri dish with a PDMS micropillar substrate inside it (Fig. 1D) was placed in the small cell incubator, and micropipette micromanipulations were performed to precisely place single cells onto the micropillar substrate. The cells physically immobilized with the micropillars were then probed by AFM (Fig. 1B).

We used the established system to investigate the mechanical changes of single cancer cells in their different states (adherent state and suspended state) during tumor metastasis. During the process of tumor metastasis, cancer cells dramatically change their states, for example, cancer cells are adherent when they detach from the primary tumor and migrate in the surrounding tissue, whereas cancer cells are suspended when they circulate in the vascular system.[1,2] Fig. 2 shows the results of the micropipette-assisted AFM of MCF-7 cells. Fig. 2(A–D) show the selection and manipulation process of a living MCF-7 cell with the use of a micropipette. the CMFDA dye was used to monitor the viability of the cell during manipulations. Under the guidance of optical microscopy, the micropipette loaded with trypsin was controlled to move towards the targeted adherent MCF-7 cell (Fig. 2A). After the micropipette tip was close to the cell, the trypsin solution inside the micropipette was delivered to the cell by applying a positive pressure in the micropipette system (Fig. 2B). After a while, the cell detached from the substrate and was adsorbed onto the micropipette tip by applying a negative pressure (Fig. 2C). The cell was then transferred to the micropillar array by moving the micropipette (Fig. 2D). Micropillars with a height of 10 μm were used for immobilizing the detached MCF-7 cells. The AFM probe was then controlled to detect the mechanical properties of MCF-7 cells. Single suspended MCF-7 cells

immobilized with the micropillars could be steadily indented using the AFM probe without any displacements, and force curves and relaxation curves were acquired from the cell surface (Fig. 2G and H). For control, the mechanical properties of living MCF-7 cells in their adherent states were also measured (Fig. 2E and F). The statistical results clearly show that the Young's modulus (Fig. 2I), relaxation time (Fig. 2J) and viscosity (Fig. 2K) of MCF-7 cells in the suspended states were all significantly larger than those in the adherent states.

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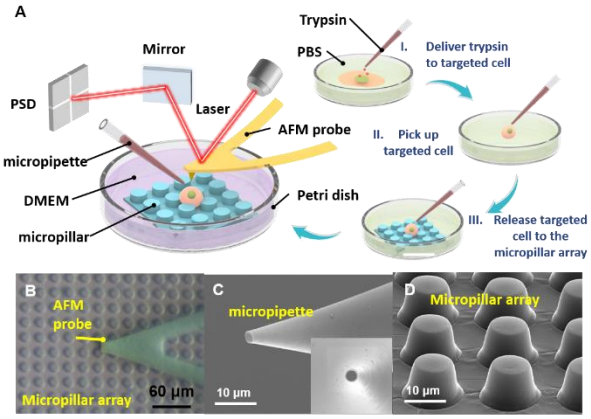

Figure 1. Experimental platform of micropipette-assisted AFM for single-cell 3D manipulations and nanomechanical measurements. (A) Schematic illustration of micropipette-assisted AFM. An example of a living adherent cell is shown. (B) Optical image of moving the AFM probe to detect a single cell which is immobilized in the micropillar array. (C) SEM image of a prepared micropipette. The inset shows the detailed aperture of the micropipette's tip. (D) SEM image of the PDMS micropillar array.

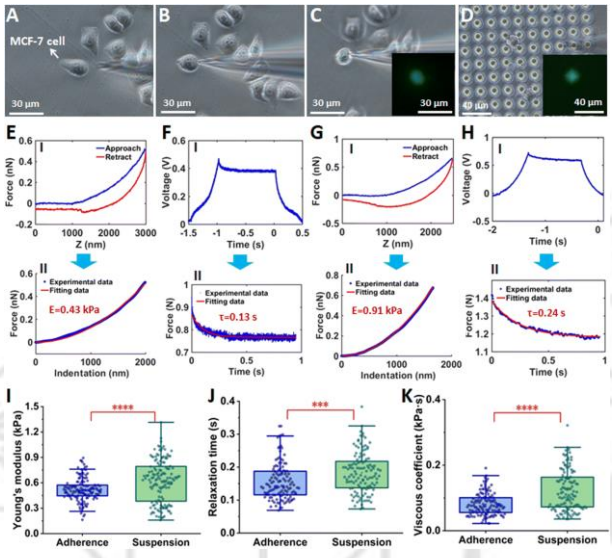

Figure 2. Micropipette-assisted AFM of single living MCF-7 cells. (A–D) Digesting and moving the targeted single MCF-7 cell onto the micropillar array by micropipette-based micromanipulations. The insets in (C and D) are the corresponding CMFDA-staining fluorescence images of the cell. (E–H) Typical force curves and relaxation curves obtained on MCF-7 cells in their adherent states (E and F) and suspended states (G and H) respectively. (I) Experimental curves and (II) corresponding theoretical fitting results. (I–K) Statistical results of the Young's modulus (I), relaxation time (J) and viscosity (K) of MCF-7 cells in their adherent states (N = 7) and suspended states (N = 6). Statistical significance was set at the following levels: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

