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SPECIAL ISSUE - REVIEW

Microfabricated substrates as a tool to study cell mechanotransduction

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Abstract Mechanical cell–substrate interactions affect many cellular functions such as spreading, migration, and even differentiation. These interactions can be studied by incorporating micro- and nanotechnology-related tools. The design of substrates based on these technologies offers new possibilities to probe the cellular responses to changes in their physical environment. The investigations of the mechanical interactions of cells and their surrounding matrix can be carried out in well-defined and near physiological conditions. In particular, this includes the transmission of forces as well as rigidity and topography sensing mechanisms. Here, we review techniques and tools based on nano- and micro-fabrication that have been developed to analyze the influence of the mechanical properties of the substrate on cell functions. We also discuss how microfabrication methods have improved our knowledge on cell adhesion and migration and how they could solve remaining problems in the field of mechanobiology.

Keywords Microfabrication · Cell mechanics · Substrate stiffness · Cell contractility · Integrins · Topography · Mechanotransduction

1 Introduction

Cells usually migrate in a 3D extracellular matrix (ECM) that is composed of fibers and glycosaminoglycans during many physiological and pathological processes such as embryonic development, tumor formation, and morphogenesis. The ability of cells to migrate within the extracellular matrix and to remodel it depends as much on the biochemical characteristics of the matrix as on its physical properties [20]. The main components of 3D tissue structures in vivo are protein fibers such as fibrillar collagen, inducing a heterogeneous network of fiber scaffolds of variable density, orientation, and mechanical strength [31, 98]. Therefore, cells encounter matrices that could have very different physical properties in terms of stiffness, protein composition, ligand density, and pore size. While migrating, cells in 3D are surrounded by ECM that acts as structural barrier. Consequently, cell migration in 3D environments during tumor formation [33, 81], immune response [64, 75], or tissue repair [42, 61] implies large deformations of cells during their penetration into interstitial tissues which pore size can be as small as a few microns, as well as a remodeling of the ECM in terms of changes in matrix stiffness and topography [69, 98, 101]. On 2D-substrates as well as in 3D environments, it is now well-known that cells respond to various parameters of the ECM such as its chemical composition [31, 74], mechanical stiffness [19, 27, 40, 49, 71, 101], and topological properties [5, 16, 22, 41, 54, 98]. As on 2D substrates, cells in 3D have to deform their surrounding matrix and thus exert forces to adhere and/or move forward [11, 13, 47, 62, 96]. However, in contrast to the 2D case, the traction forces generated by a cell moving in a 3D gel are transmitted through cell matrix attachments over all the surfaces of a cell in contact with the surrounding matrix [11, 101]. On 2D substrates, cell migration is seen as a multistep cycle which includes the extension of a membrane protrusion, the formation of stable attachments near the leading edge of the protrusion, and the translocation of the cell body forward [56, 97]. At the rear, this process leads to a release of

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adhesions and retraction. Actin polymerization governs the initial extension of the plasma membrane at the cell front [9, 83]. The interaction of the integrin family of transmembrane receptors with the extracellular matrix stabilizes the adhesions by recruiting signaling and cytoskeletal proteins [83]. Forces are thus transmitted to the substrate through these adhesion sites and thus help to move the cell body forward. Then the release of adhesions at the rear completes the migratory cycle allowing net translocation of the cell in the direction of movement.

The adhesion sites to which contractile forces are applied can be classified in different classes depending on their size and composition, mainly focal complexes, focal adhesions (FAs), and fibrillar adhesions [6, 36, 37]. At these sites, transmembrane integrin receptors are associated via their cytoplasmic domains with the actin cytoskeleton. This interaction with actin is mediated by a submembrane plaque, consisting of numerous cytoskeletal and signaling molecules. FAs are thus an important linkers between the external environment and internal cellular structures. They are typically 0.25–0.5 μm wide and 2–10 μm long. They appear to play a key role in mecanotransduction processes since they have both mechanical and biochemical signaling activities [2, 37, 48, 68]. The coupling of FA assembly with actomyosin contractility and traction forces [2, 76, 79, 90] makes FAs plausible candidates to modulate mechanosensing activity. A recent study at molecular level has shown indeed that mechanical stretching of single FA proteins can activate binding of other molecules [24]. However, the exact mechanism by which FAs are coupled to mechanical forces remains unclear.

In this context, traditional studies and standard cell culture conditions are made on 2D-substrates such as glass coverslips or Petri dishes. Compared to cells on 2D surfaces, cells in vivo or in model tissues show a drastically different morphology and behavior, including the lack of prominent stress fibers and FAs [20]. The complexity of 3D in vivo micro-environments led the scientific community to first use 2D surfaces to perform cell analysis. However, the recent development of a large panel of techniques such as traction force microscopy [72], fabrication of soft biocompatible substrates [27, 29], soft lithography [99], microfabrication [21, 86], and micromanipulation [1, 17, 63] allows us to control and uncouple the various physical parameters that cells may encounter in their natural environment.

Micro- and nano-engineered methods have thus provided new and important information about the regulation of cell–substrate interactions. For instance, micro-patterning of ECM proteins has been used to confine cell spreading on well-defined areas [16, 92, 93]. Using this method, it has been shown that the proliferation rate decreased and apoptosis increased on substrates composed of extracellular matrix-coated adhesive islands of decreasing size that restrict cell extension [16]. A similar method has succeeded in showing that ECM can guide the orientation of the cell division axis [92]. A second example is the use of microfluidic devices to locally modify the chemical environment of cells. The dimensions of standard microfluidic channels enable to induce multi-laminar flows within the main channel and thus deliver pharmacological reagents onto cells on a micrometer scale [88].

