Probing cytoskeletal pre-stress and nuclear mechanics in endothelial cells with spatiotemporally controlled (de-)adhesion kinetics on micropatterned substrates

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ABSTRACT

The mechanical properties of living cells reflect their propensity to migrate and respond to external forces. Both cellular and nuclear stiffnesses are strongly influenced by the rigidity of the extracellular matrix (ECM) through reorganization of the cyto- and nucleoskeletal protein connections. Changes in this architectural continuum affect cell mechanics and underlie many pathological conditions. In this context, an accurate and combined quantification of the mechanical properties of both cells and nuclei can contribute to a better understanding of cellular (dys-)function. To address this challenge, we have established a robust method for probing cellular and nuclear deformation during spreading and detachment from micropatterned substrates. We show that (de-)adhesion kinetics of endothelial cells are modulated by substrate stiffness and rely on the actomyosin network. We combined this approach with measurements of cell stiffness by magnetic tweezers to show that relaxation dynamics can be considered as a reliable parameter of cellular pre-stress in adherent cells. During the adhesion stage, large cellular and nuclear deformations occur over a long time span (>60 min). Conversely, nuclear deformation and condensed chromatin are relaxed in a few seconds after detachment. Finally, our results show that accumulation of farnesylated prelamin leads to modifications of the nuclear viscoelastic properties, as reflected by increased nuclear relaxation times. Our method offers an original and non-intrusive way of simultaneously gauging cellular and nuclear mechanics, which can be extended to high-throughput screens of pathological conditions and potential countermeasures.

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Introduction

The mechanical properties of the extracellular matrix (ECM) regulate important physiological processes in eukaryotic cells, such as spreading, differentiation, division or motility. An important factor in this mechanical-functional interplay is the reorganization of the actin cytoskeleton, which controls intracellular pre-stress. However, the exact molecular mechanisms that underlie substrate-dependent mechanotransduction remain elusive. Recently, Swift and co-workers have shown that the matrix stiffness increases nuclear lamin-A levels, thereby stabilizing the nucleus while also contributing to lineage determination. A-type lamins and the linker of the nucleoskeleton and cytoskeleton (LINC) complex directly transmit forces from the extracellular matrix into the nucleus. Mechanical forces lead to changes in the molecular structure and assembly of A-type lamins that in turn activate a tension-induced “inside-out signaling.” This process allows feedback from the nucleus to the cytoskeleton and the extracellular matrix in order to balance ‘outside-in’ forces. This finding suggests that the LMNA gene is mechanoresponsive to ECM elasticity and underlines the importance of studying the relationship between the cytoskeletal organization and the nucleoskeletal homeostasis.

The ability to measure mechanical properties of adherent cells relies on a toolbox of cell mechanical measurement techniques that can apply forces or deformations on adherent cells (e.g. cell-stretchers, atomic force microscopy, magnetic tweezers, parallel plates, microfluidic devices, optical tweezers), whereas the characterization of the mechanical properties of the nucleus...
requires local mechanical techniques (i.e. endogenous particles\textsuperscript{10} or a micropipette aspiration method\textsuperscript{10,11}). While all of these methods have proven extremely powerful in characterizing cellular or nuclear viscoelastic properties, none of these techniques allows to probe simultaneously and non-invasively the mechanical properties of the cell and the nucleus.\textsuperscript{12}

To address this challenge, we propose to use cell-ECM adhesion and detachment (in other terms (de-)adhesion) kinetics, for characterizing combined cellular and nuclear mechanical properties. In line with work from Wildt and coworkers, who have developed surfaces composed of RGD-functionalized arrays of microscale gold strips for studying the detachment dynamics of fibroblasts,\textsuperscript{13,14} we used culture substrates of different rigidities patterned with protein microfeatures. Our strategy allows to overcome many of the limitations associated with existing methods by controlling the matrix stiffness, the cellular morphology and the spreading area, as they are known to modulate the intracellular force balance and\textsuperscript{15} the nuclear homeostasis.\textsuperscript{16,17} Using standardized (de-)adhesion assays, we investigate how changes in matrix stiffness affect the cellular pre-stress and we show that (de-)adhesion dynamics on micropatterned surfaces can be used to investigate the modifications of nuclear mechanics.

