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Role of the Nucleus as a Sensor of Cell Environment Topography

Karine Anselme, Nayana Tusamda Wakhloo, Pablo Rougerie, and Laurent Pieuchot*

The proper integration of biophysical cues from the cell vicinity is crucial for cells to maintain homeostasis, cooperate with other cells within the tissues, and properly fulfill their biological function. It is therefore crucial to fully understand how cells integrate these extracellular signals for tissue engineering and regenerative medicine. Topography has emerged as a prominent component of the cellular microenvironment that has pleiotropic effects on cell behavior. This progress report focuses on the recent advances in the understanding of the topography sensing mechanism with a special emphasis on the role of the nucleus. Here, recent techniques developed for monitoring the nuclear mechanics are reviewed and the impact of various topographies and their consequences on nuclear organization, gene regulation, and stem cell fate is summarized. The role of the cell nucleus as a sensor of cell-scale topography is further discussed.

1. Introduction

Cells are strongly influenced by their environment, which can be described in terms of their chemical, topographical, and mechanical properties. Every cell function, including adhesion, migration, proliferation, or differentiation, is affected, ultimately affecting processes ranging from morphogenesis to cancer progression to tissue repair. For instance, the influence of mechanical stimuli on the cell fate has been extensively studied over the past two decades, with seminal papers demonstrating the potential of matrix elasticity[1] and topography[2–4] in controlling the stem cell phenotype.

The topography of the environment emerged as a relevant parameter in the control of cell behavior.[5] Specific topographical patterns can affect cell morphology, migration, mechanics, or differentiation. Topography readings work as a type of mechanosensing, where the topographical patterns affect cellular and nuclear mechanics by deforming the cell and forcing the rearrangement of its cytoskeleton.[6] The capacity of the mechanical forces or the cell-scale microenvironment to modulate cytoskeletal organization and cell contractility and induce downstream signaling events is defined as mechanotransduction and raises the question of the existence of mechanosensors structures or mechanisms that translate mechanical input into biochemical or behavioral output within the cells. While mechanotransduction through force-sensitive membrane channels, focal adhesions or cell–cell junction proteins at the plasma membrane and within the cytoplasm have been well studied,[7–9] the role of the cell nucleus as a mechanosensor has only been recently confirmed.[10–14]

Seminal work performed 20 years ago shows that the forces applied to integrins are directly transmitted to the nucleus, resulting in its elongation and deformation.[15] Actin and intermediate filaments mediate the force transfer and microtubule stabilization of the nucleus.[16] This observation sustains the cellular tensegrity model proposed by Ingber and co-workers, where tensional forces are borne by cytoskeletal microfilaments and intermediate filaments. They are counterbalanced by interconnected structural elements that resist compression, most notably internal microtubule struts and extracellular matrix (ECM) adhesions.[17,18] Because the nucleus is the largest and stiffest organelle, the tensegrity model suggests that it would eventually be affected by any morphological deformation or mechanical stress to the cell.[10] It was later confirmed that the nucleus, its nuclear membrane and chromatin are directly wired to the cytoskeleton through the linker of the nucleo-cytoskeleton complex (LINC), the nuclear pore complex, and the underlying lamina.[19] Recent papers demonstrate the existence of intrinsic nuclear mechanotransduction pathways, showing that the structural and chemical organization of the nucleus is able to sense and respond to mechanical stress by itself and finally may act as a mechanosensitive structure.[20,21]

The architecture and the mechanics of the cell nuclei are often altered in diseases. For more than a century, variations in the nuclear morphology (size and shape) of cancer cells have been considered a gold standard for the diagnosis of cancer, even if they are now associated with molecular analyses.[22] More recently, molecular analyses have shown that the composition of nuclear matrix proteins (NMPs) is altered in tumor cells and has been proposed as a biomarker for cancer lesions.[23] Among the most abundant NMPs are the lamins forming the lamina layer at the interface between the chromatin and the inner nuclear membrane. In most healthy cells, the peripheral layer

Dr. K. Anselme, N. T. Wakhloo, Dr. L. Pieuchot
University of Haute-Alsace
University of Strasbourg
CNRS UMR7361, IS2M, 68057 Mulhouse, France
E-mail: karine.anselme@uha.fr
Dr. P. Rougerie
Institute of Biomedical Sciences
Federal University of Rio de Janeiro
Rio de Janeiro, RJ 21941-902 Brazil

DOI: 10.1002/adhm.201701154

of chromatin, called heterochromatin, is transcriptionally silent. In contrast, alterations of the nuclear shape in cancer cells are often associated with altered organization of the chromatin and thus with altered patterns of gene expression, possibly contributing to transformation.[22] Moreover, an increasing number of reports have correlated lamin levels with tumor aggressiveness. This can be related to an increased deformability of the nuclei with decreased levels of lamin that could contribute to cancer progression in 3D environments. Indeed, nuclear stiffness can be a rate-limiting factor for the progression of cells through narrow constrictions smaller than the size of the nucleus.[24] Recently, a close correlation was found between tissue elasticity and lamin A/C levels in the nucleoskeleton, which implies a role for the lamina as a "mechanostat" that mirrors tissue stiffness.[25,26] Interestingly, a strong decrease of lamin A/C, SUN1, SUN2, and nesprin2 levels was recently reported in human breast tumors and breast cancer cell lines compared to normal tissues.[27] However, the causes and consequences of such lamin changes in cancer remain understudied.[26]

Aging is one of the highest risk factors for cancer but is also associated with a large variety of pathologies related to dysfunctioning mechanotransduction processes, affecting various tissues such as skeletal muscle, bone, cartilage, cardiovascular, skin, etc.[28] Interestingly, accelerating aging syndromes, such as Hutchison-Gilford progeria, is associated with mutations of the lamin-A gene that increase nuclear stiffness.[29] Like other laminopathies, it has been hypothesized that the mutations of the lamin-A gene induce a loss of structuring function, increasing the sensitivity of the nucleus to mechanical strain and resulting in increased cell death in tissues, such as muscle, that are exposed to repetitive mechanical stress. Other hypotheses have been proposed where mutations in lamin would perturb gene regulation, disturb stem cell differentiation, or disrupt nucleo-cytoskeletal coupling, with all these hypotheses not being mutually exclusive.[24]

From all these considerations, it appears that the role of the cell nucleus in mechanotransduction is becoming more and more prominent in the normal and pathological environment and in cells or tissue/material interactions. As the following discussion can only highlight a small portion of the research in this field, we deliberately restrict focus to the current knowledge on the role of the nucleus in the response of pathological and healthy cells to mechanical stress induced by the modification of their environmental topography. This question is particularly important for improving materials used in various healthcare fields, such as the development of materials-based diagnostic devices, tissue engineering, or regenerative medicine strategies.

2. Nuclear Structures and Nuclear Cytoskeletal Interactions

A cell consists of a hydrophobic lipid bilayer membrane called a plasma membrane, which serves as a boundary between its external and internal milieu. The attachment of the plasma membrane to its extracellular environment is brought about by signaling molecules embedded inside the bilayer membrane. Among them, integrins have been the most extensively studied over the past few decades. These proteins form clusters with the anchoring complexes on the inside of the cell, which are called focal adhesions. The focal adhesion complex consists of various groups of proteins that relay signals from the extracellular matrix (ECM) to the inside of the cell and finally to the nucleus. This intracellular relay of information is called indirect mechanotransduction and occurs in response to mechanical stimuli from the ECM in the form of chemical molecules. During this process, the cell "senses" its environment and brings about adaptive changes to maintain homeostasis with its surroundings. A direct mechanotransduction has also been proposed, where the major cytoskeletal components, that is, the microfilaments (actin), microtubules (tubulin), and intermediate filaments (vimentin, keratin, etc.) connect the plasma membrane to the nucleus. Among them, actin is the most abundant and most vital element, acting together with its motor protein myosin to remodel the shape of the cell.

Most of the recent studies in this regard shed light on the fact that of the various cytoskeletal elements, microtubules and intermediate filaments provide prime forces for cell and nucleus flexibility.[30–34] Several distinct types of actin stress fibers, such as ventral and dorsal stress fibers, transverse actin arcs and perinuclear actin cap fibers, have been described.[35–41] Ventral stress fibers lie on the basal side of the cell body with both ends anchored to focal adhesions.[41] Dorsal fibers and transverse actin arcs emanate from the protruding cell front and form an interconnected contractile actin network at the cell edge. They are associated with focal adhesions at one end only.[41] Perinuclear stress fibers form a so-called "actin cap" composed of several contractile actin bundles that rise from the leading edge to the dorsal side of the cell, covering the top of the nucleus and descending to the basal side at the cell rear, resulting in a "dome-like" shape.[35,36,41] This actin cap is physically connected to specific focal adhesions called actin cap associated focal adhesions (ACAFAs)[42] but is also connected to the nuclear membrane through the LINC and nuclear lamina.[35,36,39,43] Tension in the actin cap has been shown to exert an active compression on the nucleus, forming deep nuclear indentations.[38,44] Thus, the actin cap tension is able to influence nuclear movement and positioning during cell

Karine Anselme has been the CNRS Research Director at the Institute of Materials Science of Mulhouse since 2003, where she leads the Biointerface group. She received her Ph.D. in cell biology from the University of Lyon in 1989. She further established her own group at the Littoral Côte d'Opale University. Her research interests aim to develop multidisciplinary research on biointerfaces and biomaterials with a focus on topography for applications in tissue engineering and regenerative medicine.

migration[37,45] and internal chromatin architecture.[34,44,46] Although this structure has been extensively studied, it has so far only been observed in vitro.