Additionally, nano- and micro- fabricated substrates have been used to probe the reciprocal relationship between cells and their external microenvironment. The nature of the ECM can modify the ability for cells to adhere, migrate, and even differentiate. The versatility of microfabrication techniques enables to culture cells in environments with well-defined mechanical, physical, and chemical properties. Here, we will focus on different aspects of mechanotransduction at the cell–matrix interface using modulated 2D-surfaces. We will particularly highlight how microfabrication can be used as a powerful tool to study in details the regulation of cell functions through the interactions of cells with their external microenvironment. In particular, we will present how the different nano- and micro-fabricated tools (1) can be useful to study the traction forces exerted by cells on their substrate; (2) can be used to modify the mechanical properties of the matrix, especially its stiffness; (3) provide well-defined conditions to analyze the cellular response to various nano- and micro-topologies.

2 Force measurements using microfabricated substrates

Traction forces, which are essential for cell movement, are exerted by cells and transmitted to the underlying substrate through focal adhesions and close contacts. The generation of forces on the ECM is important for a large variety of cellular processes such as cell adhesion and motility. Due to the size of adhesion sites and the range of cellular forces (nanoNewton scale), it appears crucial to develop substrates composed of micro- and nano-force sensors. Such substrates should allow the detection of forces with a subcellular spatial resolution, be biocompatible and allow standard surface coatings of ECM proteins to promote cell adhesion.

In the 1980s, Harris et al. [44] were the first being able to qualitatively visualize cellular traction forces as visible wrinkles on a deformable silicone rubber substrate, reflecting the strong traction forces exerted by motile fibroblasts parallel to the direction of locomotion (Fig. 1a). These sensitive membranes were formed by pouring liquid silicone rubber onto a glass coverslip. Then a brief exposure

to a flame cross-linked a thin skin of rubber (1 μm thickness) on top of the lubrificating fluid silicone layer [10, 44, 45]. This extremely smart and powerful technique has been used with success to show that fibroblasts were able to deform the thin cross-linked layer and thus study the compressive forces exerted by those cells. However, although an elegant approach, the method has several limitations such as the complex relationship between the size of the wrinkles and the amplitude of the generated forces.

Based on the same principle, more recent techniques have been developed to achieve a better spatial resolution, especially at individual adhesion sites [26, 65]. The technique, called traction force microscopy, is based on the use of elastic gels or membranes that avoid the formation of wrinkles. For instance, a polyacrylamide (PA) gel with a variable degree of cross-linking allows easy manipulation of substrate rigidity and a surface onto which extracellular matrix proteins can be conjugated to provide optimal cell adhesion [53, 71]. Fluorescent beads are embedded into the gel and are used as markers of deformation (Fig. 1b). Quantification of forces can be made through large scale matrix computation that converts maps of substrate deformation (detected as local bead movements) into maps of traction stress (force per unit area) [25, 26]. This inverse operation does not lead to unique solutions and requires assumptions about the force distribution patterns. Several groups have thus developed various strategies to solve the problem of computing the traction field from the observed displacement field [3, 12, 25].

Since the beads are randomly distributed inside the gels, an elegant way to limit the difficulty of the inversion problem was to use regular arrays of embedded markers [2, 82] (Fig. 2a). Such substrates were prepared by microfabrication techniques based on a combination of electron-beam lithography and "soft" lithography. Briefly, the preparation of the micropatterned surfaces was carried out in different steps. First, the negative pattern was prepared using standard optical or electron-beam lithography on solid substrates (Fig. 2a). The solid substrates and their photoresist or polymethylmethacrylate (PMMA) pattern were then used as a mould for patterning the surface of the elastomer (PolyDiMethylSiloxane (PDMS)) before its reticulation. For instance, Si wafers were prepared by spin-coating photoresist polymer whose final thickness was chosen at 0.3 μm. The silicone elastomer was mixed with the silicone elastomer reticulating agent in a ratio leading to a very soft gel (Young's modulus of $\sim$15 kPa), allowing cells to significantly deform the substrate. The silicone mixture was poured on glass coverslips and the Si–resist moulds were put in contact with the elastomer in order to imprint the topographic pattern onto the upper layer of the elastomer. The thickness of the resist that determines the depth of the topographic patterns on the elastomer was thin enough to avoid a possible influence of topography on cell adhesion but allows the optical detection of the deformations of the elastomer (Fig. 1c). In this case, the calculation of the forces is similar to the previous method using embedded fluorescent beads into PA gels but the regular positioning of the markers reduces the number of solutions in the determination of the traction force field [82].