Results and discussion

Cell spreading dynamics is determined by matrix stiffness

Individual primary endothelial cells (HUVECs) were deposited on fibronectin (FN)-coated rectangular micropatterns with a 1:10 aspect ratio and a surface area of 1200 $\mu$m\textsuperscript{2}. The entire spreading process, from the contact of the cell with the adhesive micropattern ($t = t_0$) to the maximum cell spreading state ($t = t_s$), was followed with time-lapse microscopy (Figs. 1A and B, Supplementary Movie S1). In their final state, completely spread endothelial cells were characterized by focal adhesions localized at both extremities of the FN micropattern,\textsuperscript{16} and thick actin stress fibers parallel to the long axis of the rectangular pattern, as evidenced by phalloidin and vinculin stainings, respectively (Figs. 1C and D).

Using culture substrates of varying stiffnesses, we studied the effect of the matrix rigidity on the dynamics of cell spreading on 1:10 rectangular micropatterns (Supplementary Movie S2). As shown in Fig. 1E, the spreading process followed a sigmoidal behavior and was longer on 5 kPa ($t_s = 5940 \pm 170$ s) than on 3 MPa ($t_s = 3230 \pm 210$ s) substrates. The cellular deformation, $\varepsilon_s(t)$, during the spreading stage was recorded by optical microscopy in DIC mode and spreading curves for different matrix stiffnesses were fitted with a sigmoidal function (Eq. 2) to estimate the characteristic spreading time, $t_s$. We found that $t_s$ decreased with increasing matrix rigidity and was 2 times higher on 5 kPa substrates ($1582 \pm 434$ s) than on 3 MPa ($814 \pm 136$ s) substrates (Fig. 1F). Together, our results demonstrate that the spreading process of endothelial cells is significantly affected by the matrix stiffness. These findings are in agreement with the recent observations of Nisenholz et al. that claimed that both the initial spreading rate and steady state of fibroblasts increase on substrates with increasing stiffness.\textsuperscript{18}

Cellular relaxation dynamics is modulated by matrix stiffness

Endothelial cells spread on adhesive micropatterns undergo a natural strain in order to adopt the 1:10 aspect ratio imposed by the pattern geometry (Figs. 1A and B). Assuming that the spatial organization of the actin cytoskeleton in elongated endothelial cells (Figs. 1C and D) leads to a large amount of cell contractility,\textsuperscript{16} we investigated whether the matrix stiffness can modulate the tension in contractile actomyosin filaments by quantifying the relaxation dynamics after cell detachment. To do this, endothelial cells were grown 24 hours on FN-coated micropatterns and then detached by adding the proteolytic enzyme Accutase. Cell detachment leads to a fast cellular relaxation (Supplementary Movie S3), as monitored by time-lapse microscopy in DIC mode (Fig. 2A).

By fitting cell relaxation curves (Fig. 2B) with a sigmoidal function (Eq. 4), we found that for a given substrate stiffness the characteristic relaxation time, $t_R$, was 2 orders of magnitude lower than the characteristic spreading time. The slopes on DIC kymographs showed clearly that the detachment kinetics on soft 5 kPa matrix ($\sim 0.26 \mu$m/s, Fig. 2C) was slower than on stiff 3 MPa substrates ($\sim 0.73 \mu$m/s, Fig. 2D). In line with this observation, we found that the characteristic relaxation time decreased significantly with increasing matrix stiffness, from $t_R \sim 15$ s for cells spread on a 5 kPa matrix, to $t_R \sim 10$ s for cells spread on 9 kPa and $t_R \sim 7$ s MPa substrate (Fig. 2E). These results suggest that cell relaxation dynamics can be considered as an indirect readout for the rigidity of the cell microenvironment, especially in the low rigidity range. Taken together, these results suggest that the relaxation dynamics relies on internal modifications of the cytoskeletal organization, i.e., a form of pre-stress, which
were driven by changes in the matrix stiffness during the spreading stage.