The contribution of vimentin to the mechanical behavior of living cells was recognized in the late 1990s.[15,47,48] It is generally considered that vimentin forms passive scaffolding, which mechanically transmits the pulling forces generated by actomyosin but also protects the cells from fast and large mechanically induced deformation.[33,49] However, actin microfilaments and microtubules can compensate for its action when it is deficient.[50] Vimentin also plays a role in actin-driven nucleus positioning during migration.[51,52] Very recently, the distribution of vimentin, actin, and microtubules was analyzed in cells cultured on micropatterns with various shapes. Shabbir et al. observed a similar distribution of vimentin on both square- and circle-spread cells, with the vimentin network surrounding the nucleus and radiating outward. Moreover, vimentin filaments remained mostly perinuclear in patterns with sharp corners and edges, avoiding the sharp corners where actin microfilaments were prominent. Overall, these observations suggest that vimentin filaments are global cytoskeletal elements, since they do not respond to local geometry cues.[53] On these surfaces, microtubules were mainly concentrated at the microtubule-organization center (MTOC) but were homogenously distributed throughout the cytoplasm.[50] Their suppression did not modify cell morphology, confirming that microtubules have a less prominent role in resistance to deformation compared to actin and vimentin, as previously observed in force-induced cellular reorientation under stretching.[54,55] However, microtubules do partake in cell mechanics and deformation. Because of their rigid structure, they have sufficient stiffness to impart mechanical integrity on the cytoskeleton and are particularly resistant to compressive loads.[56–58] They form a rigid structural network to which actin and myosin attach to create tensile forces during cell migration.[57] They play a major role in intracellular trafficking and signaling, focal adhesion dynamics, protrusion formation, and cell migration.[59,60] Moreover, they have been shown to stabilize the nucleus against lateral compression and deformation[15,30] and to control nuclear rotation and positioning.[61–63]

The nucleus itself is compartmentalized by a double-lipid bilayer membrane that separates it from the cytoplasm (**Figure 1**). The two nuclear membranes connect themselves at nuclear pore complexes where molecular trafficking between the cyto-nucleoplasm occurs. At the interface of the nuclear envelope are LINC proteins that help integrate and bridge the nucleus with the cytoskeleton.[64] Since their discovery, there has been extensive research to understand the role of individual LINC proteins in the coupling of the nucleo-cytoskeleton and their role in direct mechanotransduction.[15]

Various mechanical signals that are initiated in the cytoplasm are transmitted to the nucleus through distinct proteins.[19]

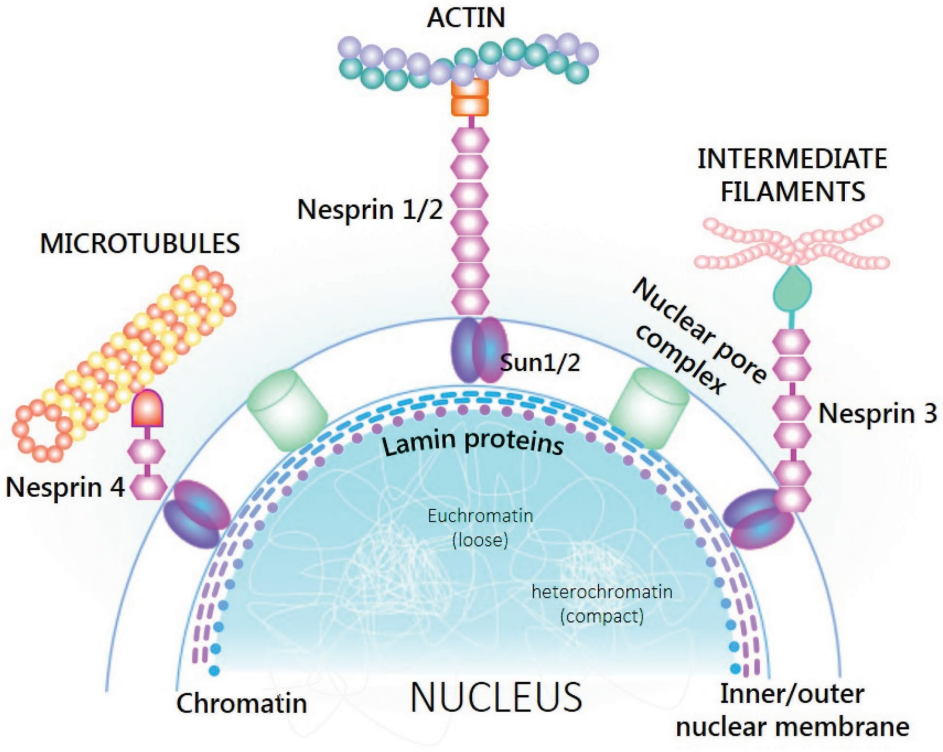

Figure 1. Nucleus organization and connection to the cytoskeletal filaments through LINC. The nucleus and its chromatin are mechanically connected to the plasma membrane and the outer microenvironment. At the nucleus, the mechanically junction is made by the LINC complex. The LINC complex is composed of nesprin proteins on the outer side of the nucleus envelope and the SUN proteins between the two membrane leaflets. The nesprins binds the cytoskeleton components whereas the SUN proteins are linked to the nucleoskeleton protein Lamins, whose distribution and dynamics can affect the distribution of heterochromatin and euchromatin. Thus, the LINC complex is one crucial element of the mechanical continuum that connects the chromatin to the exterior microenvironment.

The nesprins (nuclear envelope spectrin repeat proteins called nesprin 1, 2, 3, and 4) are the first layer of proteins creating a bridge between the cytoskeleton and the nucleus. On one hand, nesprins 1/2 are connected to the actin filaments, while nesprin 3 binds to intermediate filaments and nesprin 4 to microtubules (Figure 1). On the other hand, these nesprins are connected to the nucleus via the cytoplasmic domain of the SUN proteins (SUN1, SUN2, and SUN3 forming homodimers). SUN proteins pass through the outer and inner nuclear membrane. They also possess a nuclear domain, which is coupled to lamins. Lamins (type A and B) are type V intermediate filaments that form the nuclear matrix. Their roles in various diseases, mechanosignaling, and genome regulation have been widely appreciated.[65,66] The lamin network is connected to chromatin.[67–69] The focal adhesion and LINC complex therefore create a mechanical continuum between the cell microenvironment and the chromatin.

A growing body of evidence has highlighted the role of the LINC complex and lamin proteins in nuclear shape changes, chromatin remodeling, and gene expression in response to mechanical stimuli.[67,70,71] Although there has been extensive research in unraveling the mechanisms of direct signal transduction, the role of the LINC complex and its components in 3D environments still remains poorly understood. Recent data suggest that the repositioning of the chromatin territories, mainly the transcriptionally active euchromatin and inactive heterochromatin, has been shown to be an important process for gene regulation.[72]

Our understanding of the biophysical characteristics of the nucleus, how they are impacted by force transmission from outside the cell and how this is related to the physiology of the cell is not fully understood. Hence, there is a necessity to establish innovative procedures to precisely access the mechanical properties of the nucleus in physiological conditions and perceive the significance of nuclear mechanosensing with respect to the cell environment.

3. Methods for Applying Force/Deformation to the Nucleus

Underlying topography modifies the cellular mechanics by creating a constraint on the shape that the cell can adopt or on the localization and density of its anchoring points onto the substrate. To understand how the nucleus senses and reacts to topography-induced mechanical constraints, describing its mechanical properties has become imperative. Several methods have been developed to address this cause, some of which are purely physical, meaning that they aim at obtaining quantitative measurements of nucleus stress, strain behavior, and rheology in various contexts (**Figure 2**). The nucleus can be probed within cells or after isolation from cells as a separate organelle. Other methods consist of subjecting the cells to confinement using controlled topography.

3.1. Physical Methods

Analyzing isolated nuclei presents an advantage in allowing the measurement of nuclear mechanics in the absence of the surrounding cytoskeleton. Several methods have been used, such as direct aspiration by micropipette of cells treated with cytoskeleton inhibitors[73] or by selective disruption of the cell plasma membrane by mechanical[74] or chemical methods.[75] On the other hand, it can be interesting to measure intact cells in which the normal cytoskeletal and nucleoskeletal architectures, as well as the chemical composition of the cytoplasm and nucleoplasm, are preserved.