This approach has provided new insights into the regulation of traction forces, their relationship with the formation of focal adhesions, the role of ECM adhesiveness and stiffness. For instance, as fluorescent beads can be used as markers in the gel, dual channel fluorescence microscopy has permitted the correlation of traction stresses in relation to the spatial localization of either GFP-fused FA proteins. Surprisingly, it has been shown by using GFP–zyxin as an adhesion marker that small nascent FAs under the leading edge of migrating cells exert stronger traction stresses than large mature FAs [4]. This inverse relationship is unique to the leading edge of motile cells and is not observed in the tail (Fig. 3b). On the opposite, in relatively stationary cells, there is a positive correlation between the traction force and the size of the FAs [2]. By combining living cell imaging of GFP–paxillin and the micropatterning of elastomer substrates, the authors demonstrated that traction forces are correlated with the orientation of FAs and also linearly

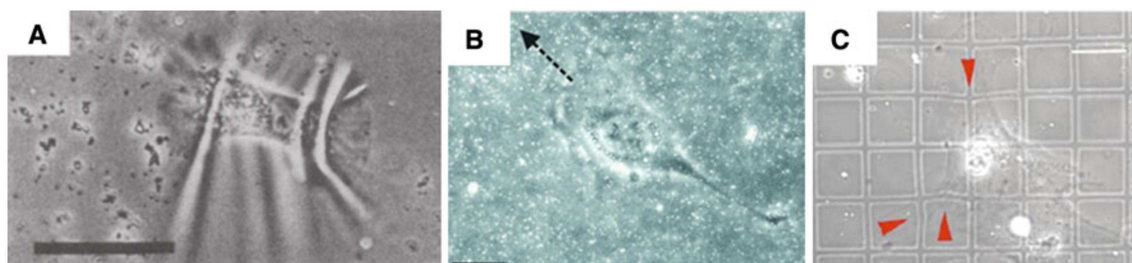

Fig. 1 Forces exerted by cells on flexible continuous substrates. **a** Wrinkles on a silicon sheet induced by fibroblast (adapted from [44], Reprinted with permission from AAAS). *Scale bar* = 50 μm; **b** Deformation of PA gels visualized by embedded fluorescent beads induced by the migration of a 3T3 fibroblast (adapted from [65], with permission from Elsevier). *Scale bar* = 20 μm; **c** Traction forces exerted by a fibroblast on micropatterned flexible substrate (adapted from [2] by permission from Macmillan Publishers Ltd.). *Scale bar* = 30 μm

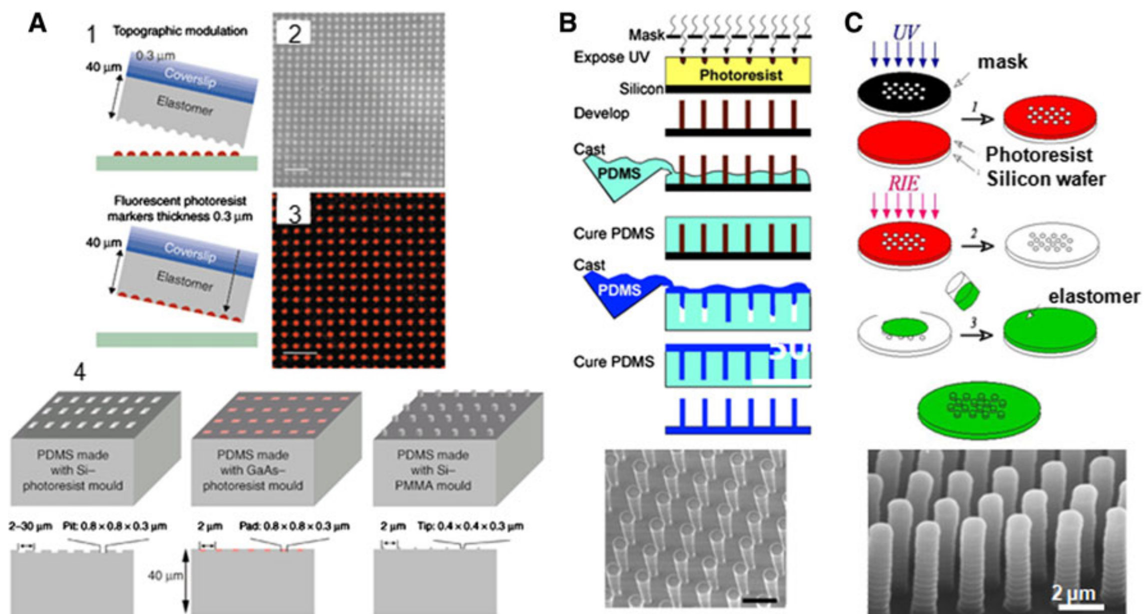

Fig. 2 Microfabrication processes to obtain deformable substrates. **a** Example of micropatterned PDMS gel with topographical features: peeling off the elastomer from the Si mould results in a topographic modulation with a depth of 0.3 μm; 2 and 3, Phase contrast and fluorescent images of the micropatterned substrates (adapted from [2] by permission from Macmillan Publishers Ltd.). **b** Schematic drawing of the method used to fabricate PDMS micropillars using soft photolithography (adapted from [90]) "Copyright (2003) National Academy of Sciences, U.S.A.". Scale bar = 30 μm. **c** Schematic drawing of the method used to fabricate PDMS micropillars using deep reaction ion etching and soft lithography

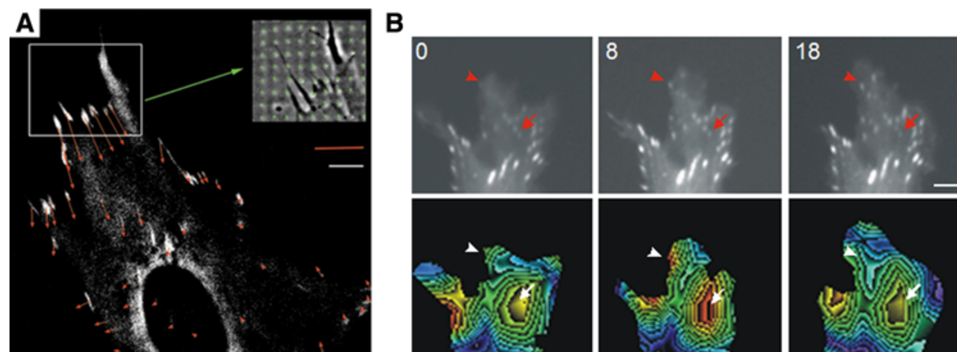

Fig. 3 Relationship between FAs assembly and traction forces. **a** Fluorescence image of non-motile fibroblast expressing GFP-vinculin. *Red arrows* correspond to the forces. *White scale bar* represents 4 μm and *red scale bar* 30 nN (adapted from [2] by permission from Macmillan Publishers Ltd.). **b** Traction forces exerted during the formation of nascent FAs at different times and color mapping of the resulting forces (*red* colors represent high forces) (adapted from [4], with permission from Elsevier)