Cell relaxation dynamics reflects the initial cellular pre-stress

To assess whether endothelial relaxation dynamics was related to the tensile state of the cytoskeleton, we decreased the intracellular pre-stress by depolymerizing the actin network with Latrunculin A (Lat-A). Our findings show that Lat-A-treated cells relaxed more than 15 times slower than control cells (Fig. 3A), supporting our hypothesis that the relaxation process reflects the state of tensile stress. In addition, micropatterned endothelial cells were treated with Y-27632, a selective inhibitor of Rho-associated kinase (ROCK). The ROCK
family of kinases has been shown to be involved in Rho-induced formation of actin stress fibers and focal adhesions and in down-regulation of myosin phosphatase. As shown in Fig. 3A, Y-27632-treated cells exhibited a significant slowing down of their relaxation dynamics. Taken together, our results suggest that the contractile actomyosin cytoskeleton (Fig. 3B) is the driving force of the cell relaxation process.

To directly assess the intracellular pre-stress, we determined the elastic modulus (E) of micropatterned endothelial cells grown on micropatterned substrates ranging from 5 kPa to 3 MPa with magnetic tweezers. Briefly, magnetic beads were coated with fibronectin and incubated with patterned cells in order to bind the magnetic beads to the cytoskeleton through formation of focal adhesions. A constant magnetic pulling force was applied to the beads during 12 s and the elastic modulus of living micropatterned cells was estimated with Eq. 6 by using bead displacements that were recorded with time-lapse microscopy. As presented in Fig. 3C, our findings demonstrated that the elastic modulus of patterned endothelial cells increased with increasing matrix rigidity, in agreement with previous reports on fibroblasts. By combining these results, we show that the cell relaxation time decreased with the cell elastic modulus (Fig. 3D), confirming that the cell relaxation dynamics reflects the internal tension of endothelial cells and can be used as a reliable parameter to characterize the pre-stress in adherent cells.

**Cell shape changes induce instantaneous and reversible adaptations of the nuclear shape**

Next, we investigated the dynamics of nuclear deformation by following H2B-GFP nuclei during spreading (Fig. 4A) and relaxation (Fig. 4B) stages with time-lapse microscopy (see Methods section). As shown in Fig. 4C, the nucleus changes its shape during the cell spreading stage, from a spherical form to an elongated shape, as a result of intracellular forces acting on the nucleus due to the remodeling of the spatial organization of the actin cytoskeleton. The nuclear deformation followed a similar temporal evolution as the cellular deformation (Fig. 4E), suggesting that nuclear and cellular deformations are coupled processes. Similarly, cellular relaxation after cell detachment is also associated with a rapid nuclear
relaxation leading to nuclear rounding. As shown in Fig. 4D, the nuclear deformations that occur during the cellular relaxation stage (Supplementary Movie S4) were at least one order of magnitude faster than the nuclear deformations during the spreading stage. Changes in H2B-GFP intensity patterns also suggested chromatin reorganization during spreading and relaxation stages, with the formation of condensed chromatin domains in deformed nuclei, as reflected by changes in the fluorescence intensity (Fig 4C and D). Modifications of the nuclear shape that take place in hundreds of minutes during the spreading stage induce marked reorganization of chromatin distribution (Fig. 4F), which are relaxed in a few dozen of seconds during cell detachment.

Nuclear relaxation dynamics is determined by the cell state

We quantified the dynamics of nuclear relaxation as a function of the matrix stiffness (E = 5 kPa, 9 kPa and 3 MPa) by performing time-lapse experiments of H2B-GFP labeled nuclei. As presented in Fig 5A, our results showed that nuclear relaxation dynamics was significantly affected by the stiffness of the substrate. Indeed, the mean characteristic relaxation time decreased from ~20 s on 5 kPa matrices to ~9 s on a 3MPa substrate, emphasizing that the nuclear relaxation is intimately related to the cell relaxation process. This was further supported by the nuclear relaxation time of Y-27632-treated cells which was 8 times slower than controls (Fig. 5B and Supplementary Movie S5), suggesting that the nuclear relaxation dynamics is modulated by substrate-induced changes in actomyosin tension.