High-Frequency Nanomechanostimulation: A New Nanoscopic Tool for Cellular Modulation

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Headline

Transmission of MHz-order nanoscale vibrational stimuli into cells is shown to be highly effective in modulating intracellular Ca^{2+} flux and hence influencing a variety of downstream cell fates, whilst maintaining very high levels in cellular viability.

Abstract

Beyond static compression, tension and shear, existing methods of mechanostimulation have, in addition, employed vibration at low frequencies—typically several Hz, not least because these are characteristic of physiological frequencies experienced by cells in the human body that is in motion. Where higher frequencies have been used, these have been at hundreds of Hz and no more than a few kHz, at most (Ref. [1] and references therein). These studies (see, for example, Ref. [2]), in particular, have suggested that there is no significant advantage in utilizing higher frequencies.

Contrary to such, and counterintuitive to the general perception that higher frequencies should not significantly induce appreciable mechanoresponses in cells, we show, for the first time, that exposing cells to nanometer-amplitude vibrations in the form of surface-reflected bulk waves (SRBW) [3] at 10 MHz order frequencies can indeed influence their transcriptomic behaviour in different ways, yielding cellular responses that can often be distinct to those reported at lower Hz and kHz order frequencies. Moreover, as cavitation—which typically results in poration and, at times, irreversible damage, of the cell membrane—is essentially non-existent at these frequencies, significantly higher retention of cell viabilities (>95%) are observed.

We show, in particular, that short bursts of the 10 MHz SRBW nanomechanical vibration (typically several minutes per day over a few days) were sufficient to induce either transient or permanent transcriptomic change due to its ability to effectively modulate Ca^{2+} flux into, out of, and within the cell, and hence influence downstream cellular fates. Transient increases in intracellular Ca^{2+} led to activation of the ESCRT-dependent signalling pathway, whereas longer-term changes to cellular mechanoresponses were associated with by Ca^{2+}-dependent activation of cAMP signalling cascades [4-6].

For instance, we observe that the SRBW nanomechanostimulation results in a significant increase in exosomal yield in mammalian cells via transient changes in ESCRT signalling [4], or triggers early and persistent osteogenic differentiation in human mesenchymal stem cells—without the need for exogenous osteogenic factors—through piezo channel activation and Rho-associated protein kinase signalling [5]. Additionally, we also demonstrate the possibility for modulating the barrier integrity in endothelial cells, with up to fourfold increases in barrier integrity that persist over several hours [6].

These riveting examples therefore not only foreshadow an array of exciting implications of the high frequency nanomechanostimulation in determining cell fate, but also opens up new possibilities in its use in fundamental mechanobiology to further our understanding and control of molecular and cellular systems.

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Characterizing the Inhibition Mechanisms of Gasdermin D Pore Formation by the Drug Disulfiram

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Headline

Characterizing the Inhibition Mechanisms of Gasdermin D Pore Formation by the Drug Disulfiram

Abstract

Gasdermin D (GSDMD) is a pore-forming protein identified as the executioner of pyroptosis (a programmed cell death) and serves as conduit to release inflammatory mediators, including inflammatory cytokines (interleukins IL-1β, IL-18, and IL-6) and alarmins. The key role of GSDMD in infection and inflammation has prompted the investigation of therapeutics targeting GSDMD. Previous work showed that disulfiram (DSF), an FDA-approved drug for treating chronic alcoholism, inhibits human GSDMD (hGSDMD) pore formation by covalently modifying Cys 191. We investigated by force-distance curve–based (FD-based) atomic force microscopy (AFM) and limited proteolysis coupled to mass spectrometry (LiP-MS) the effect of DSF on hGSDMD pore formation. We find that DSF inhibits most of the GSDMD-processing proteases that activate the auto-inhibited full-length hGSDMD to the lytic hGSDMD N-terminus, thus reducing the proteolytic activation of hGSDMD. Our data also show that DSF inhibits both wild-type and C191A mutant oligomerization and pore-formation, therefore, suggesting that DSF may target a different cysteine other than Cys 191 or act via additional alternative mechanisms. Indeed, we observe that DSF causes both hGSDMD wild-type and mutant C191A aggregation thereby reducing the protein available for pore formation. Besides, we find that DSF increases the mechanical stiffness of the supported lipid membrane, which appears to hinder hGSDMD membrane insertion. In summary, our data show that DSF inhibits hGSDMD pore formation through different mechanisms, namely - by inhibiting hGSDMD proteolytical activation, by covalently modifying hGSDMD cysteines, by causing hGSDMD aggregation, and by stiffening the membrane.