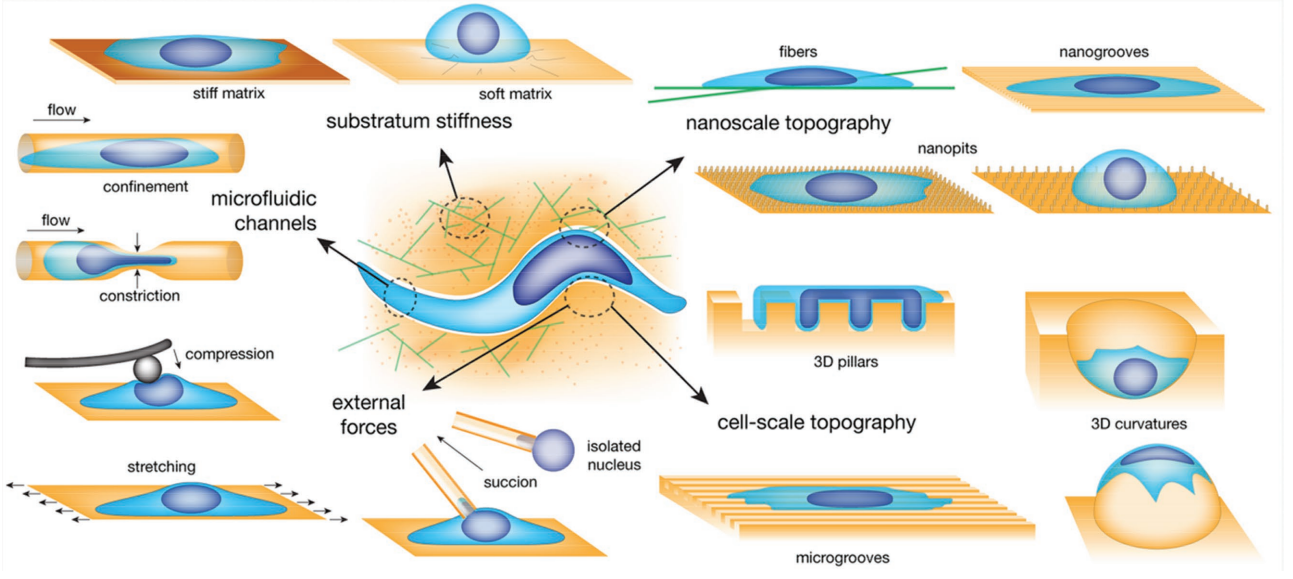

Figure 2. Schematic of different approaches developed to characterize mechanical and topographical stimuli undergone by cells in their natural environment. Cell mechanics or topography sensing can be studied using various biophysical techniques. Cell reaction to substrate stiffness is assessed by controlling the specific modulus of artificial matrices. The mechanoresponse and the rheology of cells can be quantified through the application of mechanical forces. The cell sensibility to topography can be analyzed at several scales (nanoscale or cell-scale) using topographically structured surfaces. Finally, microfluidics addresses the specific question of 3D cell confinement.

3.1.1. Micropipettes

Micropipettes have been the most common tool for measuring the mechanical properties of the isolated nucleus. Indeed, it is easy to directly visualize the deformation due to aspiration inside the micropipette by microscopy. From this deformation and knowing the applied pressure, the effective stress versus strain behavior of the nucleus can be determined. In the isolated nuclei, Guilak et al. determined that they behave as viscoelastic solid materials similar to the cytoplasm but are 3–4 times stiffer and nearly twice as viscous as the cytoplasm.[73] Micropipette aspiration also showed that the elastic nuclear lamina can serve as a "shock absorber" in cells, thereby protecting the nuclear interior.[74] Later, thanks to micropipette aspiration of whole cells, it was demonstrated that the nuclei of naive stem cells are physically plastic and sixfold more pliable than the nuclei of differentiated cells. Moreover, human adult stem cells possess an intermediate stiffness and deform irreversibly.[76] More recently, micropipettes were used to displace and deform the nuclei of adherent, viable 3T3 fibroblasts. To accomplish this, the application of a pulling force of a few micronewtons, far greater than the typical intracellular motor forces, was needed. The original shape and position was restored quickly within a few seconds after forced removal, demonstrating an elastic response. Using drugs that inhibit different elements of the cytoskeleton, it was demonstrated that F-actin or microtubules were not essential for this response, as opposed to vimentin, lamin A/C, and SUN molecules.[77]

3.1.2. Atomic Force Microscopy (AFM) and Microindentation

Nuclear mechanical properties can also be probed by AFM. AFM can either be performed on isolated nuclei or on intact cells. In the case of AFM, the probe is a cantilever with a microscopic pyramidal, conical, or spherical tip. In the case of microindentation, the cell or the nucleus is probed using a cantilever with an attached spherical microbead of several micrometers in diameter using a flexible plate.[78] The great advantage of the AFM technique is that it provides direct high-resolution measurements of the applied forces and the induced deformation in isolated nuclei. In contrast, the advantage of the microindentation compared to AFM is that it applies a uniform compressive force to the entire nucleus.[78] Thanks to microindentation using glass microplates, Caille et al. determined that the elastic modulus of the nucleus is on the order of 5000 N m^{-2} in the cell and 8000 N m^{-2} for the isolated nuclei, while the elastic modulus of the cytoplasm is 500 N m^{-2}.[75] Similarly, the elastic modulus of the isolated nuclei fixed by poly-L-lysine to a glass slide was determined at various levels of swelling using a borosilicate sphere-tipped cantilever.[74] Mechanical characterization of the cell nucleus by AFM showed that the isolated nuclei from fibroblast-like cells exhibited significantly lower Young's moduli than intact nuclei in situ.[79] Recently, AFM was used to apply forces on the nuclei of living 3T3 fibroblasts, revealing their anisotropic deformation. This nuclear anisotropy is regulated by the cytoskeleton in intact cells with actin and microtubules that are resistant to orthonormal strains. However, the nuclear anisotropy is intrinsic and is also observed in isolated nuclei.[80]

3.1.3. Substrate Strain Experiments

Similarly, strain applied on cell substrates was used to demonstrate the distinct role of actin and microtubules in governing the anisotropic deformation of the cell nuclei.[30] In these experiments, uniform uniaxial and biaxial strain was applied to living cells cultured on transparent elastic substrates coated with the extracellular matrix protein to promote cell adhesion and proliferation, while monitoring-induced nuclear deformations on a microscope. Compared to the two previously described techniques, one major interest of this approach is that it allows simultaneous probing of many cells at once and the further collection of cell lysates and mRNA to study mechanotransduction events in cells exposed to mechanical strain.[78,81] This approach has notably been used by Lammerding et al. to measure nuclear mechanics in mouse embryo fibroblasts lacking specific nuclear envelope proteins, such as emerin or lamins.[82,83]

3.1.4. Magnetic Tweezers

For all the techniques described above, external forces are applied on the cell and its nucleus. However, it can sometimes be desirable to measure local mechanical (rheological) properties of the interior of the nucleus. This can be achieved by active or passive microrheology. In passive microrheology, sub-micrometer particles are injected into the nucleus, and their Brownian motion is analyzed to determine the stiffness of the nuclear material. In this way, it was calculated that the mean nucleoplasmic viscosity and elasticity of 3T3 fibroblasts is ≈500 Poise and 200 dyn cm^{-2}, respectively.[84] In active microrheology, magnetic microparticles are microinjected into the nucleus and subjected to precisely controlled forces thanks to magnetic or optic tweezers.[78] Recently, magnetic tweezer experiments on isolated nuclei showed that they are able to respond to force by adjusting their stiffness to resist the applied tension. This response does not involve chromatin or nuclear actin but requires intact nuclear lamina and emerin.[20]

3.2. Nuclear Deformation Thanks to Topography

In their natural environment, cells are submitted to diverse boundary conditions due to the neighboring cells or extracellular matrix, which limits their spreading, migration, and proliferation. This confinement imposes reorganization of their internal architecture, particularly the focal adhesions and cytoskeleton that impacts the nucleus morphology (Figure 2). Because the cell environment is complex, it has been necessary to develop a well-controlled artificial cell environment. Initially, 2D cell-adhesive patterns with controlled size and morphology were developed thanks to microcontact printing (μCP) techniques.[85] Later, a third dimension was added to the patterns using photolithography techniques for developing cell-scale 2.5D or 3D environments based on microgrooves, micropillars, or microchannels.[86] Some selected results of the nuclear positioning, confinement, or deformation obtained using these artificial cell-scale microenvironments are reviewed below.

3.2.1. 2D Patterns

Thery et al. developed extensive μCP-based 2D cell-adhesive patterns to control cell morphology and, more specifically, cell division.[87–89] By changing the shape of the micropatterns, they manipulated cell adhesion and the associated location of the polarity cues, finally guiding the orientation of the mitotic spindle.[89] The μCP technique, which is now commercially available, is in demand in biomedical research and has led to groundbreaking studies in nuclear remodeling of cells that are confined in the 2D environment.[90]

Recently, interesting results were obtained using these tools on the spatial coordination between the cell and nuclear shape in endothelial cells.[91] Circular, square, and various rectangular adhesive micropatterns mimicking elongated bipolar shapes observed in vivo were developed. Analysis of the orientation and deformation of the nucleus on these patterns demonstrated that this process was regulated by lateral compressive forces driven by tension in central actomyosin fibers. Combining this approach with other micromanipulation tools revealed that tension in lateral stress fibers is gradually generated by anisotropic force contraction dipoles as the cell elongates and is strongly dependent on the cell spreading area.[91] Similarly, the combination of the analysis of adhesion and de-adhesion of endothelial cells on 2D patterns with different stiffness allowed the probing of nuclear mechanics without applying external forces and without any alteration of the nucleo-cytoskeletal interactions such as those observed in micropipette aspiration experiments on isolated nuclei.[92] Interestingly, differences in terms of regulation of the nuclear shapes on various rectangular 2D patterns were observed between fibroblasts and a model cancerous cell.[93] Finally, it was observed that these large-scale cell shape changes induce a drastic condensation of chromatin and dramatically affect cell proliferation.[91] These chromatin modifications were shown to be at the origin of gene expression and cell fate modifications by several groups using 2D adhesive patterns (developed more extensively in the last chapter of this review).[94–96]