Our results demonstrate that cell detachment from adhesive protein micropatterns allows to probe the nuclear mechanics without applying external forces and without any alteration of the nucleo-cytoskeletal interactions, such as observed in micropipette aspiration experiments on isolated nuclei.11

Lamin A impacts on nuclear relaxation dynamics

The balance of A-type and B-type lamins has been shown to affect the rheological properties of the nucleus and alteration of the composition of the nuclear lamina are encountered in different diseases such as laminopathies.26,27 Indeed, micropipette aspiration experiments have shown that nuclei with a low expression of lamin A were more compliant than those with a high expression of lamin A.2,28 Cells lacking A-type lamins have a

Figure 3. Role of the actomyosin cytoskeleton on cell relaxation dynamics. (A) Cellular relaxation times (mean ± SD) for control endothelial cells (black bar, n = 18), Latrunculin A-treated cells (white bar, n = 14) and Y-27632-treated cells (dashed bar, n=15) deposited on 3 MPa micropatterned substrates. Data are expressed as mean ± SD and ***p ≤ 0.001. (B) Typical fluorescent images of the spatial distribution of the actin cytoskeleton (in green), the nucleus (in blue) and the myosin (in white) of endothelial cells plated on 1200 μm² and 1:10 aspect ratio rectangular micropatterns deposited on 3MPa substrates. Scale bars are 10 μm. (C) Semi-logarithmic evolution of the cell elastic modulus of endothelial cells spread on elongated patterns deposited on substrates with increasing stiffnesses (5 kPa, 9 kPa, 45 kPa, 110 kPa and 3 MPa). (D) Evolution of the characteristic relaxation time as a function of the cell elastic modulus.
reduced nuclear stiffness and an increased nuclear fragility, leading to more cell death under mechanical strain\textsuperscript{29,30}. In contrast, accumulation of farnesylated prelamin A, as encountered in restrictive dermopathy and Hutchinson–Gilford Progeria Syndrome (HGPS), may lead to a lower deformability of the nucleus under mechanical stress\textsuperscript{30,31}.

We investigated the role of the nuclear lamina in the nuclear shape relaxation dynamics by treating HUVECs spread on 3 MPa micropatterned substrates with the HIV protease inhibitor saquinavir, which inhibits ZMPSTE24, the protease that catalyzes conversion of farnesylated prelamin A to prelamin A\textsuperscript{32}. As shown in Fig. 6A, the nuclei of saquinavir-treated cells were statistically less deformed (NSI \textasciitilde 0.86) than the nuclei of control cells (NSI \textasciitilde 0.79). Our results are in agreement with the observation of Dahl and coworkers that the altered nuclear lamina in HGPS induces an increased resistance of the nucleus to mechanical stress\textsuperscript{27}. As shown in Fig. 6B, immunostaining of the actin network and lamin A/C shows that saquinavir-treated cells present an intact actin cytoskeleton. Saquinavir-treated cells present a broader distribution of their characteristic nuclear relaxation times, with 70% of nuclei relaxing slower than the...
mean relaxation time of control nuclei (Fig. 6C). In contrary, saquinavir treatment did not influence the cell relaxation time. Together, our results indicate that accumulation of farnesylated prelamin A in saquinavir-treated cells leads to a modification of the viscoelastic properties of the nucleus, which leads to less deformed nuclear shapes and an increase of the nuclear relaxation dynamics. Our results are consistent with the hypothesis that the characteristic relaxation time of the nucleus ($t_N$) reflects the viscoelastic properties of the nucleus, that depend on the ratio of lamin A:B isoforms. Modifications of the ratio between lamin-A which contributes as

![Figure 5](image1.png)

**Figure 5.** Dynamics of nuclear relaxation. Evolution of the characteristic nuclear relaxation time as a function of (A) the matrix stiffness ($E = 5$ kPa $n = 5$, $9$ kPa $n = 6$ and $3$ MPa $n = 7$) and (B) a Y-27632 treatment that affects the actomyosin contractility (control cells: $n = 7$ and Y-27632 treated cells: $n = 5$). Data are expressed as mean ± SD, **p < 0.01 and n.s. non significant.