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Rheological properties of monodiperse alginate microgel beads with a rigid core prepared by microfluidic picoinjection

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Headline

We use the picoinjection technique to fabricate the cell model (soft shell with a rigid core) from alginate and polystyrene beads. We are using constriction channels to compare the rheology of the model cells with the rheology of living WM35 cells.

Abstract

Alginate hydrogels are used for numerous applications in biomedical fields such as drug delivery, tissue engineering, or cell culture [1]. This wide range of applications of alginates is due to their biocompatibility, biodegradability, non-toxicity and mild gelation conditions [2]. The simplest way to prepare alginate beads is through the process of ionic crosslinking. This occurs when a drop of alginate water solution interacts with a solution of divalent cations such as Ca^{2+}. However, the main drawback of this method is that the size range varies from hundreds to thousands of microns. To produce alginate beads of tens of micrometers in size, we use different microfluidic devices and a picoinjection technique [2]. The addition of polystyrene (PS) microspheres results in formation of alginate beads with a rigid core mimicking the cell body and nucleus, respectively.

We use these to compare rheological properties of the cell model and living melanoma WM35 cells under compression in constriction channels.

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Mechanical Interplay of the cytoskeleton with *in cellulo* crystal

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Headline (max 400 characters including spaces)

In cellulo protein crystals are intrinsically entangled with the cytoskeleton and has great impact onto the wholistic elastic behaviour of the cell. We quantitively characterize the intracellular crystals organization and their mechanical relation to the cytoskeleton with cTFM and propose a method using FluidFM in measuring the flexus modulus of crystals, revealing the overall cellular stress.

Abstract (max 2 pages for the whole document)

To maintain internal stability and protect cellular components from external fluctuations, ensuring optimal conditions for biochemical reactions, cells must function as closed systems restricted by the cell membrane and control their shapes throughout their life cycles. The interplay between cellular mechanical forces and interactions with the extracellular matrix (ECM) dictates cell shape, influencing critical processes such as cell migration, tissue homeostasis, and cellular differentiation. Therefore, the quantification of cellular forces plays a crucial role in determining cell states. One strategy involves assessing the dynamic changes in cytoskeletal elasticity. This assessment can be performed through techniques such as indentation using Atomic Force Microscopy (AFM), pulling via micropipette aspiration and more recently manipulating the cytoskeleton using optical tweezers [1]. However, these techniques focus on the local measurements of cytoskeleton and could not address the origin of the intracellular force as a whole. Alternatively, another approach centers on bidirectional cell-extracellular matrix (ECM) interactions. This can be achieved by measuring extracellular traction forces using traction force microscopy (TFM) [2] or studying cell-ECM adhesions with single cell force spectroscopy obtained by FluidFM (FFM) [3]. However, these methods rely on extracellular anchoring, thus they are limited by the presence focal adhesion and actomyosin clutch and provide only a partial representation of intracellular forces.

In this study, we employ an intracellular protein crystal setup, focusing on the mechanical quantification of the impact of such crystals on the cytoskeleton. For the first time, the cytoskeleton is experimentally considered as a three-dimensional elastic network. The phenomenon of protein crystallization within living cells has been frequently observed as a natural assembly process over the past decades. [4] Crystalline states of recombinant proteins are also reported to grow in animal cells, in which metazoan specific kinase PAK4 crystalizes within the cytosol in the presence of its potent endogenous inhibitor Inka1. (Inka-PAK4) [5] The Inka-PAK4 aggregates and spontaneously nucleates resulting in the formation of long rod-shaped crystals. It offers engineering flexibility through different designed plasmids during the transfection procedure. [6] [7] Inka-PAK4 in this work is treated as a form of intracellular stress, with rigid ends protruding internally onto the cell cytoskeleton.