3.2.2. 2.5D Pillars

In 2009, we showed for the first time that several lineages of cancer cells have the ability to significantly distort their nucleus (cross-shaped nuclei) when the cells are grown on adhesive polymer surfaces patterned with square micropillars.[97] Nuclear deformation spontaneously occurs upon cell adhesion and displays a trend similar to that of metastatic cancer cells migrating in tissues. This deformability is an intrinsic property of these cells, and it was possible to discriminate three human osteosarcoma cell lines by the ratio of deformation of their nuclei as a function of the spacing between the pillars. These deformations are directly related to the composition and organization of the cytoskeleton.[98] Even more striking, the cells continue to divide and differentiate despite the deformation of their nucleus. In contrast, healthy cells do not deform, while immortalized cells from the same tissue origin generally do.[99,100] We recently extended these results to transformed cells of epithelial origin (colon carcinoma). We showed that the nucleus of the colon cancer cells characterized by a stronger polarization than osteosarcoma cells were also capable of deforming on surfaces with pillars, although the cells were not able to pass through the porous membranes used in conventional invasion tests (unpublished data). The high capacity of the nucleus of adhesive cancer cells to deform between micropillars may thus reveal specific cellular mechanical properties. Consequently, these micropillared surfaces may be the basis for new assessment tools of invasive ability of cancer cells based on their ability to deform their nuclei. With this aim, Hasirci and co-workers developed a high-content image analysis algorithm, the micropillar-induced nuclear deformation (MIND) platform, to quantify changes in nuclear morphology at the single-cell level. Therefore, nuclear deformation can be used as a physical parameter to evaluate cancer cell transformation and to compare them to noncancerous cells originating from the same tissue type. This platform could be exploited for the systematic study of mechanical characteristics of large cell populations in complement with previously described conventional physical methods.[101,102] The comparison of nuclear deformation of various healthy and cancer cells on micropillared surfaces showed that nuclear stress-induced inhibition of cell proliferation was stronger for healthy cells compared to cancer cells. The authors did relate this behavior to a greater expression of Lamin A/C and thicker nuclear lamina in healthy cells, which increase the elastic modulus of their nuclei and their mechanical resistance to nuclear deformation.[103]

Vertical nanopillars or nanowires have also been developed to support long-term cell culture and have emerged as a unique platform for probing live cells. Adherent cells engulf nanopillars, and the tight membrane–nanopillar attachment affords highly sensitive nanopillar-based electrical and optical sensors.[104] Nanowires also induce a significant nuclear deformation that could alter the gene expression as a result of the increase in proximity between the chromosomes and nuclear membrane.[104] Recently, Hanson et al. proposed using these vertical nanopillars for probing the nuclear mechanics in adherent cells.[105] For micropillars, they showed that the curvature of nuclear deformation can be controlled by varying the geometry of the nanopillar arrays as determined by nuclear stiffness. Additionally, this study confirmed the main role of actin microfibers in deformation compared to microtubules. More surprisingly, they observed an increased deformation when inhibiting intermediate filaments, demonstrating that these could balance the contractile force provided by the actomyosin network and pull the nucleus toward the cell membrane.[105]

3.2.3. 3D Microenvironments and Cell Migration

During cell locomotion, the displacement of the nucleus, along with the rest of the cell body, is achieved in different ways depending on the cell migration mode. The mesenchymal migration characterizes strongly adherent cells. It is associated with a spindle-like shape, strong adhesions, and actin-rich protrusions such as lamellipodia or filopodia. In mesenchymal migration, integrin-dependent actomyosin contraction, possibly in combination with vimentin intermediate filaments[51] and microtubule-associated motors, pulls the nucleus forward during 2D- and 3D-migration, respectively.[62,106] In contrast,

amoeboid migration is characterized by cycles of expansion and contraction of the cell body mediated by the cortically localized actin and myosin, which allow cells to squeeze through gaps in the extracellular matrix.[107,108] In amoeboid cells, actomyosin contraction at the back of the cells, which specifically involves nonmuscle myosin IIB but not myosin IIA, applies a squeezing force, propelling the nucleus forward.[109–111] This categorization is not rigid, and cells can switch between these migration modes depending on the intrinsic (protrusions, contractility) and extrinsic parameters (environment porosity, rigidity).[112–114]

To better approximate the in vivo context, many studies have investigated cell migration through porous and/or fibrillary complex 3D microenvironments containing multiple extracellular matrix components and other cell populations. Different approaches have been explored to reach this objective, such as culturing cells inside 3D artificial ECM, hydrogels, porous scaffolds, or inside microchannels in microfluidic systems. In all models, the objective is to recapitulate the physical characteristics of the ECM and study their impact, in particular on the cell migration capacity. Depending on the degree of ECM alignment, porosity, and stiffness, cell movement can be guided, hindered, or prevented.

Migration through an artificial ECM lattice requires drastic morphological deformations of the cell body and depends on the nucleus stiffness and pore sizes.[115,116] To reduce the complexity of cell–matrix interactions in ECM-based migration assays, artificial 3D systems mimicking native cell surroundings have been developed to analyze the behavior of single cells in defined microenvironments. For example, direct laser writing was used to fabricate 3D cell culture scaffolds with adjustable pore sizes (2–10 µm) on a microporous carrier membrane for applying diffusible chemical gradients.[117] Thanks to these scaffolds, it was demonstrated that nuclear stiffness constitutes a major obstacle to matrix invasion for fibroblasts. However, chemotaxis signals are not essential, while the contrary was observed for epithelial cells.[117] The development of microfluidic systems has allowed a precise assessment of the behavior of cells migrating within channels and encountering narrow constrictions. As cells migrate through the channel bottleneck, distinct phases of nuclear translocation can be described, such as buckling of the nuclear lamina and severe intranuclear strain. Thanks to a specific device designed to quantify the dynamics of intranuclear deformations, it was shown that lamin A/C-deficient fibroblastic cells exhibit increased nuclear deformations compared to wild-type cells.[118] The comparison of in vitro nuclear deformation of cancer cells in this device and inside a collagen matrix with MMP inhibitors showed rupture in the nuclear envelope during deformation. In vivo, similar nuclear envelope ruptures were also observed, particularly in individually migrating cells, while these ruptures were less prevalent in cells moving as multicellular strands.[116] Nuclear envelope rupture was shown to increase exponentially with decreasing pore size.[119–121] Nuclear envelope rupture occurred predominantly at the leading edge of the nucleus. It was preceded by the formation of nuclear membrane protrusions called "blebs" that formed when the nuclear membrane detached from the nuclear lamina and bulged into the cytoplasm. The capacity of nuclear deformation is increased in cancerous cells[27,29,112,116,122] or cells deficient/lacking in lamins, such as in laminopathies[24,29,82,123] or the immune system.[124–127]

The case of the immune system is particularly interesting for the study of nuclear deformation during migration. Many cell types of the immune system travel great distances throughout the body and encounter various microenvironments. For example, T lymphocytes circulate in the blood stream and regularly cross capillary endothelia to enter lymph nodes. In the absence of antigen-driven activation, they exit the lymph nodes through the lymphatic vessels and recirculate into the bloodstream. However, upon encountering pathogens, they proliferate and subsequently exit the lymph nodes, circulating through the blood and entering the inflamed peripheral tissues (skin, lung, etc.). Therefore, T cells are able to migrate through 3D matrices of different physicochemical and topographical organizations (lymph nodes vs infected tissues), over 2D surfaces (blood vessel walls), and through highly constricted spaces (in between endothelial cells to exit the blood flow). It has been shown that the topography of the environment can orient the trajectory of migrating T lymphocytes within both lymph nodes[128] and tumors.[129] Other leukocyte types, such as neutrophils or dendritic cells, also display this striking migration adaptability. It appears that the nucleus of dendritic cells mechanically hinders migration through narrow topographical patterns due to its size and rigidity. A seminal paper showed that the migration of dendritic cells through an artificial collagen lattice or in vivo extracellular matrix heavily relies on myosin II contractility to squeeze the nucleus through narrow matrix pores.[109] Inhibition of myosin II activity results in highly stretched cells with a front end that still advances through the matrix and a rear end containing the nucleus that is stuck behind. Other than the rear myosin II contractility, it was recently shown that perinuclear actin could also help squeeze the nucleus through tight spaces.[127] When dendritic cells migrating through artificial channels encounter a sudden bottleneck, the actin nucleator Arp2/3 induces the polymerization of the actin network around the nucleus. The perinuclear actin then forces the nucleus to deform through constriction. Interestingly, it was shown in this case that myosin II was dispensable for nuclear deformation. The relative importance of myosin contractility and perinuclear actin might depend on the cell type, nuclear rheology or topography, and both are not necessarily or mutually exclusive. The general conclusion remains that the nucleus acts as a mechanical limiting factor for dendritic cell progression through narrow spaces. This limitation is surpassed by localized actin or myosin activity to squeeze the nucleus. The observation that nuclei depleted in lamin A/C (hence, softer) can stretch through constrictions without the help of Arp2/3-mediated perinuclear actin confirms the role of the nucleus as a hindrance. Interestingly, Thiam and co-workers observed that a coating of actin forms around artificial beads swallowed by dendritic cells when they are trying to pass through the constriction. This suggests that the cytoskeleton-based squeezing of the nucleus is a general cell mechanism arising due to spatial confinement and not a nucleus-originated system. Therefore, it is possible that, in this context, the nucleus acts as a passive sensor of topography that is dependent on the cytoskeleton activity. Further work extended the idea of nuclear hindrance to other types of leukocytes and highlighted the importance of myosin II contraction. The migration of resting or activated T lymphocytes through the endothelia necessitates

myosin II contraction as well.[130,131] In activated T cells, myosin II is accumulated at the rear of the cell. Its depletion leads to highly deformed cells with the front end extending across endothelia, while the rear and nucleus remain stuck on the other side.[131] Interestingly, the mechanical hindrance caused by the nucleus can work as a selection system for permissive topography, as myosin II-inactivated resting T cells are able to cross large pores and loose endothelia but not narrow pores or tightly joined endothelia.[130]

Few questions in the above context remain unanswered. First, leukocytes and macrophages have been shown to switch their modes of migration.[132] How does the nucleus, and in particular its ability to be squeezed through matrix pores, participate in this adoption of one mode of migration over another? Second, the extreme deformation of the nuclei during leukocyte migration during normal circulation or through artificial channels can lead to nuclear envelope rupture.[127] How the process of nuclear envelope rupture, deformation, and repair affects the gene expression in the leukocyte is still poorly understood. Topographically induced regulation of gene expression could thus integrate with the other already known mechanisms controlling the immune system.

