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Current Opinion in Cell Biology

Transduction of cell and matrix geometric cues by the actin cytoskeleton

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Abstract

Engineered culture substrates have proven invaluable for investigating the role of cell and extracellular matrix geometry in governing cell behavior. While the mechanisms relating geometry to phenotype are complex, it is clear that the actin cytoskeleton plays a key role in integrating geometric inputs and transducing these cues into intracellular signals that drive downstream biology. Here, we review recent progress in elucidating the role of the cell and matrix geometry in regulating actin cytoskeletal architecture and mechanics. We address new developments in traditional two-dimensional culture paradigms and discuss efforts to extend these advances to three-dimensional systems, ranging from nanotextured surfaces to microtopographical systems (e.g. channels) to fully three-dimensional matrices.

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Current Opinion in Cell Biology 2021, **68**:64–71

This review comes from a themed issue on **Cell Architecture**

Edited by **Pekka Lappalainen** and **Pierre Coulombe**

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.ceb.2020.08.016

Keywords

Cell geometry, Cytoskeleton, Micropatterning, Nanotopography, 3D, Confinement.

Introduction

Cells in tissue reside within a complex niche containing many components, including other cells and the surrounding extracellular matrix (ECM). The ECM biophysically instructs cell behavior through its three-dimensional structure, mechanics, and geometric placement of adhesive sites. The resulting constraint on cell shape regulates a wide variety of processes, including proliferation and differentiation [4–6], as well as morphogenetic processes in development, wound healing, and disease [7–9]. Engineered ECM substrates have long been recognized as a powerful experimental paradigm for controlling cell shape and investigating shape-dependent phenomena, starting with the retrofitting of electron microscopy grids in the 1960s, continuing to microcontact printing in the 1990s, and progressing to today's sophisticated surface engineering technologies capable of controlling ECM geometry at the nanoscale [1–3]. Although the biological and technical details of these studies have varied widely, a remarkably common theme is the essential role of the cellular cytoskeleton—particularly the actin cytoskeleton—in transducing geometric cues into phenotype, with changes in cytoskeletal organization and mechanics driving activation of mechanotransductive signals that profoundly influence cell biology [10–13].

In this mini-review, we briefly highlight a selection of recent studies that have deepened the field's understanding of the relationships between cell/ECM geometry, actin cytoskeletal structure and mechanics, and the cell phenotypes that lie downstream. First, we discuss recent studies that have used 2D micropatterning to create new mechanistic insight into the role of the actin cytoskeleton in governing shape-dependent biology. Next, we cover efforts to extend these patterning technologies to three dimensions [14,15], starting with the introduction of microtextured and nanotextured surfaces to create 3D architectures within 2D topologies, including the increasing use of microchannels to simulate tissue confinement. Finally, we discuss efforts to extend geometric control to "true" 3D environments in which cells are fully encapsulated within a 3D network such as a polymer hydrogel [14,16].

New mechanistic insights from two-dimensional culture systems

There is a rich tradition within cell biology of using single-cell micropatterning to control cell shape in 2D culture. Although single-cell patterning is sometimes viewed as a relatively recent innovation, the concept dates back to at least 1967, when electron microscopy grids were repurposed to constrain single hepatocytes within "haptotactic islands." In some cases, hepatocytes spread to adopt the geometry of the island, whereas in others, cells were observed to migrate and divide, offering the opportunity to track clonal populations [1]. Even this early study did not overlook the power of micropatterning to facilitate parallelization and standardization, with the author noting the

ability to track more cells over longer periods of time than traditional time-lapse approaches. More than two decades later, alkanethiol-based microcontact printing was famously applied to produce cell-sized ECM islands to constrain spreading, leading to the seminal observation that cells can be switched between proliferative and apoptotic programs by controlling spread area [6,17]. More recently, cell shape has been used to control stem cell differentiation, with lineage trajectories dependent on seemingly minuscule details such as whether the cell perimeter consists of convex or concave segments [10,18].

