Electronic Supplementary Material (ESI) for Integrative Biology

Online supplemental material includes:

- One text file describing the optimization of the device, description of cortical blebs dynamics upon confinement and a comment on induction of histones expression
- 5 Supplementary figures
- 3 Supplementary movies

Supplementary Figures legends

Sup. Figure 1: Numerical simulation and experimental characterization of the microstructured slides. (**a**) Simulation of sagging between micro-pillars under a 10 kPa pressure. Left diagram represents the displacement field along the vertical direction in the simulated structure, in the case of a glass slide supporting the PDMS structures. Right graph shows the simulated sagging (the profile of the roof between two pillars) with the supporting glass slide (red) or with a 5 mm-thick PDMS pad instead of the glass slide (blue). (**b**) Simulation of the deformation of small pillars allowing pressure-controlled variable height of confinement. Displacement field along the vertical direction is shown for 10 kPa pressure. In this case, a cavity surrounds the pillars, increasing its height to allow more deformation (higher aspect ratio) and channels have been added to allow the fluid to move more easily when the height is changed. (**c**) Diagram showing the designs of pillars used in the experiments. The small design on the left represents a slide intended for fixed height confinement (pillars are shown in purple). Larger design on the right represents a slide intended for variable confinement (pillars are shown in red and isolation channels are shown in green) (**d**) Pictures showing examples of microstructured slides intended for a 28 µm fixed height (left) and for variable height (right). (**e**) Position of actual confinement height measurements shown in Figure 1b. Yellow crosses correspond to measurements made on the 3.5 µm height slide, blue crosses to measurements made on the 5.5 µm height slide and purple crosses to measurements made on the 28 µm height slide. (**f**) Measurement of the thickness homogeneity along a line between 2 pillars. A 5.5 µm height slide loaded with a 10 kPa pressure has been used for this characterization.

Sup. Figure 2: Modified 12-well plate for fixed confinement. (**a**) Principle of operation: a pressure is applied using large molded soft PDMS pillars holding the microstructured slides. Height of the large holding pillars is designed so slides were held over the cells cultured in the wells with a moderate pressure when the cover of the well-plates was closed with a clamp. (**b**) Picture showing a modified 12 well plate.

Sup. Figure 3: Cell cortex behavior after dynamic change in confinement. (**a**) Fluorescence images of a HeLa cell expressing MyrPalm-GFP (green) and cytoplasmic mCherry (red) with DNA stained using HOECHST. Images show the cell confined at 3.5 µm, 1 min before, 1 minute after and 60 min after application of the confinement. (**b**) Equatorial section and side view extracted from confocal stacks of fluorescence images of a HeLa cell expressing LifeAct-GFP (filamentous actin reporter). Images show cortex reassembly after two successive compressions starting from suspended spherical cells. Time zero is the first confinement at 7 µm. Further confinement at 3.5 µm is applied after 6 minutes (bottom-left). Scale bars are 10 µm.

Sup. Figure 4: Relative change of the nuclear volume in the 90 min following bleb formation for 2 independent experiments. The volume change was deduced from the top view of Lamin A-GFP staining 20 min after and 110 min after application of a confinement at 3.5 µm. In the first experiment, volume change was not significant (0.95%±2.2% s.e.m. increase, n=25),

whereas a significant decrease of 3.9%±1.4% s.e.m (n=23) was observed in the second experiment.

Sup. Figure 5: Relative expression change of 4 genes identified with microarrays. Expression levels were quantified by qPCR on 5 independent experiments. The confidence of changes were evaluated using t-test (* means $p < 0.05$, ** means $p < 0.01$, *** means $p < 0.001$). Error bars represent S.E.M.

Sup. Figure 6: Cell cycle-related gene expression. (a) Expression levels for genes known to be induced in S-Phase, relative to the control experiment. We found no evidence of enrichment of cells in S-phase, when cells were confined in a 3.5 µm gap. Relative expression levels of the same genes in cells synchronized in M-phase by a mitotic shake is shown for comparison. (b) Expression level of cyclins relative to the control experiment. We found no evidence of synchronization in a specific phase of the cell division cycle when cells were confined in a 3.5 µm gap. Relative expression levels of the same genes in cells synchronized in M-phase by a mitotic shake is shown for comparison. (c) Expression levels of all histones of the HIST1 cluster relative to the control experiment. In contrast with the S-phase induction of histones, only cell-cycle dependent histone H3.1 and H4 were induced in cells confined in a 3.5 µm space.

Electronic Supplementary Material (ESI) for Integrative Biology

Supplementary movies legends

Sup. movie S1: HeLa cells under a confinement with a 3 µm spacer, showing nuclear bleb growth in S-phase. The cell expressed alpha-tubulin-GFP, staining the microtubules (green), and H2B-mCherry, staining the chromatin (red).

Sup. movie S2: HeLa cells showing nuclear blebs after a confinement with a 3.5 µm spacer. The Lamina-GFP appeared progressively on the bleb surface in the first 30 minutes after the nuclear cortex breakdown. The HeLa cell expressed laminA-GFP (green) and was stained with HOECHST for DNA (red). Time zero is the confinement.

Sup. movie S3: Detail of the Sup. movie S2.