The interactions of Inka-PAK4 crystals with the cytoskeleton are quantified with variosu techniques, elucidating the wholistic elastic behavior of the actin cortex. Firstly, the growth and the contact of the intracellular crystal with the cytoskeleton are recorded with fluorescent imaging time lapses. We then compared the states of the crystals with intact and depolymerized actin cortex. The intracellular forces

during crystal growth are also interpreted with confocal reference free imaging (cTFM) [8]. Interestingly, we observe a “soft” feature of the Inka-PAK4 crystal manifesting as bending and fracture. This phenomenon is further investigated through live actin imaging upon depolymerization treatment via the FFM technique [3]. In order to quantify the modulus of the “soft” crystal, we propose a convenient method that measures the flexus modulus of the protein crystal rod with FFM.

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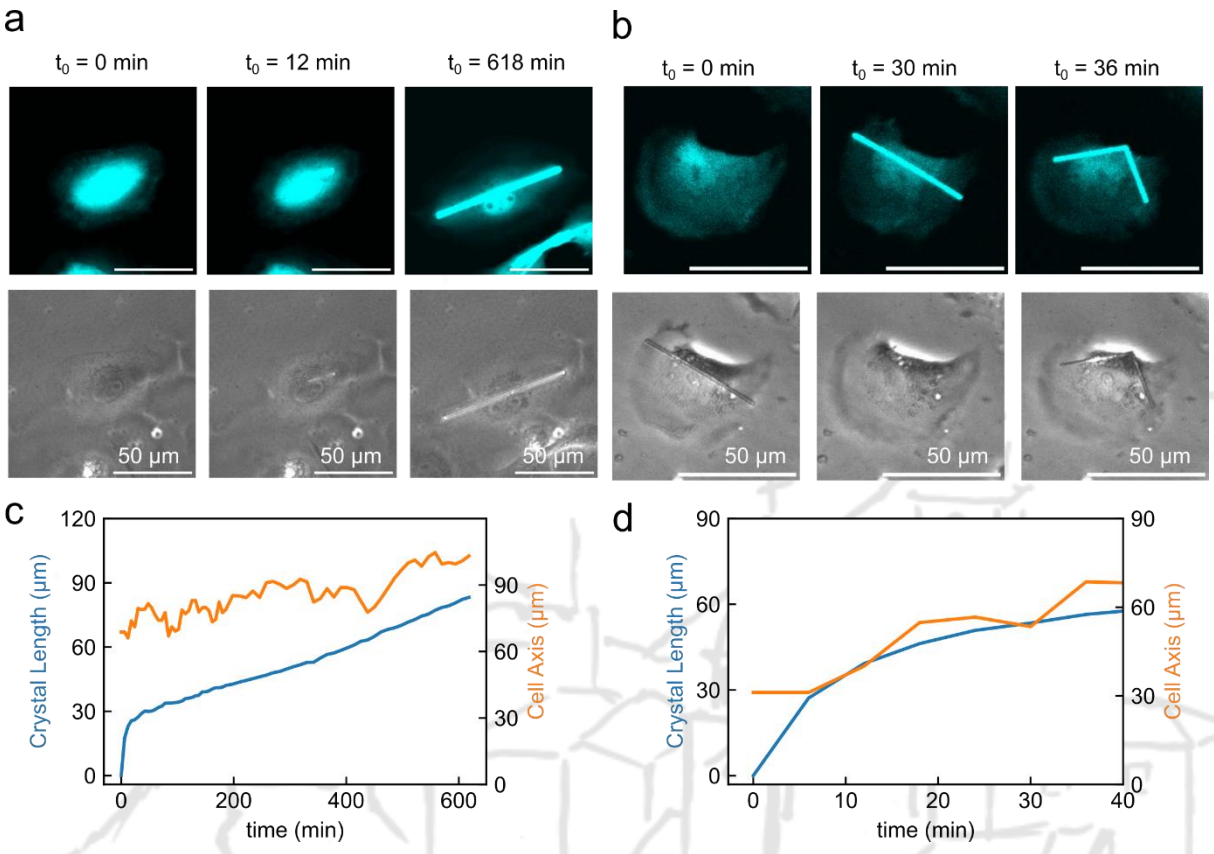

Figure 1. GFP-tagged Inka-PAK accumulates and forms crystals under two conditions: (a) where the crystal length is shorter than the cell axis along the crystal, and (b) where the crystal contacts the cytoskeleton during growth. This process is visualized in the GFP channel (first row) and bright-field (BF, second row) images. The corresponding changes in Inka-PAK4 crystal length and cell axis are depicted over time in (c) and (d) for condition (a) and (b) respectively. In d), crystal and cell axis length after 36 min is the sum of the two resulting crystals.