A common and consistent theme throughout a large subset of these studies is the central role played by the cytoskeleton in sensing and integrating geometric cues, with shape-induced changes in actin cytoskeletal structure in particular altering front-back polarity, traction force generation, and placement of lamellipodia and other adhesive processes [11,12,19]. Recently, new mechanistic insight into this regulatory relationship has been gained by the integration of micropatterning approaches with other single-cell biophysical tools to more precisely dissect molecular and physical mechanisms through which adhesive geometry and cell shape regulate cytoskeletal organization and mechanics. As one example, we recently combined single-cell micropatterning, subcellular laser ablation, and mathematical modeling to investigate how actomyosin stress fiber (SF) viscoelastic properties depend on SF length. Here, micropatterning enabled us to standardize cell shape while also prescribing the location and length of specific SFs by compelling fibers to form across defined gaps in the ECM. We then measured SF viscoelastic properties by laser-severing individual SFs and tracking fiber retraction. We found that the elastic energy stored by a SF depends not only on its length but also the degree to which the fiber is physically networked to other SFs in the cell. Our experimental measurements could be accurately predicted by a computational model depicting the SF network as connected elastic cables [20]. In a subsequent study, we combined single-cell micropatterning and laser ablation to measure viscoelastic properties of dorsal SFs, transverse arcs, and ventral SFs, which are found in polarized, migratory cells. The use of crossbow patterns produced cells with standardized morphologies with clear front-back polarity and a tight range of SF lengths. We found that dorsal fibers bear little to no intrinsic prestress and that ventral SF mechanics depend on the mechanism through which these fibers are assembled. Specifically, when ventral SFs formed through the fusion of dorsal SFs and transverse arcs, they incorporated actin crosslinkers that function as "brakes" to increase viscous drag during retraction. This viscous drag fell significantly when ventral SFs assembled de novo from smaller actomyosin subunits, such as when cells were cultured on microlines that are too narrow to support arc assembly [21].

2D micropatterns have also been used to study the effects of geometric constraints on actin organization and dynamics during cell spreading and migration. In several past studies, cells had been observed to respond to cell-scale curvature in the extracellular environment by forming protrusions at positive curvatures and actin cables at negative curvatures [22–24]. However, it had remained unclear whether or how these principles operated at a larger, multicellular length scale. A recent study addressed this question using geometrically defined wound-closure assays and multicellular flower-shaped patterns. Notably, multicellular structures adapted their actin flow according to the sign of curvature encountered. Whereas convex curvatures promoted standard retrograde flow of actin, concave curvatures promoted an anterograde flow of actin toward the leading edge, culminating in the assembly of SFs that span nonadhesive regions and enable cells to spread across regions that lack underlying matrix (Figure 1a) [25]. In complementary work also aimed at understanding how cells spread across nonadhesive substrates, we used micropatterned rectangular frames to create defined spreading trajectories reminiscent of how cells engage 3D collagen fibers. The spreading path was found to depend both on the geometry of the pattern and the initial cell adhesive position. As cells spread, they assembled SFs that ran parallel to the leading edge and bridged nonadhesive area of the pattern. Because the trajectory of the leading edge was dependent on the initial cell adhesive position, the final arrangement of SFs in the spread cell reflected its spreading trajectory and encoded a sort of history or memory [26].