Thus, available data highlight the hindrance caused by the nucleus during migration through a confined environment. While this is not the case in topography sensing per se, it still suggests that the morphological and rheological properties of the nucleus create some kind of selectivity. Some cells will be able to penetrate microenvironments with a given topography, while others may not. In addition, reported cases of nuclear rupture while passing through narrow spaces may have a repercussion on cell homeostasis and gene expression, thereby linking nuclear mechanical properties, cell locomotion, and topography reading.

4. Nuclear Architecture and Gene Regulation

Taken together, all the previously described cell migration experiments and the strong nucleus deformation through confining spaces could challenge the integrity of the nuclear envelope and DNA content. In the absence of efficient repair, this deformation could promote DNA damage, aneuploidy, and genomic rearrangements.[120]

4.1. Role of Chromatin Position Relative to Nuclear Membrane

The possibility of regulating gene expression by changing nuclear morphology was already demonstrated 15 years ago using 2D confining adhesive micropatterns.[70] At the same time, it was shown that the repositioning of genes to nuclear lamina induced a repression of their transcription. This confirmed the observations that the electron-dense heterochromatin enriched at the nuclear envelope in higher eukaryotic cells is associated either with gene silencing[133] or having a direct repressive effect on certain genes.[134] Therefore, the position of genes inside the nucleus could participate in the regulation of their expression. Altering the nuclear shape by plating cells on microfabricated substrates or by forcing their migration through constrictions will increase the ratio of genes localized at the nuclear envelop and logically modify their expression (**Figure 3**). However, nuclear deformation alone is not sufficient for influencing gene expression. Specific signaling intermediates, such as Erk signaling, might also accompany nuclear deformation for modulating gene expression.[95]

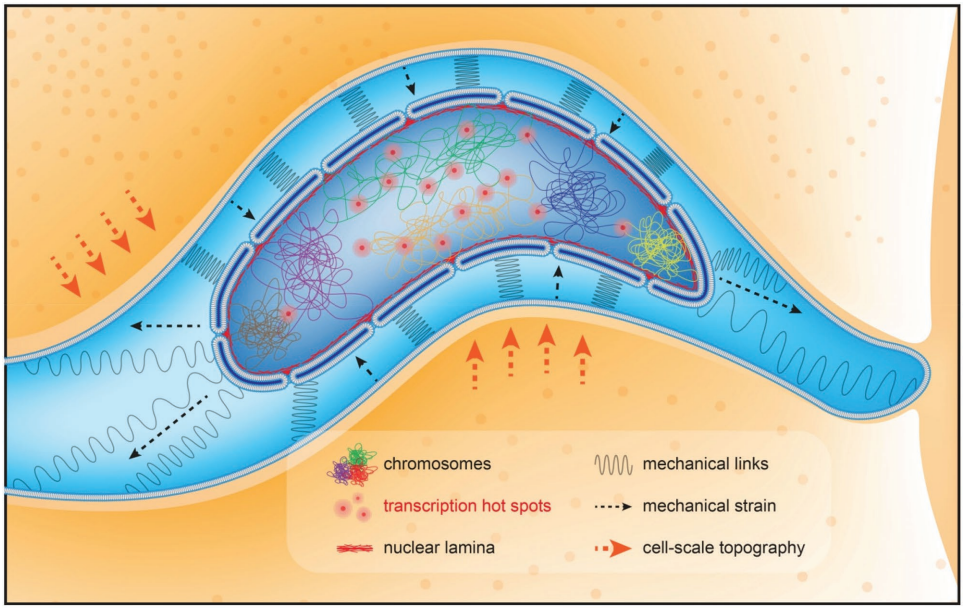

Figure 3. Nuclear architecture and gene regulation. The chromatin is heterogeneous: The transcription is more active where the chromatin is loose. The cytoskeleton and adaptor complexes (such as the LINC complex, see Figure 1) ensure that the nucleus is constantly mechanically connected to the rest of the cells. Any deformation through microenvironment topography or mechanical stress affects the shape and the structure of the nucleus and modifies the distribution of the chromosomes. Thus, sectors of the genetic material can reach the transcription hotspot and others can be excluded.

4.2. Role of Nuclear Lamina Damage

As seen before, confined migration can result in nuclear envelope rupture,[135,136] as this was observed spontaneously in cultured cells from patients with laminopathies[137] or cancer.[138] More recently, it was demonstrated that the spontaneous nuclear envelope ruptures in cancer cells occur in the weakest membrane areas because of an increase in nuclear pressure induced by the actin cytoskeleton.[139] Intriguingly, cells remain viable even after nuclear envelope rupture while the exchange of nuclear and cytoplasmic content, including organelles, transcription factors, or nuclear fragments, occurs.[32,136] The consequences of nuclear envelope rupture and how it could contribute to genomic instability have recently been studied.[32,136] HeLa cells subjected to high compression exhibited abundant nuclear envelope ruptures and displayed increased expression of genes involved in DNA damage response.[135]

The incidence of nuclear envelope rupture dramatically increased with cancer cell confinement, and the depletion of nuclear lamins requiring DNA damage repair was also shown. It was recently shown by several groups that members of ESCRT (endosomal sorting complexes required for transport) were involved in DNA damage repair.[120,126] However, several important questions still persist, such as the precise mechanisms leading to DNA damage, how cells detect and recruit ESCRT proteins to the site of rupture and the long-term consequences of rupture.[32,136] Nonetheless, the long-term effect of nuclear envelope rupture on genomic integrity has been explored very recently in cancer cells and on mesenchymal stem cells (MSCs) after migration through a Transwell filter with 3 µm pores.[140] The authors confirmed that confined migration causes multiple DNA breaks, segregates repair proteins that were mislocalized into the cytoplasm away from DNA delaying its repair, and favoring damage accumulation. Additionally, confined migration also causes many gains and losses in large segments of chromosomes in diverse cellular clones. Interestingly, stable clones with stable genomic differences arose from the constricted migration. In particular, one clone exhibited a highly elongated MSC-like shape associated with an ≈2-fold upregulation of GATA4, which is a transcription factor that drives endothelial-to-mesenchymal transition (EMT), demonstrating for the first time that heritable changes can be provoked by confined migration.[140]

4.3. Role of Mechanical Forces on Chromatin

Mechanical forces applied on cells were shown to change nuclear rheology.[141–143] Fluid shear stress of 2 Pa (20 dyn cm^{-2}) applied to endothelial cells induced an elongation and a vertical compression on their nuclei. The stiffness of the stressed nuclei measured with micropipettes revealed a higher elastic modulus compared to control nuclei, suggesting a modulation of chromatin density.[141] Further studies confirmed subnuclear movements of the chromatin and nuclear bodies of various cell types under shear stress and compression forces.[142] The application of forces on the plasma membrane of single cells using magnetic tweezers revealed the propagation of forces directly to the nucleus, resulting in changes to chromatin organization followed by nuclear deformation.[143] Similarly, stretching applied on isolated nuclei using LINC complex coated beads triggered remodeling of the nuclear lamina and nucleus stiffening. Interestingly, emerin was involved in this nuclear mechanical response to tension.[20]

Therefore, a novel concept is emerging where a direct mechanical stress can propagate from the plasma membrane to the inside of the cell and alter the nuclear architecture and chromatin state.[144] However, little is known about how these perturbations regulate chromatin remodeling and gene expression. Recently, Tajik et al. exerted shear stress using 3D magnetic tweezers to the apical surface of living CHO cells expressing a GFP-tagged transgene, enabling its live visualization. A correlation was found between the transgene transcription rate and the chromatin stretching, both depending on the magnitude and direction of the loading. By disrupting actomyosin contraction and knocking down lamins, emerin, and LINC complex proteins, they demonstrated that local stress applied on integrin can be propagated through the cytoskeleton and LINC to the chromatin, thus upregulating transcription.[145] The operative molecular machinery through which mechanical stimuli induce chromatin remodeling in MSCs was explored recently.[146] Dynamic tensile loading was shown to induce marked chromatin condensation mediated by acto-myosin action and ATP signaling. The increase in the chromatin condensation stiffened the nucleus, which became resistant to deformation. Interestingly, the increased chromatin condensation persisted for different amounts of time depending on the duration and amplitude of the original stimulation. Moreover, the condensed state of the chromatin was sustained for longer periods of time after cessation of loading, establishing a "mechanical memory" in the MSC nuclei encoded in the chromatin architecture.[146]