![Figure 6](image2.png)

**Figure 6.** Role of the nuclear lamina on nuclear relaxation dynamics. (A) Characterization of the nuclear shape of control (in black, $n = 44$) and saquinavir-treated cells (in gray, $n = 41$) plated on a 3 MPa micropatterned substrate. Mean ± SD and ***p < 0.001. (B) Immunostaining images of the actin cytoskeleton (in green), the nucleus (in blue) and the lamin A/C (in white) of rectangular micropatterned endothelial cells treated for 3 d with the protease inhibitor saquinavir after plating and then treated with fresh saquinavir for 24 h before fixation and immunostaining. Scale bar is 10 μm. (C) Characteristic nuclear ($n = 11$, in red) and cellular ($n = 11$, in black) relaxation time of endothelial cells grown on rectangular micropatterns and treated for 4 d with the protease inhibitor saquinavir (20 μM). **p < 0.01 and n.s. non significant.
a highly viscous fluid (η) and lamin-B which acts as an elastic solid (G) lead therefore to changes of the nuclear relaxation time (1/\(\eta G\)). This illustrates the performance of our approach and positions (de-)adhesion dynamics as a powerful technique to differentiate living cells based on the composition of their nuclear lamina.

Conclusions

We have developed a simple and efficient assay to probe cellular and nuclear mechanical properties based on (de-)adhesion kinetics. By combining protein micropatterns with culture substrates of different stiffnesses, we standardized the shape and the spreading area of single endothelial cells, while modulating their internal pre-stress. The dynamics of detachment reflects the amount of pre-stress accumulated in the actomyosin network during the spreading stage. In addition, our technique allows characterizing the dynamics of nuclear deformations during cell spreading and detachment. Large deformations of the nucleus occur in hundreds of minutes during the spreading stage, whereas the nuclear shape and condensed chromatin can be relaxed in a few dozens of seconds after the cell detachment. Finally, our results indicate that chemically induced accumulation of farnesylated prelamin A causes a nuclear stiffening, which is reflected by less deformed nuclei and an increase of their relaxation dynamics. We envision that this simple and robust technique can be suitable for investigating the role of complex structures such as LINC complexes that link the actin cytoskeleton and the nuclear lamina and for the diagnosis of human diseases involving cytoskeletal and/or nuclear modifications.

Materials and methods

Rigidity-controlled substrate preparation

Culture substrates in polydimethylsiloxane (PDMS) silicone elastomer, (Sylgard 184 Silicone Elastomer Kit; Dow Corning, Midland, MI) of 3.0 ± 0.3 MPa Young’s modulus were prepared from 1:10 cross-linker to monomer ratio. The uncured solution of PDMS was mixed and degassed in a conditioning mixer and then inked for 1 h at room temperature with a structured surface of the PDMS stamps were made temporarily hydrophilic in an UV/O3 cleaner for 8 min and then inked for 1 h at room temperature with a 25 μg/ml fibronectin (FN) solution from human plasma. FN-coated stamps were dried under filtered nitrogen and gently deposited on a flat rigidity-controlled substrate for 1 hour (hydroxy-PAAm hydrogels) or 15 seconds (PDMS elastomers). Uncoated regions were then blocked

Microcontact printing

Rectangular (1:10 aspect ratio) micropatterns of 1200 μm² were drawn with the Clewin software (Wie-Web Software, The Netherlands) and generated to a silicon master by deep reactive-ion etching (FH Vorarlberg University of Applied Sciences, Microtechnology, Dornbirn, Austria). The silicon surface was passivated under vacuum with vapors of fluoro-silane (tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane) for 30 min, and then molded with a thick layer of PDMS (Sylgard 184 Silicone Elastomer Kit; Dow Corning, Midland, MI). After 4h of curing at 60°C, the PDMS membrane was peeled off and stamps of 1 × 1 cm² were cut manually. The structured surface of the PDMS stamps were made temporarily hydrophilic in an UV/O₃ cleaner for 8 min and then inked for 1 h at room temperature with a 25 μg/ml fibronectin (FN) solution from human plasma. FN-coated stamps were dried under filtered nitrogen and gently deposited on a flat rigidity-controlled substrate for 1 hour (hydroxy-PAAm hydrogels) or 15 seconds (PDMS elastomers). Uncoated regions were then blocked
by incubating hydroxy-PAAm hydrogels overnight in a 5 mg/ml BSA solution at 4°C and elastomers for 5 min in a 1% solution of Pluronic F-127.

**Cell culture**

Primary human umbilical vein endothelial cells, HUVECs (Cells Applications, San Diego, CA) were grown in complete endothelial cell growth medium (Cells Applications, San Diego, CA) supplemented with 1% Penicillin/Streptomycin and antimycotics and maintained at 37°C in a humidified atmosphere with 5% CO₂. Cells between passages 2 and 8 were cultured on micropatterned PDMS coverslips at a concentration of about 15,000 cells/ml.