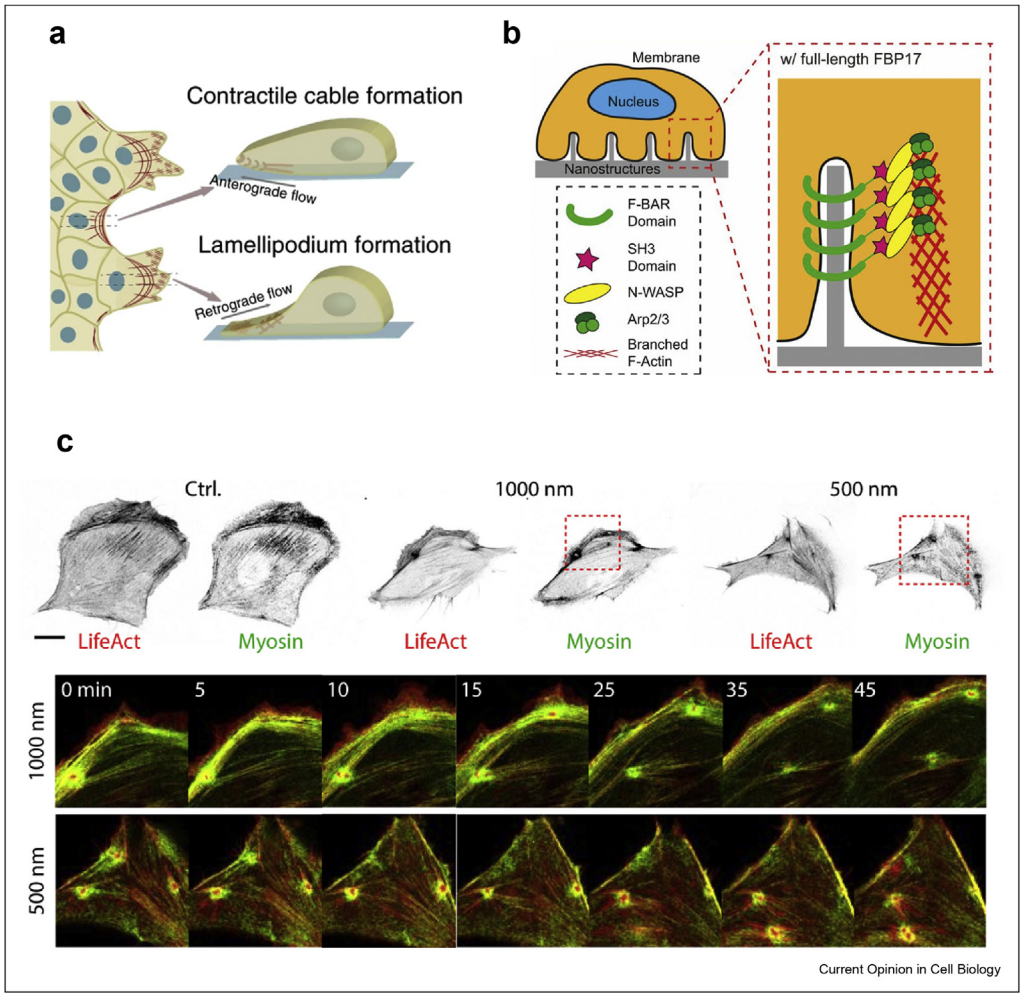
Control of cell and matrix geometry in three dimensions

While much continues to be learned from traditional 2D patterning strategies, a major acknowledged goal in the field is to define cell and matrix geometry in three dimensions, a topology that better represents many tissues [15]. We now discuss efforts to extend geometric regulation of cell behavior in 3D, progressing from 2D nanotextured and microtopographical substrates to "true" 3D matrix environments in which cells are fully embedded within ECM.

Nanotextured surfaces

At the molecular scale, cells encounter many ECM components as 3D, nanoscale structures, such as collagen fibers and fibronectin fibrils. Although these structures are much smaller than the cell, they can strongly influence biology by driving the assembly of supramolecular structures that govern adhesive signaling, such as integrin clusters [27–29]. Many studies have utilized patterning technologies to add nanotexture to simulate the nanotopography formed

Figure 1



Mechanisms of cell sensing of 2D substrate geometric cues. (**a**) Multicellular structures sense curvature by adapting actin flow at the leading edge, promoting actomyosin flow toward the cell edge at negative curvatures and retrograde actin flow at positive curvature. (**b**) Illustration of F-actin assembly around membrane curved by a nanotopographic feature. The protein FBP17 senses the curved membrane and recruits N-WASP and Arp2/3 to nucleate the assembly of branched actin around the feature. (**c**) Contractile myosin rings regulate the cytoskeleton and sensing of substrate topography. Top panel: On flat control surfaces, F-actin is organized into bundles, and there are no puncta. On electrospun surfaces featuring fiber diameters of 500 nm and 1000 nm, myosin rings form around actin foci as cells respond to the underlying topography, visualized as puncta in the images (red boxes). Bottom two panels: Time lapse imaging shows the formation and disassembly of myosin (green) and actin (red) foci. Reproduced with permission from Chen et al. [25] (**a**), Lou et al. [38] (**b**), and Di Cio et al. [39] (**c**).