5. Control of Stem Cell Fate Thanks to Topography

Stem cells are classified into embryonic stem cells (ESCs) and adult stem cells such as MSCs. ESCs are pluripotent stem cells derived from the inner mass of blastocysts with the potential to maintain an undifferentiated state. They are able to regenerate into all cell types of the three germ layers (ectoderm, mesoderm, and endoderm).[147] Recently, the technique for reprogramming adult cells into pluripotent stem cells, referred to as induced pluripotent stem cells (iPSCs), has been established. These iPSCs exhibit properties similar to ESCs.[148] Adult stem cells, on the other hand, are derived from adult tissues and are considered multipotent since they can generate progeny of multiple distinct cell types, such as osteoblasts, chondrocytes, adipocytes, or myoblasts, for MSCs.[149] In some conditions, they can be transdifferentiated into neurons using neuronal induction medium and/or cell contact with neurons.[150] This is the reason why MSCs have a great potential in tissue engineering for regenerative medicine, in addition to their immunomodulatory properties and low immunogenicity.[151,152]

Besides the biochemical cues classically used for regulating in vitro stem cell differentiation, such as growth factors or cytokines, the physical cues of the cellular environment can also control stem cell commitment. However, a better understanding of the relative influence of the biochemical and

biophysical cues during the differentiation process is still needed. Moreover, it is interesting to compare the biophysical properties of the pluripotent and adult stem cells along the time course of their differentiation.

5.1. Nuclear Mechanics of Stem Cells

The rheology of hESCs and hiPSCs was compared using the particle tracking method, revealing significant differences between them. The cytoplasm of the hiPSCs was essentially viscous, while the parental fibroblasts were both viscous and elastic. The cytoplasm of hESCs was viscous with some subcellular elastic regions.[153] The same technique was applied on MSCs along the time course of their osteogenic differentiation, which showed an increase in both the elastic and viscous moduli but with a conversion of the intracellular viscoelasticity from viscous-like to elastic-like.[154] In parallel, the nuclei of the differentiating hMSCs become stiffer and more resistant to deformation because of increased heterochromatin content and lamin A/C relocalization to the nuclear periphery.[155] These observations are coherent with a transcriptional activity shift for pluripotent stem cells from the whole genome toward tissue-specific genes with a lineage commitment. Indeed, this shift needs a chromatin reorganization that is favored by the viscosity of the stem cell nucleoplasm. During differentiation, elastic heterochromatin foci grow, inducing a progressive transition to a "frozen" chromatin conformation and a global stiffening of the cell, which is mediated by chromatin and cytoskeleton.[156] In contrast, human ESCs display very low prestress compatibility with the high plasticity of their nucleus as measured by micropipette aspiration.[76] By looking more specifically at the actin cytoskeleton organization in hESCs, Wirtz's group observed that actin stress fibers of the actin cap are completely absent from undifferentiated hESCs, while conventional actin stress fibers at the basal surface of the cells are present along the hESC differentiation time course. Similarly, the perinuclear actin cap is absent from human-induced pluripotent stem cells (hiPSCs). Moreover, the formation of the actin cap follows the expression and proper localization of the nuclear lamin A/C and LINC complexes at the nuclear envelope and strongly correlates with differentiation.[36]

Studies of nuclear stiffening during the differentiation of epidermal multipotent stem cells showed that emerin, nonmuscle myosin IIA, and F-actin control chromatin compaction and gene silencing. This resulted in the accumulation of H3K27me3 (a histone mark indicative of compacted chromatin) in heterochromatin and the attenuation of transcription. Interestingly, this mechanism is mediated by the availability of the free nuclear G-actin, which is an important transcriptional cofactor that could further adjust transcription and subsequent progenitor cell lineage commitment.[157] YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif) are transcriptional coactivators that are classically part of the Hippo pathway, which regulates organ growth and development but also plays a role in mechanotransduction. Nuclear localization of YAP/TAZ in MSCs increases on stiff matrix and requires RhoGTPase activity and actomyosin contractility.[158] A high nuclear:cytoplasmic ratio of YAP/TAZ correlates with increased osteogenic potential in MSCs in culture.[159]

5.2. Mechanobiology of Stem Cells

As previously noted, the mechanoresponsive behavior of adult stem cells, and in particular MSCs, has been well explored.[1] The investigation on the mechanosensitive and responsive behavior of pluripotent stem cells is more recent.[160–162] Evans et al. demonstrated that the spreading and growth of mESCs on polydimethylsiloxane (PDMS) substrates of varying stiffness (0.041–2.7 MPa) increased with substrate stiffness, while cell attachment was unaffected. The expression of genes involved in early mesendoderm differentiation was upregulated on stiff substrates compared to soft ones.[163] Additionally, it was shown that mESCs can remain pluripotent for 15 passages, even in the absence of leukemia inhibitory factor (LIF), when they are cultured on soft polyacrylamide substrates (≈0.6 kPa).[164] In contrast, hESCs cultured on rigid micropost arrays functionalized with vitronectin displayed a significantly higher percentage of undifferentiated cells compared to the ones on soft micropost arrays.[165] This was related to the higher expression of E-cadherin-mediated cell–cell contacts, favoring mechanical-mediated cytoskeleton tension and the maintenance of pluripotency.[166] While the mechanoresponsive behavior of mESCs and hESCs appears to be contradictory, it is still unclear if these differing results reflect differences in the cell lines or different experimental conditions. Since, as noted previously, the effects of substrate stiffness are just beginning to be explored in the context of hESCs, further studies are required in the future to elucidate this point.[162]

These studies have focused on the effect of the mechanical properties of the local cell microenvironment on stem cells. As extracellular topography also regulates cell stemness, Lu et al. have tested the morphology, proliferation, and stemness of mESCs on microfabricated polyacrylamide hydrogel substrates with two elasticities and three topographies.[167] They observed that stiffness was predominant in preserving mESC stemness on soft substrates, while a combined effect of stiffness and topography on stemness preservation was found at a higher stiffness.[167]

We propose now to focus more specifically on the control of stem cell shape and fate by modulating the topography of their microenvironment at the subcellular, cellular, and supracellular scales.

5.3. Topography Influence on Stem Cell Fate

Chen et al. showed for the first time that cell shape can regulate cell survival.[168] Later, his group demonstrated the possibility to control the stem cell lineage commitment of MSCs using 2D adhesive patterns of different sizes (**Figure 4**).[2]

By varying cell spreading, they showed that MSCs did differentiate into osteoblastic cells when they were sufficiently spread, while unspread cells became adipocytes. Later, Kilian et al. directed MSC differentiation by modifying the aspect ratio or shape of the patterns. The osteogenesis yield increased with the aspect ratio of the rectangular patterns. Regarding the patterns with pentagonal symmetry, but with different types of curvature, the balance between adipogenesis and osteogenesis changed. On a flower shape with large convex curves,