**Live cell spreading**

Cells were detached from a routine culture flask with a solution of Accutase, then suspended in fresh culture medium to a final concentration of 15,000 cells/ml and then transferred to a micropatterned rigidity-controlled substrate. The cellular spreading process on elongated micropatterns was recorded in DIC mode via time-lapse microscopy with a Roper QuantEM:512SC EMCCD camera (Photometrics, Tucson, AZ) controlled by NIS Elements Advanced Research 4.0 software (Nikon, Japan). Images were recorded every 500 ms up to complete cell detachment.

The dynamics of the cellular relaxation, $e_R(t)$, was determined by normalizing the temporal evolution of the cellular length, $L(t)$ with the long axis of the micropattern, $L_M$, as:

$$e_R(t) = \frac{L(t)}{L_M}$$

Considering that $L_M = 109.5$ μm for a 1:10 micropattern of 1200 μm² and an initial mean cell diameter of 17.78 ± 2.62 μm, $e_R(t)$ varied between 1 (full spreading state) and 0.16 (total cell relaxation).

The temporal evolution of the normalized cell deformation, $e_R(t)$, during the relaxation stage was fitted with a sigmoidal curve, according to:

$$e_R(t) = A_2 + \frac{A_1 - A_2}{1 + e^{(t-t_c)/t_h}}$$

where $A_1$ is the maximal cell spreading area at $t = t_f$, $A_2$ is the minimal cell spreading area at $t = t_0$, $t_c$ is the center of the sigmoid and $t_h$ corresponds to the characteristic spreading time.

**Live cell relaxation**

Cells grown on rigidity-controlled micropatterned (1:10 aspect ratio) substrates for 24 hours were observed with a Nikon Eclipse Ti-E motorized inverted microscope equipped with ×10 Plan Apo, ×40 Plan Apo (NA 1.45, oil immersion), ×60 Plan Apo (NA 1.45, oil immersion) and ×100 Plan Apo (NA 1.45, oil immersion) objectives and with a temperature and CO₂ level controller. The relaxation process of the elongated cells was induced by adding 1 ml of Accutase after a gentle wash of warm PBS (37°C) and recorded in DIC mode via time-lapse microscopy with a Roper QuantEM:512SC EMCCD camera (Photometrics, Tucson, AZ) controlled by NIS Elements Advanced Research 4.0 software (Nikon, Japan). Images were recorded every 500 ms up to complete cell detachment.

The characteristic spreading time was determined by fitting $e_s(t)$ with a sigmoidal curve, as:

$$e_s(t) = A_2 + \frac{A_1 - A_2}{1 + e^{(t-t_f)/t_h}}$$

where $A_1$ is the maximal cell spreading area at $t = t_f$, $A_2$ is the minimal cell spreading area at $t = t_0$, $t_f$ is the center of the sigmoid and $t_h$ corresponds to the characteristic cell relaxation time.

**Nuclear deformation**

To observe nuclear DNA in live imaging, HUVECs were transduced with the BacMam 2.0 CellLight Histone 2B-GFP according to the manufacturer’s protocol (Life Technologies, Molecular Probes). Routine culture flasks
were incubated with approximately 20 BacMam particles/cell for at least 24 hours. Labeled cells were then detached and plated on rigidity-controlled micropatterned substrates to perform spreading and detachment experiments. The nuclear shape index (NSI) was defined as NSI = 4 × area/(perimeter)². The nuclear morphology was characterized with time-lapse fluorescence microscopy and the nuclear deformation, \( e_N(t) \), during spreading and detachment experiments was fitted with a sigmoidal curve, according to:

\[
e_N(t) = A_2 + \frac{A_1 - A_2}{1 + e^{(t-t_c) / \tau_N}} \quad (5)
\]

where \( A_1 \) is the maximal nuclear deformation, \( A_2 \) is the minimal nuclear deformation, \( t_c \) is the center of the sigmoid and \( \tau_N \) corresponds to the characteristic relaxation time of the nucleus. The mean nuclear diameter was characterized by FACS as 12.42 ± 2.42 µm.