by supramolecular ECM structures. These studies have systematically varied nanotopographical patterns and characterized the resulting cellular responses, with cellular responses generally depending on pattern geometry, periodicity, and other features. For example, parallel patterns such as gratings and grooves are frequently observed to induce cellular and cytoskeletal alignment with the patterned features [30,31]. Isotropic nanopost and nanopit topographies have also been observed to influence cell spreading and adhesive complex formation [32–34]. In a particularly notable recent study, Puliafito et al. utilized polarized laser beams to spatiotemporally deform otherwise static nantopographies to mechanically stimulate cells. In this system, the polarization pattern of the laser deforms photosensitive polymer micropillars beneath attached cells into an anisotropic pattern. The resulting deformation pattern induced cells to orient and align SFs along the direction of deformation. This system showed light-responsive materials could be used to observe and study biological response to dynamic topographies [35].

Previous work has postulated that nanotopography influences cell behavior through many different mechanisms, including through increased cell-ECM interaction along the surfaces of the features and

through cortical actin rearrangement from the curving of the cell membrane around nanotextured features [36]. Despite these hypotheses, the precise molecular mechanisms that underlie cell sensing of nanotopography have remained elusive [34]. Toward this end, one study examined cells cultured on nanopillar arrays of varying density to identify key mechanistic length scales. At high nanopillar densities cells attached and formed adhesions only on the nanopillar tops, as they would on a flat surface. Lowering the density led to engulfment of the nanopillars and more adhesions along the sides of the nanopillars, resulting in increased contact area and F-actin colocalization along the sides of the nanopillars. The authors associated these regimes with length scales of integrin clustering and migratory process formation [37]. In another study, cells cultured on nanopillars were found to assemble branched F-actin along the curved membrane adjacent to the features, leading to reduced assembly of SFs and mature focal adhesions throughout the cell. The altered F-actin assembly was accompanied by localization of Arp2/3 and formin-binding protein 17 (FBP17), which has a curvature-sensing F-BAR domain, at the ends of the nanopillars (Figure 1b). Cells expressing a truncated FBP17 mutant lacking the actin polymerization-inducing domain did not accumulate F-actin at the nanopillars, indicating that FBP17-actin binding senses the curved cell membrane and mediates the nanotopography-induced actin polymerization [38].

In a complementary study, cells cultured on electrospun nanofibers of varying diameters exhibited disrupted actin bundle assembly on increasingly narrow fibers. Time lapse imaging revealed that cells on narrow fibers (<1000 nm diameter) initially assembled actin bundles during early phases of spreading. However, during later, more contractile phases of spreading, these bundles coalesced into punctate actin foci as the narrowly confined adhesions failed to stably support parallel arrays of actin bundles. These foci were found to be surrounded by myosin rings and to sequester the actin-severing protein cofilin, leading to disassembly of the foci (Figure 1c). In other words, sensing of nanotopography is governed both globally by the cellular actomyosin network and locally by individual adhesions [39]. Combined, these studies suggest that nanotopography influences cell behavior by modulating the spatial organization of the actin cytoskeleton, through engulfment of features and curving of the cell membrane around features.

While the incorporation of nanotopographically textured substrates represents an important step toward creation of 3D architecture, such surfaces are incapable of capturing a number of defining features of 3D tissues, including steric hindrance of spreading and migration along all axes and the absence of externally imposed apical-basal polarity [14]. We now discuss efforts to overcome these limitations through the development of microtopographically patterned substrates and fully 3D matrices.