increased with their area, indicating a constant stress (force divided by the FA area) of around 5.5 nN/μm^2 (Fig. 3a). Assuming that traction forces are correlated with acto-myosin contractility, these differences in the traction force–FA size relationship under both conditions may be related to the connection of FAs with actin stress fibers. In non-motile cells, if actin filaments are distributed over several mature FAs, the force generated by the actin filaments may be distributed to multiple FAs whereas actin filament may be related to less adhesion sites for a nascent FA. This could explain why a nascent FA may sustain more force transmitted through the actin filament. Furthermore, as ECM adhesiveness and stiffness modulate traction forces, it would be interesting to directly compare motile and non-motile cells on substrates sharing similar chemical and physical properties.

Assuming that forces are linked to FA formation, it might be difficult to use flexible continuous susbstrates since forces propagate within the substrate whereas FAs make discrete adhesion sites. Alternative methods can thus be useful to directly measure traction forces exerted by individual FAs. Instead of using uniformly flexible substrate, an innovative approach has been the use of micromachined cantilevers as force transducers on silicon wafers [34]. Cells

adhere and exert forces on micrometer-sized pads at one end of the flexible cantilever, causing displacements that are detected with high precision on a light microscope. Unlike flexible sheets in which strain propagates across the surface and requires sophisticated computational analysis for the calculation of traction forces, strains are confined to individual cantilevers and forces can be easily calculated by multiplying the spring constant of the cantilever by the distance of movement. However, the device is difficult to construct and the surface topology can exert some effects on cell migration. Moreover, the spatial resolution is limited by the density of cantilevers and the detection of forces is limited to one dimension—perpendicular to the axis of the cantilever. To overcome these limitations, a similar approach has been developed using substrates composed of flexible arrays of micropillars [28, 73, 77, 78, 90]. Those pillars can be fabricated in various materials such as silicon wafers [73, 78] or PDMS [40, 66, 77, 90]. In the case of silicon wafers, the basic microfabrication process consists first on a deposition of a thin layer of photoresist on a silicon wafer to create a temporary etching mask, and then the uncovered paths of the silicon wafer are etched up to the desired length. High aspects ratios ($\sim$1:100) can be achieved with this method in a close packing geometry. Since PDMS has a 10^4 times lower Young's modulus than silicon, the use of PDMS micropillar substrates permits to fabricate pillars with lower aspect ratio to detect nano-Newton forces exerted by cells and makes the fabrication process easier and more rapid than silicon substrates. Various methods can be used to fabricate such substrates. These devices are made from PDMS rubber formed from a microfabricated mold. A negative replica of the array was fabricated either by standard photolithography [90] or by deep reactive ion etching after a photolithography step [28], was then silanized (to make them anti-adhesive) and covered with (?) PDMS (Fig. 2b, c). After curing, PDMS replicas were peeled off.

In these cases, the deflection of each post gives a direct measurement of the local force exerted by the attached cells independently of the forces acting on the neighboring posts. The pillars act as simple independent springs and the linear theory of elasticity gives their deflection. For a cylinder of radius r and length L bent by the application of a force F, it leads to the following formula:

$$F = k.\Delta x = \left({}^{3}\!/_{4}\pi E\frac{r^4}{L^3}\right)\Delta x \quad (1)$$

where E, k, and Δx are, respectively, the Young's modulus, the spring constant and the deflection of the post. For instance, pillars of 1 μm in diameter and 100 μm in height for silicon substrates lead to $k \sim 0.022$ N/m. As a comparison, PDMS pillars of around the same diameter and a height of 10 μm have a lower spring constant of 0.0045 N/m.

Cells are able to deform the micropillar substrates as they spread onto the top of the pillars (Fig. 4). Such devices permit to confirm previous observations made on flexible continuous gels such as the linear relationship between FA areas and traction forces [90] but it also leads to new insights into the regulation of forces through other ligands such as cadherin proteins [35, 55] or within a group of cells [28, 66, 75, 80] instead of individual cells. Using this technique, Nelson et al. [66] have shown that gradients of mechanical stresses generated within multicellular aggregates are correlated with cellular proliferation and can induce spatial organization of cell assemblies. In addition, force mapping within epithelial cell sheets showed that the strongest deformations were always localized at the edges. Hence the largest forces were mostly due to the mechanical activity of the edge of the monolayer and they were in average oriented normally to the monolayer edges.