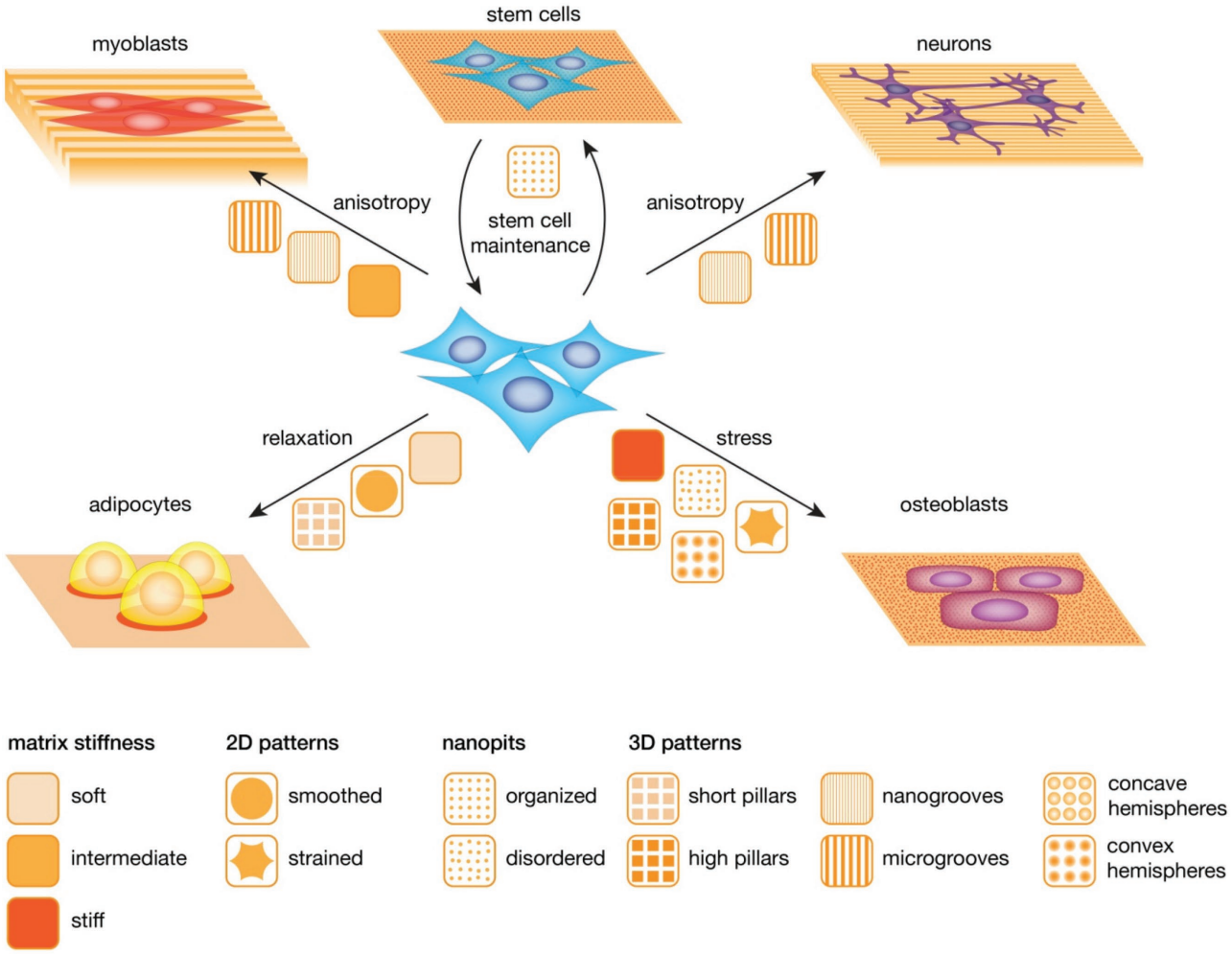

Figure 4. Matrix stiffness and topography influence stem cell fate. Various mechanical and topographical parameters can affect the fate of stem cells. Osteoblast differentiation is generally associated with high cell deformation or tension. This can be obtained using stiff gels, strained 2D patterns, convex surfaces, or disordered nanopits. On the contrary, stem cells are maintained in low tension conditions using, for instance, ordered nanopits. Similarly, adipocytes also differentiate on low tension/low strain surfaces such as short pillars, smooth 2D pattern, or soft matrix. Anisotropic topographies such as nano- or microgrooves favor cell elongation and differentiation into myoblasts or neurons.

MSCs differentiated more in adipocytes, while on a star shape with concave edges and sharp points at the vertices, MSCs differentiated more in osteoblasts. On a pentagon shape with straight lines for edges, an even distribution of adipocytes and osteoblasts was observed.[169] All these studies highlighted the role of actomyosin contractility in the commitment of MSCs. In a recent study, a possible role of the nucleus mechanics in ESC gene expression during the onset of differentiation was demonstrated using a single-cell micropatterned array. This study highlights the role of mechanical heterogeneity of the stem cell nucleus in the spatial modulation of transcription factors, enabling distinct gene expression programs and hence modulating the differentiation potential.[170]

Pioneering papers highlighting the influence of 2D nanotopography on nuclear organization and stem cell commitment were initially published by Dalby et al. They used surfaces with an 80 nm diameter and 100 nm deep nanopits organized in a hexagonal arrangement with a 300 nm pitch.[171,172] First, MCSs cultured on the surfaces with slightly disordered nanopits were found to have increased focal adhesion size and an upregulation of osteogenic differentiation markers such as osteopontin (Figure 4).[3] In contrast, on the surfaces with ordered nanopits, MSCs underwent self-renewal and prolonged growth as multipotent stem cells.[4] This demonstrated the possibility to control MSC differentiation by controlling the spatial organization of these nanopits. The results confirmed that morphological changes of the cells in response to nanotopography modify the cytoskeletal tension and interphase nuclear organization, hence directly influencing the cellular gene expression profile.[3,4,173]

Several studies have focused on the role of nano/micrograttings for the differentiation of stem cells toward the neuronal lineage (Figure 4).[174–177] Initially, this was demonstrated with MSCs cultured on 350 nm deep gratings, 350 nm to 10 µm in width and 700 nm to 20 µm pitch.[174] On these surfaces, the cell bodies and nuclei of MSCs were significantly aligned and elongated. The novelty of this work was the application of nanotopography to direct stem cell differentiation in a nondefault pathway. Later, the same studies were performed using

ESCs[175,176] or iPSCs.[176,177] Again, the contact guidance of the cells or colonies was significantly increased on the surfaces with a smaller pitch in relation to the highest neuronal marker expression.[176,177] More recently, the stimulating potential of the topographical cues was confirmed on fibroblast-to-neuron reprogramming[178] or cardiac progenitor differentiation in cardiomyocytes on micrometer-size features.[179] The possibility of reprogramming fibroblasts into pluripotent stem cells using nano- and microscale features was also demonstrated.[180] Microgroove substrates had a similar effect to valproic acid and tranylcypromine hydrochloride, chemical compounds known to promote transcription factor-free reprogramming. They promote the initiation of a mesenchymal-to-epithelial transition in fibroblasts, which relies on an increase of H3 acetylation and methylation marks, which are regulated by histone deacetylase 2 and WD repeat domain 5.[180]

As previously observed with cancer cells,[97,98] MSCs from rat bone marrow also displayed a nuclear deformation capacity on poly(D,L-lactide-*co*-glycolide 85:15) PLGA micropillars, irrespective of the height of the micropillars.[181,182] The consequences of this nuclear deformation on MSC differentiation were studied after culturing the deformed cells in osteogenic or adipogenic differentiation culture media for 7 d between pillars measuring 0.8, 4.6, and 6.4 μm in height.[182] Osteogenic differentiation was enhanced on higher pillars, while adipogenic differentiation was promoted on flat and smaller pillars. Surprisingly, osteogenic differentiation was associated with a lower cytoskeletal tension, which was evaluated by myosin light chain 9 (MYL9) and the F-actin intensity coupled with a lower projected cell area but a higher cell aspect ratio. Indeed, this observation appeared to contradict the previously described on 2D patterns, where geometric features that increase actomyosin contractility promoted osteogenesis.[2,169] The interpretation of this discrepancy is a potential role for the severe changes in nuclear shape due to chromatin reorganization and gene expression, but this hypothesis remains to be demonstrated.[182] In a recent paper, this group observed that the nuclear deformation of MSCs was actually not maintained over time, contrary to cancer cells.[183] Indeed, the MSCs nuclei were able to partially recover after their initial deformation, as observed previously in healthy osteoprogenitor cells derived from hMSCs.[99] This discrepancy between cancer and healthy cell nuclei deformability maintenance over time must be explored more extensively in the future.

All previously described studies demonstrate that surface topographies are important tools for future tissue engineering strategies associating stem cells with structured materials. However, there is a need for a screening approach in which the reactions of living cells to a large set of topographies could be evaluated. This was initially done thanks to a topographically microstructured surface library composed of 504 different topographical microstructures made of squares or round micropillars called the biosurface structure array (BSSA).[184,185] The best microstructures for expanding undifferentiated ESCs or for inducing their differentiation on BSSA were defined by measuring colony number and colony spreading, respectively.[186] For the expansion of undifferentiated mESCs, smaller pillars sizes (1 or 2 μm) with an optimal distance between pillars of 2 and 4 μm were best for colony formation and growth, respectively. For enhancing ESC differentiation, the best surfaces had large pillars (6 μm) with gaps of 1 μm and a limited vertical height (0.6 μm).

More recently, a similar high-throughput screening approach based on a library of 2176 topographically microstructured surfaces called "TopoUnits" forming a "TopoChip" has been proposed.[187,188] This approach gave birth to the field of "Materiomics," a holistic study of biomaterial systems, which combines materials, biological and computational sciences.[189] In the TopoChip, the surface features that are 5 μm in height are constructed by an algorithm using three types of primitives, namely, circles, triangles, and lines. Circles can create large smooth areas, triangles can generate angles, and thin rectangles can result in stretched elements. By changing the primitive type, primitive size, and angle of the rotation in the algorithm, an unlimited number of topologies can be prepared.[187] In recent papers, TopoChip was used to define the best topographies for maintaining the pluripotency of hiPSCs[190] or inducing the osteogenic differentiation of MSCs.[191] Using computational analyses, it was demonstrated that a small feature size was the most important determinant of the pluripotency of hiPSCs, followed by high wave number (fraction of the total energy in the signal in sinusoids) and high feature density.[190] Similarly, surfaces with a small feature size and large spacing were shown to induce low osteogenic differentiation of MSCs. In contrast, large features of 10–30 μm with a moderate spacing of 5–10 μm induced MSC confinement and high nuclear deformation between the structures, which was associated with high osteogenic marker expression.[191] Again, the pattern area, feature density, and wavenumber parameters were the most important cell shape features to predict MSC osteogenic differentiation. Interestingly, these results were confirmed in vitro on larger surfaces using more conventional molecular and cellular assays as well as in vivo with titanium implants onto which the best TopoChip features were reproduced using micromachining technologies.[191]

In the TopoChip, the features are fabricated using photolithography and etching, which are then used for imprinting the topographies onto polymer films. However, the presence of sharp edges and right angles between the feature wall and flat bottom render these surfaces very different from the natural cell environment, which is composed only of curved surfaces. The influence of curvature was analyzed at the subcellular, cell scale, and tissue scale using nano- or microfabricated substrates. Historically, the initial paper dealing with curvature used cylindrical fibers on which fibroblasts and epithelial cells were cultured. Cells containing pronounced straight parallel actin bundles, such as fibroblasts, became elongated and oriented along the cylinder, while cells with circular or no actin bundles, such as epithelial cells or transformed fibroblasts, respectively, bent around the cylinder with much less orientation along its axis.[192]