Microtopographical confinement

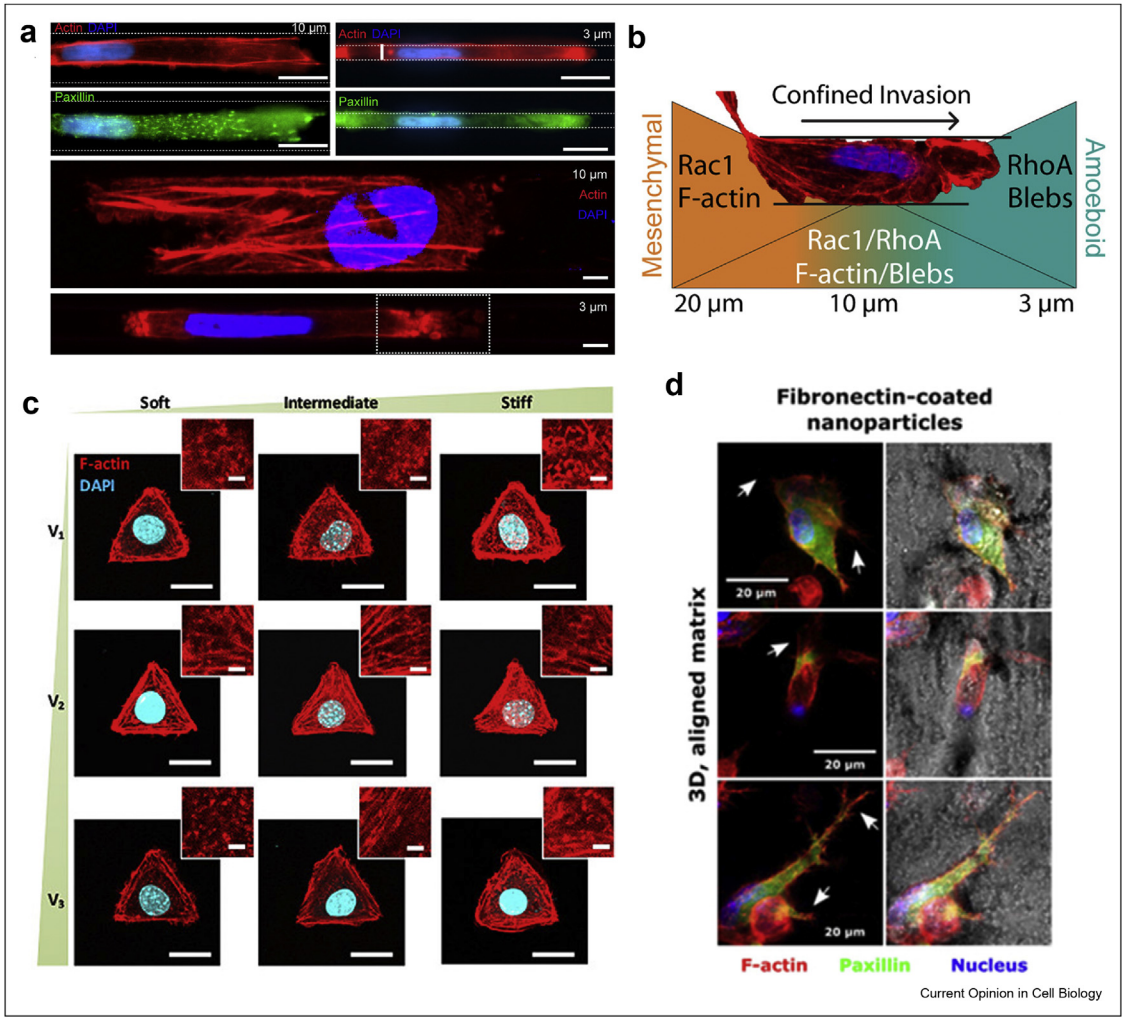
There are many physiological contexts, including development, inflammation, and cancer, in which cells must migrate through confined spaces in tissue. For example, tumor cells must squeeze through tight physical barriers imposed by cells and ECM to invade and metastasize. Because these confined features cannot be captured with 2D patterning tools and are challenging to standardize in 3D systems, microchannels have emerged as a valuable paradigm for studying confined migration. These devices model cells squeezing through tight spaces, with 3 or 4 walls physically confining cells and only allowing migration along the axis of the microchannel. Previous studies have shown that cells, especially tumor cells, reorganize their cytoskeleton to a primarily cortical distribution of actin to squeeze through the channels. The cells traversing the channels also have decreased numbers of focal adhesions and SFs compared with fully spread cells on 2D surfaces [40–43].