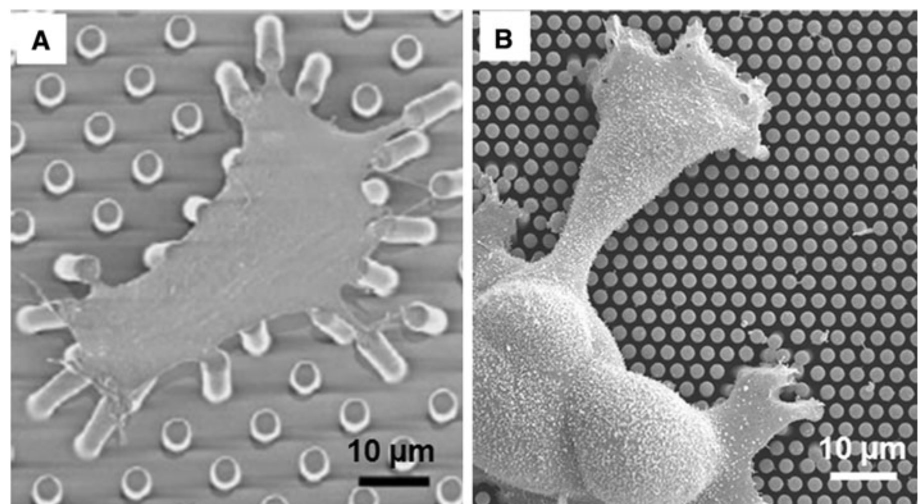

Fig. 4 Scanning electron micrographs of cells on PDMS micropillar substrates. **a** Fibroblast spread on the top of micropillars (adapted from [90]) "Copyright (2003) National Academy of Sciences, U.S.A.". Scale bar = 10 μm. **b** Cellular island of epithelial cells on PDMS micropillars. (adapted from [28]) "Copyright (2005) National Academy of Sciences, U.S.A."

A recent improvement of the traction force microscopy technique enabled to visualize the forces exerted by a growing epithelial cell sheet as well [94].

Micro-pillar technique appears as a versatile tool for various applications: (1) surface chemistry on PDMS pillars can be easily modified using micro-contact printing technique [16, 84, 90]; (2) different proteins can be delivered on the PDMS pillars [35]; they can be used in different configurations to measure forces exerted by tissues or cellular assemblies suspended between the pillars [52, 57]; and (3) different polymers can be used to tune the stiffness of the micropillars such as softer PA gels than PDMS [7].

The development of these recent tools to map the traction forces exerted by cells led to a deeper understanding of the regulation of cell–matrix interactions as well as the coupling between mechanics and biochemical signalling. Up to now, subcellular mechanical measurements at the size of adhesion sites have been performed. To bridge the gap between single molecule experiments and mechanical studies within living cells, the fabrication of mechanical nano-devices would be of particular interest to analyze forces exerted at single molecule levels and the impact of protein recruitment on force dynamics. Furthermore, as cells evolve in vivo in 3D environments, similar approaches using force sensors embedded in 3D matrices would allow us to better understand the mechanisms of cell migration in vivo.

3 Rigidity sensing during cell adhesion and migration

Most of the cells do not only apply forces but also respond through cytoskeleton organization to the resistance that they sense with regard to the mechanical properties of the matrix. Cell migration may be directed toward increased substrate adhesiveness [14], stiffness [27], and cell adhesion depends on substrate rigidity [43, 71]. Cellular tension can also be affected by changes in the mechanical properties of the environment [17, 59, 70, 76, 79].

In this context, the different methods previously described are also useful to modify substrate stiffness. In particular, the rigidity of PA-based hydrogels can be easily manipulated by varying the concentration of the bis-acrylamide crosslinker [50]. By controlling the extent of polymer cross-linking in the gels, their Young's modulus, E, can be adjusted over several orders of magnitude, from extremely soft to stiff, respectively from 1 to 100 kPa. Interestingly, these values are compatible with the stiffness of in vivo tissues [27]. Seminal studies on epithelial cells and fibroblasts exploited inert PA gels with a thin coating of covalently attached collagen [71]. This adhesive ligand allows the cells to attach to the substrate. Images of adhesion proteins such as vinculin revealed that on soft, lightly cross-linked gels ($E \approx 1$ kPa), diffuse and dynamic adhesion complexes were observed whereas stiff, highly crosslinked gels ($E \approx 30$–100 kPa) induced stable focal adhesions, close to those observed in cells cultured on glass substrates [71] (Fig. 5a).

Additionally, traction force mapping of fibroblasts on PA gels proves that they are able to pull harder on relatively stiffer substrates [59]. If a cell can generate higher traction, it has some internal structure that can sense the stiffness of the matrix on which it resides. Fibroblasts also preferentially move toward stiffer areas of a gel as well as areas of a gel that are compressed, a phenomenon analogous to chemotaxis in which a cell can move directionally toward a chemical signal. The movement has been so called "durotaxis." This finding may have relevance to areas from development to disease where cells migrate to various regions of tissue systems that may differ in material characteristics [69]. Moreover, theoretical models have been recently developed to predict the dynamics and the orientation of cells in response to changes in the matrix elasticity [8, 23, 67]. Let us notice that not all cell types appear to be sensitive to substrate stiffness, and not all mechanosensitive cell types respond similarly to changes in substrate stiffness. For instance, neurons appear to survive better on soft materials and exert less traction forces on stiffer substrates [15, 38]. A recent study has shown that mesenchymal stem cells can differentiate toward neurons, myoblasts, and osteoblasts on PA gels of different stiffnesses under identical serum conditions [30].