**Pharmacological treatment**

In order to assess the effect of the cytoskeletal organization on cellular and nuclear relaxation dynamics, endothelial cells were treated with ROCK-inhibitor Y-27632 (Sigma-Aldrich, 30 µM) or the actin polymerization inhibitor Latrunculin-A (Sigma-Aldrich, 0.5 µM) 1 hour prior to detachment with Accutase. The HIV protease inhibitor Saquinavir (Sigma-Aldrich, 20 µM) was used to selectively block the human ZMPSTE24 enzyme, leading to accumulation of farnesylated prelamin A. Endothelial cells were treated during 4 d with a 20 µM solution of saquinavir.

**Cell fixation and immunostaining**

Endothelial cells were fixed with a solution of 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) for 10 min, permeabilized with 0.05% Triton X-100 (Sigma) in PBS (PAA Laboratories) for 15 min at 37°C and washed 3 times in PBS. Cells were incubated for 45 min at 37°C with 4,6-diamidino-2-phenylindole dihydrochloride 1/200 (DAPI, Molecular Probes, Life Technologies, Cergy Pontoise, France), Alexa Fluor 488 labeled Phalloidin 1/200 (Molecular Probes, Invitrogen), with 4,6-diamidino-2-phenylindole dihydrochloride 1/200 (Molecular Probes, Life Technologies), monoclonal mouse anti-vinculin antibody (1/200, Sigma-Aldrich), mouse anti-lamin A/C antibody (1/200, Sigma-Aldrich) or rabbit anti-myosin light chain antibody (1/300, Sigma-Aldrich) washed 3 times in PBS, and incubated for 45 min at 37°C with a goat anti-mouse antibody labeled with tetramethylrhodamine (Molecular Probes, Invitrogen) or with a goat anti-rabbit antibody labeled with tetramethylrhodamine (Sigma-Aldrich, T6778). Both secondary antibodies were used at a concentration of 1/200 for 45 min at 37°C. Slides were mounted in Slow Fade Gold Antifade (Molecular Probes, Invitrogen).

**Measurement of cell stiffness by magnetic tweezers**

BcMagTM Epoxy-Activated Magnetic beads (Bioclonne Inc., San Diego, CA) of 4.5 µm in diameter were coated with human fibronectin (FN) by following the bead manufacturer’s protocol for bead functionalization. Briefly, beads were first rinsed in phosphate buffered saline (PBS) and subsequently incubated on ice in 10 µg/mL human FN overnight under a gentle (0.5 Hz) agitation to disperse beads without precipitating FN still in solution. After rinsing in PBS, FN-coated beads were seeded onto cells plated on micropatterned rigidity-controlled substrates in culture medium for 20 minutes at a mean ratio of 4 beads per cell. A home-made magnetic tweezer mounted on an inverted Nikon Eclipse Ti microscope (Nikon, Japan) and connected to a LabVIEW software (National Instruments, Austin, Texas) was positioned at a 35-degree angle to the microprinted culture substrate by using an automated micromanipulator system (Inject-Man N12, Eppendorf, Hamburg, Germany). The magnetic forces were calibrated using the Stokes’ law by quantifying the beads displacements in a 99% glycerol solution as a function of the current intensity and the bead-tip distance. A constant pulling force, \( F \), was then applied to paramagnetic beads of section \( S \) and radius \( R \) bounded to the cytoskeleton of the micropatterned endothelial cells. The cell elastic modulus, \( E \), was estimated from the beads displacements, \( \partial \), as

\[
F = \frac{1}{2} ESU
\]

where \( S = 4\pi R^2 \) is the bead surface and \( U \) is bead translation \( \partial \) normalized by the bead radius \( R \), as \( U = \partial / R \).

**Statistical analysis**

Differences in means between groups were evaluated by 2-tailed Student’s t-tests performed in Origin 8.5 (OriginLab, Northampton, MA). For multiple comparisons the differences were determined by using an analysis of variance (ANOVA) followed by Tukey post-hoc test. * \( p \leq 0.05 \), ** \( p \leq 0.01 \) and *** \( p \leq 0.001 \). Unless otherwise stated, all data are presented as mean ± standard deviation (SD).

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.
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