Recently, new mechanistic insight into how tumor cells navigate confined spaces in tissues has been obtained by measuring cellular viscoelastic properties during confined migration. For example, we recently used an open microchannel system in which tumor cells could be subjected to confined migration while still being accessible to atomic force microscopy measurements. Cells softened as they experienced confinement, with the actin cytoskeleton deploying away from the basal surface and toward the channel walls, potentially to support lateral traction forces. Confinement-induced softening was also accompanied by exclusion of YAP from the nucleus [44]. Confinement-induced changes in cell mechanics were also reported in a study with optical tweezer–based microrheology. In a spheroid invasion assay in a biomimetic collagen matrix, tumor cells at the tip of invasion stalks were found to have a higher viscosity than the cells in the interior of the stalk. Inhibition of FAK or actin polymerization diminished mechanical differences between leader and follower cells [45]. Both studies directly demonstrate that tumor cells can mechanically adapt to confined geometries through coordinated cytoskeletal rearrangements that produce cell-scale changes in viscoelastic properties.

Additional microchannel studies have incorporated more complete confinement, either by manipulating channel geometry or introducing a fourth wall. Cells fully confined within collagen microtracks displayed more adhesive contacts, faster migration, and greater matrix strains than their more partially confined counterparts. The increased migration speed of fully confined cells suggests that contractility is key in migration under full confinement

[46]. In narrow (3 μm width) PDMS microchannels, cells also exhibited increased migration speeds and displayed strong blebbing behavior at both their leading and trailing edges (Figure 2a). As cells squeezed into the channels, they underwent an ECM-induced transition from actin protrusion-driven mesenchymal to faster RhoA contractility-driven ameboid migration, accompanied by a loss of F-actin organization in the cell body (Figure 2a and b) [47,48]. Similarly, when *Dictyostelium* cells cultured in a sandwich paradigm were subjected to external pressure, the cells switched from actin polymerization-based pseudopod migration to myosin II contractility-driven bleb migration with recruitment of myosin II to the cortex [49]. Collectively, these studies indicate that increased confinement of cells because of either a smaller pore size or increased mechanical pressure stimulates cells to reorganize their cytoskeleton to a primarily cortical distribution and utilize an amoeboid, contractility-dependent mode of migration.

Figure 2



Effects of 3D geometry on cytoskeletal organization and cell behavior. (**a**) Confinement of cells within increasingly narrow microchannels significantly disrupts the organization of F-actin and focal adhesions. *Top 2 rows of images*: Cells traversing 10 μm-wide channels exhibit bundled F-actin and well-defined focal adhesions, as visualized by localization of paxillin (green) and actin (red). Conversely, cells confined within 3 μm channels lack clear focal adhesions or F-actin bundles. *Bottom 2 images*: Higher magnification imaging of F-actin reveals an organized actin in cells in 10 μm channels (Top), whereas cells in 3 μm channels have disorganized F-actin networks and display blebs at both ends of the cell (Bottom). The dashed box in the bottom image highlights multiple blebs at the leading edge of the cell. (**b**) Schematic illustrating how increasingly confined geometries can trigger a transition from mesenchymal migration to RhoA contractility-based amoeboid migration. 20 μm, 10 μm, and 3 μm indicate the width of the confining microchannel. (**c**) In microwells of independently controlled volume and stiffness, medium volume V2 promoted robust SF assembly while larger and smaller volumes (V1 and V3) did not. (**d**) In 3D hydrogels with aligned fibronectin-coated nanoparticles-based fibers, cells preferentially extended multiple protrusions parallel or perpendicular to aligned fibers. Left column: maximum intensity projections of confocal slices, with F-actin displayed in red, paxillin in green, and the nucleus in blue. Right column: brightfield with superimposed fluorescent images to show particle alignment. Arrows indicate cell protrusions in the plane of the fibers. Reproduced with permission from Holle et al. [47] (**a, b**), Bao et al. [52] (**c**), and Paul et al. [55] (**d**).