Micropillar substrates can be used as well to quantify the effects of substrate stiffness on cell traction forces [40, 75]. According to Eq. 1, surfaces of different spring constants can be designed by changing the geometrical parameters of the pillars (length and radius). For instance, the dimensions of the posts from 1 to 2 μm in diameter and from 1.6 to 6 μm in height lead to a wide range of spring constants, from 2 to 130 nN/μm. Such devices permit to confirm some of the previous observations made on flexible continuous gels such as the correlation in the size of FAs and the rigidity of the substrate [79] as well as the development of higher traction forces on stiffer substrates (Fig. 5b, c). Using this technique, the force-rigidity relationship of different cell types can be determined [40]. A first regime observed for low rigidities exhibits a linear variation of the traction forces exerted by either epithelial cells or fibroblasts on their substrate (Fig. 5c). Beyond this regime, a saturation plateau is observed for the highest spring constants of the pillars, characterizing the upper limit of traction forces that cells could exert on the substrate. These results showing a linear increase of the forces in a certain range of rigidity clearly imply that cell traction forces are regulated to achieve a certain deformation of the matrix. Additionally microfabricated substrates are helpful to

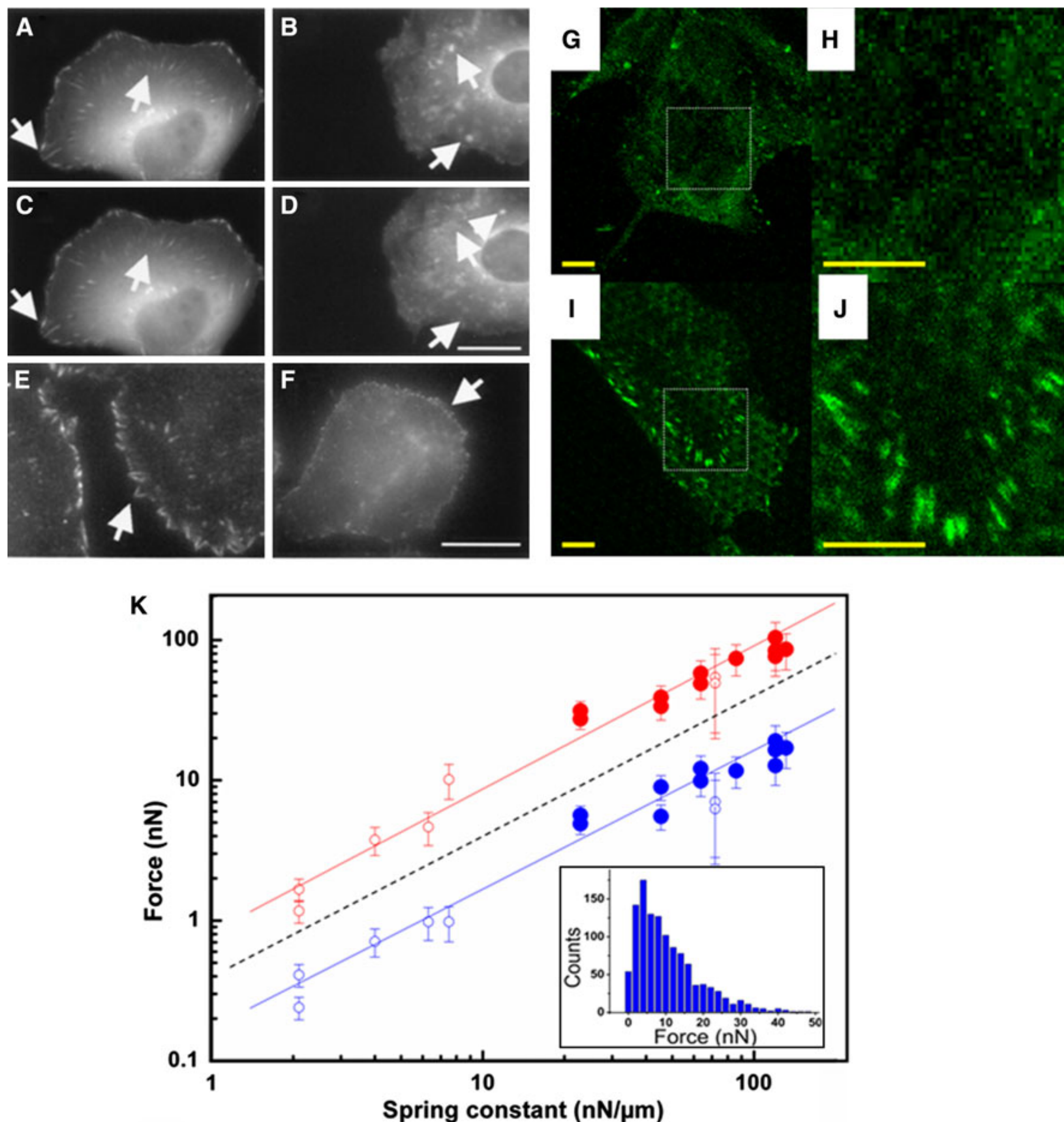

Fig. 5 Influence of rigidity on FA dynamics and forces. (**a**–**d**) Distribution of vinculin and phosphotyrosine in NRK cells cultured on PA stiff substrates (**a**, **c**, **e**) or soft substrates (**b**, **d**, **f**). Cells were injected with rhodamine-labeled vinculin and imaged over a period of 10 min. On more rigid substrates (**a**, **c**), vinculin is incorporated into elongated focal adhesions, which show only minor changes during the period of observation. On highly flexible gels (**b**, **d**), vinculin is localized at punctate structures of irregular sizes and shapes, many of which appear and disappear over a period of 10 min (*arrows*). **e** and **f**, Immunofluorescence of phosphotyrosine. Phosphotyrosine is localized at elongated focal adhesions in cells cultured on more rigid gels (**e**), and at punctate structures in cells cultured on highly flexible gels (**f**). (adapted from [71]) "Copyright (1997) National Academy of Sciences, U.S.A.". Scale bar = 30 μm. **g** and **i** Confocal images of immunofluorescence staining of the focal adhesion protein vinculin for epithelial cells on micropillar substrates with various rigidities, respectively, soft and stiff. **h** and **j** Details corresponding to the indicated regions in **a** and **c**, respectively. Scale bars correspond to 10 μm. **k** "Log–Log" plot of the force as a function of substrate rigidity for epithelial cell islands cultured on micropillar substrates. Mean and maximal forces (resp. <*F*> and F_{max}) within an island of cells are represented (resp. *blue* and *red* plots). *Hollow* and *filled symbols*, respectively, correspond to pillars of 1 and 2 μm in diameter. The slope of the *dashed line* is 1. (*Inset*) Typical histogram of force distribution (spring constant 64 nN/μm; adapted from [79], with permission from Elsevier)

engineer substrates that exhibit local anisotropic stiffnesses [80, 90]. By making substrates that consist of a dense array of micro-pillars of oval cross-section, one direction is made stiffer than the other. Such an anisotropic rigidity induces directional epithelial growth and guides cell migration along the direction of highest rigidity [80].

Cell-to-substrate interactions are controlled by a multitude of biochemical processes within the cell, which