Electronic Supplementary Material (ESI) for Integrative Biology

Supplementary Information

1. Device shape optimization

Simulation of the sagging of the microstructured slides. Experiments showed that the usage of a soft material such as PDMS and the application of a moderate pressure (10 kPa) was necessary to erase the effect of small dust particles and irregularities, to achieve reliable confinement as small as 3.5 µm. However, if the micro-pillars were spaced too far apart, the low rigidity of PDMS induced a sagging of several micrometers between the pillars. Simulating such a chamber using FEM, we found that a 10 µm thick chamber made in a 5 mm thick PDMS block and sustained with pillars spaced by 1 mm, sagged by more than 5 µm between 2 pillars (Supplementary Fig. 1a, right graph, blue curve). Introducing a 160-µm-thick rigid glass coverslip 40 µm above the structures dramatically decreased sagging down to 100 nm (Fig. Supp. 1a, left diagram and right graph, red curve). We chose the distance between pillars to be as large as possible while keeping this sagging effect bellow 100 nm with glass slides 160 µm thick. However, it is possible to increase the distance between pillars if less homogeneity is needed or if using thicker glass slides to hold the structures.

Simulation of the deformation of small pillars. For the variable height chamber, the pillars sustaining the confining surface can deform under the applied pressure. This effect is a drawback to keeping a desired confining height independent of the applied pressure. It can, however be used advantageously to tune the confining height by adjusting the pressure. The sensitivity of pillars to the applied pressure increases with the pillars' height and decreases with the pillars' radius, according to the elastic deformations laws of rigid bodies. We simulated the deformation of pillars of different diameters to find the appropriate geometry. In these simulations, the pressure applied to the slide was corrected according to the surface ratio of the pillars over the whole surface of the confiner, to take into account the difference between the 3D device and the 2D simulations regarding the pressure applied to the pillars. We found that pillars with a radius larger than 10 µm showed almost no change in their height under pressure. However, we chose to use a larger radius of 110 µm. This allowed us to optimize the available space by siting pillars to 1 mm apart, thereby reducing the surface they occupied (5 % of the surface in this case). In this situation, we experimentally measured a very small change in pillar height of 23 nm/kPa, as shown in Figure 1a. Conversely, increasing the height of the pillars can increase this sensitivity. We introduced a cavity around each pillar to increase its height while maintaining a given confinement. We optimized the pillars' geometry to cover a large range of heights with moderate pressures. This avoided the propagation of the PDMS deformation around the pillar, ensuring a good homogeneity of confinement (Supplementary Fig. 1b). We found that slides that included pillars of 150 µm radius and 120 µm height, spaced by 1 mm, and surrounded by channels 100 µm thick and 100 µm wide were optimal to control the height of confinement in the 0-20 µm range with a moderate pressure (experiments and simulation in Fig. 1c). It would be possible to increase the sensitivity of the device to the applied pressure by increasing pillar height, decreasing the pillar radius or increasing the space between pillars.

2. Cortical Blebs

To better understand how the cell cortex behaves when the cell is dynamically confined, we recorded the dynamics of the actin cortex. We confined cells using a confinement slide that enabled the confining height to be altered dynamically. To start with spherical cells of about 20 µm in diameter (Supplementary Fig. 3), we used cells that were not adhered to the substrate. Just after the cell was confined down to a height of 7 µm, we observed that the cell membrane detached from the actin cortex, forming large and numerous blebs. These blebs were very likely caused by a pressure overload in the cell because the cell cortex surface was not elastic enough to accommodate the large change in surface/volume ratio imposed by the change of shape from a sphere to a pancake, while cell volume remained constant. These induced blebs have already been described in cells after a hypo-osmotic shock (1). After 1 minute, a new actin cortex was already rebuilt on the bleb surface. As was described for osmotically induced blebs, the blebs were retracted and a new continuous actin cortex formed within 5 minutes. However, since the cortex had to increase its surface to accommodate a constant cell volume, the cortical contraction pulled the cell surface toward the new circular cortex with a bigger diameter, instead of merging the bleb in the former cortex structure as was observed for osmotically induced blebs (2) or laser ablation-induced blebs (3). This cortex break and reconstruction could be induced repeatedly in the same cell after further confinement as shown in Figure S3.

3. Histones induction:

The genes whose expression increased the most at 3.5 µm were histones H4 (H4A-D) and H3 (H3F), while the expression level of other histones did not change (Fig. 5c, Supplementary Fig. 6). We checked whether the over-expression of this subset of replication-dependent histones was related to the synchronization of confined cells in S-phase. No specific synchronization under 3.5 µm confinement was observed, based on the expression of cyclins or genes known to be induced in S-phase for HeLa cells (4) (Supplementary Fig. 6).

Importantly, quantitative PCR experiments were not able to confirm the increase in expression of the histone H4 and H3.1 genes. We checked that it could not be due to an artifact of the array, as the probes were dispersed all over the array. We cannot at that point explain the discrepancy between both techniques and thus consider that the results on Histone gene expression have to be considered potentially artefactual.

References

1. Charras GT, Yarrow JC, Horton MA, Mahadevan L, & Mitchison TJ (2005) Non-equilibration of hydrostatic pressure in blebbing cells. *Nature* 435(7040):365-369.
2. Charras GT, Coughlin M, Mitchison TJ, & Mahadevan L (2008) Life and times of a cellular bleb. *Biophysical Journal* 94(5):1836-1853.
3. Tinevez JY, *et al.* (2009) Role of cortical tension in bleb growth. *Proc. Natl. Acad. Sci. U. S. A.* 106(44):18581-18586.
4. Whitfield ML, *et al.* (2002) Identification of genes periodically expressed in the human cell cycle and their expression in tumors. *Mol. Biol. Cell* 13(6):1977-2000.