Thanks to PDMS surfaces, which presented wavy continuous features, Jiang et al. showed that the orientation of fibroblasts was similar to those of cells described in previous studies of contact guidance on surfaces with square-shaped grooves. These observations and comparisons indicate that sharp edges in the features defining the grooves are not essential in eliciting contact guidance, suggesting that cells are able to feel

and respond to the anisotropy of the curvature.[193] On similar sinusoidal wavy structures, it was recently observed that the majority of T cells migrated along the concave surfaces. This preference to concave surfaces decreased as the wavelength increased (or curvature decreased), suggesting that cells were able to appreciate the amplitude of the curvature.[194] Park et al. were able to prepare concave and convex microstructures, with base diameters of 200–300 mm and a depth (or height) of 50–150 mm (aspect ratios up to 1:0.5) with a controlled flat area between the curved hemispheres.[195] They cultured L929 mouse fibroblast cells and human MSCs and studied their migration on these microstructures, highlighting for first time the different cell behavior between concave and convex cell-scale hemispheres. Interestingly, the cells clearly sensed the 3D microscale curvature. When cells contacted the convex structures, they occasionally moved on top of them, while when they encountered the concave structures, they appeared to avoid entry. After 3 d of culture, the majority of cells stayed around the edge of the concave wells, while few cells were observed in the middle of concave surfaces. Moreover, the cells remaining in the concave wells were not able to spread, contrary to those on the flat and convex surfaces. When the cells were initially placed into the concave wells, they escaped more rapidly than the cells migrating on flat or convex surfaces. Based on these results, it appears that microscale concave structures suppress cell adhesion and proliferation.[195] These results suggest that cells are able to differentiate between convex and concave surfaces with the same curvature. The mechanism underlying this capacity still remains to be elucidated. Malheiro et al. observed variation in the shapes of human macrophages on concave and convex cell-scale surface features. However, these shape variations did not correlate with any shift in macrophage polarization state, illustrating that the sensitivity to curvature is also cell-type dependent.[196]

The mechanics of cells on curved surfaces has not been evaluated experimentally but was modeled by several authors.[197–200] In a recent paper, Vassaux and Milan used a mechanical cellular model that features a geometrical description of all the main intracellular components (cell membrane, FAs, cytoskeleton intermediate filament networks, actin stress fibers, microtubules, nucleus membrane, nucleoskeleton) to analyze the mechanical behavior of intracellular components on convex and concave cell-size cavities with a different curvature radii, R_{curv}: ±75, ±100, ±150, ±200, and ±500 µm. An increase in the convexity is accompanied with an increase of stress fibers, cell membrane extension, and compression of the cytosol and microtubules, altogether leading to a stiffer cell. Conversely, more concave structures make the nucleus more stable and more round.[200] He and Jiang showed in their model that cell migration was more persistent on concave surfaces than on convex surfaces (Figure 4).[199] This was recently confirmed experimentally with hMSCs. Interestingly, the migration mode changed between convex surfaces, on which a typical 2D mesenchymal migration mode with protrusion formation, cell body translocation, and rear retraction at a constant speed was observed. In contrast, concave surfaces cells produced very long and thin extensions spanning over the concave pit, attaching far from the cell and further pulling the cell body upward at an increased speed. This difference in migration mode was sustained by a different organization of the cytoskeleton and focal adhesions with formation of arc-like actin filaments at the periphery of the concave features and accumulation of vinculin-positive focal adhesions at their ends.[201] Differentiation of hMSCs was also studied by immunocytochemistry on these surfaces. Osteocalcin, a marker of osteoblastic differentiation, was more expressed on convex surfaces compared to flat or concave structures. Surprisingly, this higher osteocalcin level was correlated with a lower F-actin intracellular level. This is in contradiction with the higher osteogenesis observed in cells with higher cytoskeletal tension.[2] Moreover, this higher osteocalcin expression was obtained after culture of MSCs both in the growth medium and in the osteogenic medium, demonstrating that convex surface features larger than the cell size can promote osteogenic differentiation of MSCs, even in the absence of osteogenic growth factors.[201] An explanation of this observation could be the modification of nuclear morphology observed on the convex surface features. Indeed, the nucleus was stretched and flattened over the convex surface, while on the concave surface, the nucleus kept its spherical morphology. On flat surfaces, the nucleus was also stretched and flattened, but its upper membrane was flat, although it appeared indented by actin cap fibers on the convex surface. This was associated with a 2.5× higher lamin-A content of the nuclear membrane on convex surfaces compared to concave surfaces, confirming higher intracellular tension on the convex surfaces.[201]

Beside these basic studies on the effect of curvature on cells, interesting experiments were recently performed to develop cell-imprinted substrates presenting concave cavities mimicking cell morphology with the aim of controlling stem cell differentiation.[202–205] By pouring PDMS on a confluent cell layer cultured in a Petri dish and removing it after curing, the authors obtain a replica of the top cell layer's morphology. Depending on the cell origin or their differentiation state, different cell imprints and thus concave shapes were obtained. For example, round- or spindle-shaped cell imprints were obtained by replicating mature or dedifferentiated chondrocyte layers. Culture on these replicas of adipose-derived mesenchymal stem cells (ADSCs) showed that they adopted specific cell-type shapes, which have been used as templates, but more interestingly, they also displayed their molecular characteristics.[202] This observation was confirmed using epithelial cell layers,[203] ADSCs, chondrocytes, and tenocytes.[204] Immunofluorescence and gene expression analyses confirmed that cell-imprinted substrates could modulate stem cell differentiation but also redifferentiation (from fibroblasts to chondrocytes) and transdifferentiation (from chondrocytes to tenocytes).[204] On the basis of the observation that the nucleus shape changes when the cell's fate is altered, a computational model was developed to investigate the effect of nucleus geometries on chromatin arrangement. This model confirmed that the local organization of the chromatin fibers inside the nucleus is affected by the deformation of the confining nucleus and that the change in adjacency is not reversible.[203] This so-called "virtual cell model" was recently used to predict the cell response to any substrate topography obtained by microfabrication or the cell-imprinting method and to substrates with various elasticities.[205]

6. Conclusion

The discovery of the role of the nucleus in mechanotransduction dates to only two decades, and its involvement in the cellular response to topography has been demonstrated even more recently. Previously, the nucleus was considered a passive object preventing, for example, the migration of cells in confined environments. However, it now appears that it can itself be considered as a rheostat that regulates the distribution of intracellular forces by modifying its own compliance and resistance to strength. The respective roles of the various cytoskeletal elements and the LINC complex in the force exerted on the nucleus are beginning to be elucidated, but many open questions still remain unanswered. It is now clear that the deformation of the nucleus due to the topography of its environment or mechanical stresses regulates gene expression, which is particularly important for pluripotent or adult stem cells. The control of stem cell reprogramming by topography has a very important potential in tissue engineering and regenerative medicine, considering the need of these strategies in the future. In particular, the need to expand these cells in vitro without inducing their differentiation before using them in regenerative medicine strategies, will require the development of cell culture substrates with adapted topographies. Most surfaces used today are prepared by nano- and microlithography techniques and produce geometric patterns with angles and edges. This is far from the natural cellular environment, which is only composed of convex or concave curved surfaces. The use of surfaces with curvatures at the cellular and subcellular scale has recently begun. Apart from the fact that these techniques better represent the cellular environment, they also render possible the control of nuclear mechanics and therefore the expression of genes, making the reprogramming of cells feasible. Additional research in this area is still needed to confirm the interests of the curved surfaces, for example, to simulate the stem cell niche. The transition toward 3D artificial cellular environments will be the next step in this area. However, the experimental difficulty of characterizing the cell fate in 3D environments will justify the development of even more sophisticated experimental techniques that must be used in combination with advanced numerical models.

The recent initiative called the 4D nucleome project[206] aims to map the structure and dynamics of the human and mouse genomes in space and time. This ambitious international program will doubtlessly help us to better understand nuclear mechanics, leading hopefully to the development of a robust quantitative model for spatial genome organization. Such a tool will be extremely useful for developing approaches that cross-correlate topography patterns, cell shape changes, nuclear reshaping, chromatin reorganization, gene transcription modulation, and stem cell differentiation. This will lead to the development of new topographically imprinted environments that are able to fine-tune stem cell differentiation through the manipulation of nuclear homeostasis.

Acknowledgements

The authors thank the Centre National de la Recherche Scientifique (CNRS) (PICS Biointerfaces Grant No. 272116) and the Agence Nationale de la Recherche (Grant No. ANR-12-BSV5-0010-02) for funding the research. K.A. was also funded by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) PVE fellowship (Grant No. 406407/2013-4). N.T.W. was funded by a Region Alsace Graduate Research Fellowship. P.R. was funded by CNPq PDJ fellowships (Nos. 150145/2015-2 and 150033/2016-8) and by Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ) PD Nota 10 fellowships (Nos. 202.820/2016 and 202.821/2016).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

cell differentiation, cell mechanics, cell migration, cell nucleus, stem cells, topography

Received: September 29, 2017
Revised: November 6, 2017
Published online:

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