comprise an overall regulation mechanism. This mechanism can work in two alternative ways: it maintains either certain deformations or certain forces. Theoretical modeling shows that the regulation of cell adhesion under stress can also lead to a linear relationship between forces and stiffness [67]. However, the regulation of forces and adhesion sites on substrates of various stiffnesses remains an open question. In particular, the determination of mechanosensing components within cells is crucial to better understand the contractile probing of cells. We need to bridge the gap between single molecule experiments exploring for instance the interaction of individual myosins with actin filaments and the collective behavior of myosins on actin stress fibers to sustain and exert traction forces within living cells. Furthermore, special attention should be given to rearrangements of proteins under mechanical forces such the opening and exposure of cryptic sites [24] as well as reorganization of ECM proteins [58, 85].

Future experiments on rigidity sensing should also take advantage of micro-structured substrates to fabricate rigidity gradients or pillars of various stiffnesses on the same chip [39].

4 The influence of topography on cell adhesion and migration

Extensive studies that are important to understand cell–matrix interactions have been done on traditional 2D surfaces. However, cells in vivo experience complex environments that have various geometric and physical properties. By creating precise surface structures, micro-nano-fabrication is helping in understanding cell interactions with topographical features. For instance, the spacing between mechanical obstacles has been found to be a critical parameter in cell migration [89]. It has been shown that these topographical cues, such as lines [21, 95], ridges [51], columns [32, 87], or pits [5] can guide cell adhesion and migration.

The development of numerous strategies to analyze the cellular response to substrate topography has provided new insights into the interactions of cells with their micro-environments, especially in terms of cell shape, cytoskeleton organization, and FAs remodeling [5, 32]. Cells on substrates composed of pillars or pits exhibit spindle shape and pseudopodial protrusions, more akin to the in vivo situation. It has been suggested that these pseudopods can insert into gaps in 3D matrices and serve as anchoring points to pull the cell body [60]. The ability of cell penetration in 3D-environments as well as the success of tissue engineering scaffolds require multiple balances between integrin activity, cell contractility, proteolysis, and matrix rearrangements and depend on matrix pore size [46, 101]. However, our knowledge concerning how cells detect, move over long time scales and respond in terms of cytoskeleton reorganization and formation of adhesive contacts in a 3D environment is still limited. Systematic studies of cell migration in well-defined topographical substrates appear as a useful tool to mimic some of the cellular processes involved in a 3D matrix.

Micro-scale features have an impact on cell migration. For instance, cell motion dynamics depends on the geometrical parameters of the substrate within 10 μm size micro-pillar substrates (Fig. 6a, b). The analysis of cell trajectories points out that substrate topography and physical constraints slow down cell movements but increase their persistence [41]. Furthermore, recent studies have shown that the organization of the actin cytoskeleton and FAs is crucial for the cell to orient and migrate through micron-sized patterns [5, 32, 41]. In particular, the life-time of FAs increases on micropillar substrates and myosin II activity and cell contractility play an important role in the migration processes. Moreover, Clark et al. [18] demonstrated that using a step could inhibit the progression of fibroblasts depending on height comprised between 1 and 10 μm (Fig. 6c, d). Consequently, as previously observed for rigidity sensing, microfabricated substrates permit to induce topographical changes within the same substrate and thus to study transition states in cell shape and migration between a 2D surface and a micro-textured substrate. As micro-patterned surfaces aim at mimicking a basic structural element of a 3D environment, such studies may provide information on mechanisms governing 3D migration and relevant pore sizes in future tissue engineering scaffolds that encourage cell migration and penetration.