Fully 3D systems

While microtopographical systems have offered valuable insight into geometric regulation in 3D, an important goal for the field remains the development of engineering strategies amenable to "true" 3D geometries in which cells are fully encapsulated within ECM. Hydrogels composed of either synthetic or natural ECM materials allow for encapsulation of individual cells or spheroids to recapitulate the dimensionality of 3D tissues [16,50]. We conclude by highlighting efforts to extend geometric regulation of cell behavior to 3D hydrogel ECMs.

Micropatterning has recently been used to investigate the effects of 3D cell geometry and volume on cell behavior in a fully encapsulated setting. In one study, a silicon mold was used to create different prism-shaped wells of defined geometry and size on a hyaluronic acid hydrogel. Single cells were seeded into each well, and the wells were covered with a thick slab of hydrogel to achieve 3D encapsulation. The study identified an optimal range of niche volumes that promoted clear SF assembly and cytoskeletal organization, irrespective of niche projected area or height. Volumes smaller or larger than this range resulted in few SFs. In niches of equal volume but different geometries, cells with anisotropic geometries such triangular and rectangular prisms exhibited more SFs, focal adhesions, and actomyosin contractility compared with cells with isotropic geometries (cylinder, cube) [51]. In a subsequent study, the both the stiffness and volume of the niches were varied to perturb the combined effects of cell volume and substrate stiffness on cell behavior. Interestingly, irrespective of the stiffness of the hydrogel, cells cultured in the optimal volume range identified in the previous study always formed stable focal adhesions and SFs, while cells in volumes smaller than the optimal range did not (Figure 2c). In this setting, cell volume overrides substrate mechanics in guiding adhesion and mechanics [52].

Most 3D tissues are fibrous with structural heterogeneity and anisotropy. The orientation of ECM fibers affects ECM mechanics and cell-ECM interactions [53]. Hydrogels do not, in general, capture such highly structured topologies, necessitating the development of new materials to control fibril alignment and other topographies in 3D hydrogels [15,16]. A recently developed nanocomposite hydrogel system exploited embedded cellulose nanocrystals that align in response to magnetic fields to create an anisotropic topography. Myotubes cultured in these matrices showed strong co-alignment of F-actin and the nanocrystals [54]. Using a related approach, Paul et al. created aligned fibrils in 3D hydrogels using ECM protein-conjugated magnetic colloidal particles to study the effects of fibril alignment on cell behavior. Under the influence of an external magnetic field, the beads aligned to create parallel fibrils. Fibroblasts seeded in aligned matrices produced longer actin-based protrusions than cells in unaligned matrices, and the protrusions were aligned either parallel or perpendicular to the fibril direction (Figure 2d). Inhibition of myosin II in fibroblasts in the aligned matrices abrogated the alignment of protrusions, indicating that myosin II-regulated cell contractility may play an important role in cell topography sensing [55]. Jointly, these studies provide tools for creating aligned topographies within hydrogels and highlight the importance of the actin cytoskeleton in mediating these responses.

Conclusion

Engineered culture systems continue to advance our understanding of how cell and ECM geometry controls cell behavior, while reinforcing the central role played by the actin cytoskeleton in transducing geometry into phenotype. As described in this brief review, the past several years have seen important advances in the field's mechanistic understanding of how 2D matrix geometry acts through the cytoskeleton to influence phenotype. There has also been exciting progress toward advancing these ideas to 3D. In addition to the areas discussed here, a number of other, equally interesting challenges are being pursued. As one example, significant effort is being devoted to extend geometric regulatory concepts to multicellular structures, including those containing heterogeneous cell populations [56,57]. In these settings, it will be important to understand how cytoskeletal structure and mechanics are regulated not only in a cell-autonomous fashion but as coordinated units across multiple cells [58]. When married to the advanced fabrication strategies described here, it may soon be possible to create microscale, three-dimensional tissues with a variety of component cell types positioned as they might be in living tissue. These "engineered organoids" should lend great new insight into biological processes such as development, morphogenesis, wound healing, and cancer and provide powerful new enabling tools to tissue engineering and regenerative medicine.

Conflicts of interest

The authors declare that there is no conflict of interest.

Acknowledgements

This work was supported by awards from the National Science Foundation (Graduate Research Fellowship to VDT) and The National Institutes of Health (R01GM122375 to SK).

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