Furthermore, nanoscale features have also an influence on cell adhesion and shape. In particular, nano-grooves within the substrate that mimic surfaces encountered by cells in vivo induce an alignment of epithelial cells along the preferential direction of the lines (Fig. 6e, f). It appears that nanoscale features can serve as substrate guidance because cell adhesion as well as cellular protrusions such as filopodia (Fig. 6f) follow the orientation of the substrate. Moreover, smooth muscle cells on poly(methyl methacrylate) (PMMA) and poly(dimethylsiloxane) (PDMS) surfaces comprising nanopatterned gratings with 350 nm linewidth, 700 nm pitch, and 350 nm depth are also significantly oriented along the lines [100]. Interestingly cytoskeletal structures are correlated to the orientation of the cells as well as the nuclei. Nanoimprinting technology could be a useful tool to study in cell–substrate interactions and for the development of medical devices with nanoscale features. Another interesting technique to better control ECM architecture is the electrospinning of nanofibrous structures [102]. This enables to engineer cellular scaffolds and control their architecture in terms of geometry,

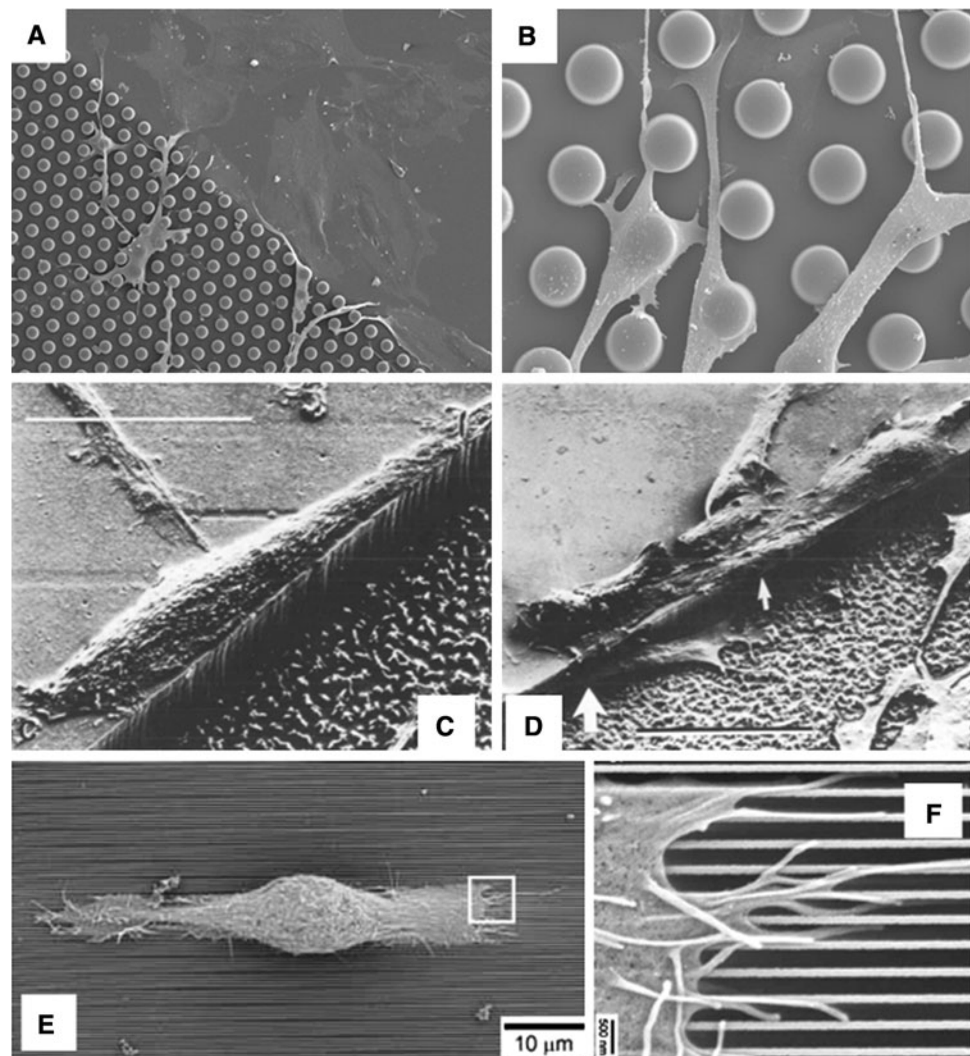

Fig. 6 Scanning electron micrographs showing the influence of micro- and nano-scale patterns on cell adhesion. **a** and **b** 3T3 fibroblasts spread on rigid PDMS micropillar substrates (pillar diameter = 10 μm). Spindle shapes are observed on micro-textured substrates as compared to flat PDMS substrates (adapted from [41], with permission from Elsevier). In **c** and **d**, cells at 10 μm steps are seen to align at both upper and lower surfaces, *small arrow* in **d** indicates the cell margin at the upper surface folded over the step edge, *large arrow* shows cell at lower surface in contact with the step wall. *Bars* = 40 μm (reproduced from [18]). Cells cultured on patterned lines with 400 nm pitch. **e** Cell aligned along nanostructured substrate. **f** Filopodia extend along the top of ridges and bottom of grooves (reproduced from [91])

morphology or topography. However, the cellular responses in terms of cytoskeleton dynamics and protein clustering are still far to be understood. Combining well-controlled surface chemistry, drug delivery, and topological features would be helpful to understand the respective influences of the physical properties of the substrate and biochemical cues.

5 Conclusion

By using nano- and microfabrication tools, we can better understand the role of physical properties of the substrate, such as topography and rigidity, on cell adhesion, migration and even differentiation. Since cell adhesion, migration, and differentiation implicate different time scales, adapting the properties of the substrate could lead to define optimal conditions for operating specific cellular functions. Given the profound effects on cell shape, adhesion, and migration, microfabricated features also represent an important factor in the engineering of artificial tissues.

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