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Single-cell manipulation by two-dimensional micropatterning

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Cells are highly sensitive to their geometrical and mechanical microenvironment that directly regulate cell shape, cytoskeleton and organelle, as well as the nucleus morphology and genetic expression. The emerging two-dimensional micropatterning techniques offer powerful tools to construct controllable and well-organized microenvironment for single-cell level investigations with qualitative analysis, cellular standardization, and *in vivo* environment mimicking. Here, we provide an overview of the basic principle and characteristics of the two most widely-used micropatterning techniques, including photolithographic micropatterning and soft lithography micropatterning. Moreover, we summarize the application of micropatterning technique in controlling cytoskeleton, cell migration, nucleus and gene expression, as well as intercellular communication.

Keywords: Two-dimensional micropatterning; cytoskeleton; cell migration; extracellular matrix; intercellular communication; gene expression.

1. Introduction

In multicellular organisms, extracellular microenvironments create diverse conditions that exert spatial extension/confinement on single cells *in situ*.[1–3] In recent years, growing evidence highlights that extracellular physical features, e.g., extracellular matrix (ECM) geometry,[4–7] adjacent cells extrusion,[8,9] substrate stiffness,[10–12] extracellular fluid viscosity,[13–15] and extracellular forces,[16,17] are determinants of cell behavior and fate, which are considered equally important as acknowledged biochemical factors. These extracellular physical cues act on the membrane receptors, mechanical sensing proteins and proximal-membrane cytoskeleton to trigger downstream signaling pathway.[18–22] Conventional two-dimensional (2D) homogeneous substrates were generally applied for cell culture, on which cells spread and migrate without any confinement, which is distinct from the crowded and complicated microenvironment *in vivo*.

The emergence of 2D micropatterning technique has provided unique opportunities to create a specific ECM environment for standardization of cell morphology,[23–26] simulation of physiological environment,[27–29] and high-throughput analysis.[30–34] A common principle of cell-patterning technique is the fabrication of adhesive region and non-adhesive region on the 2D substrate.[35,36] Cells cannot spread across the adhesive–non-adhesive boundaries, instead, they are confined in adhesive regions. Varieties of strategies have been developed to realize the adhesive-nonadhesive alternate substrates with 2D spatial confinement, which not only regulate the cell morphology but also affect the intracellular cytoskeleton, organelles and nucleus.[37,38]

In this review, we first introduce the principle and properties of general 2D micropatterning techniques, including photolithography-based micropatterning and soft lithography micropatterning. We then focus on the application of 2D micropatterning techniques in single-cell manipulation, cytoskeleton targeting, cell migration, nucleus morphology and gene expression, and intercellular communication. Lastly, we summarize the current technical challenges and future progress in micropatterning technique in biological applications.

2. Micropatterning Technique

2.1. *Photolithographic micropatterning*

The principle of micropatterning in cell culture is to fabricate a substrate to which cells can adhere on specific areas. Photolithography, as the workhorse of microfabrication and semiconductor industry, plays a crucial role in 2D micropatterning for biological applications.[39–41] Photolithography is often carried out by projecting a pattern on a photomask onto a photoresist film spin on a substrate.[42,43] UV light is exposed through the mask to modify the photoresist on specific regions. Combined with the following surface modification process, i.e., surface passivation and protein coating, photolithographic micropatterning creates a surface where cells adhere to protein-coated regions and are repelled by non-adhesive regions. The fabrication process is shown in Fig. 1(a). Polyethylene glycol (PEG) and its derivatives (e.g., poly-lysine-PEG and fluorescence probe-labeled PEG), which are inherently cell repellant due to a low level of non-specific binding of proteins and other macromolecules,[44–48] have been used from general non-fouling coatings to cell patterning, biosensing, microfluidics, etc. Photolithography micropatterning provides a fast, easy-to-use, and high-resolution 2D micropatterning strategy. To further extend the application of micropatterning from 2D to 3D, two-photon laser scanning (TPLS) lithography technology uses two-photon excitation to confine focus to a micron scale, while leaving the area outside the focus unchanged. Using this technology, 3D patterns of biochemical signals can be generated in PEG hydrogels, overcoming the limitation that traditional photolithography technology can only construct 2D micropatterns.[49] Indeed, photolithography is an intrinsically expensive process because of the equipment, such as the highly demanding processes required for the fabrication of microelectronic devices.[50,51]

2.2. *Soft lithography micropatterning*

Soft lithography, also known as micro-contact print (μCP), is another widely used 2D micropatterning technique (Fig. 1(b)), which provides access to generate specific surface modification for single-cell manipulation.[52] Vary from photolithography-based

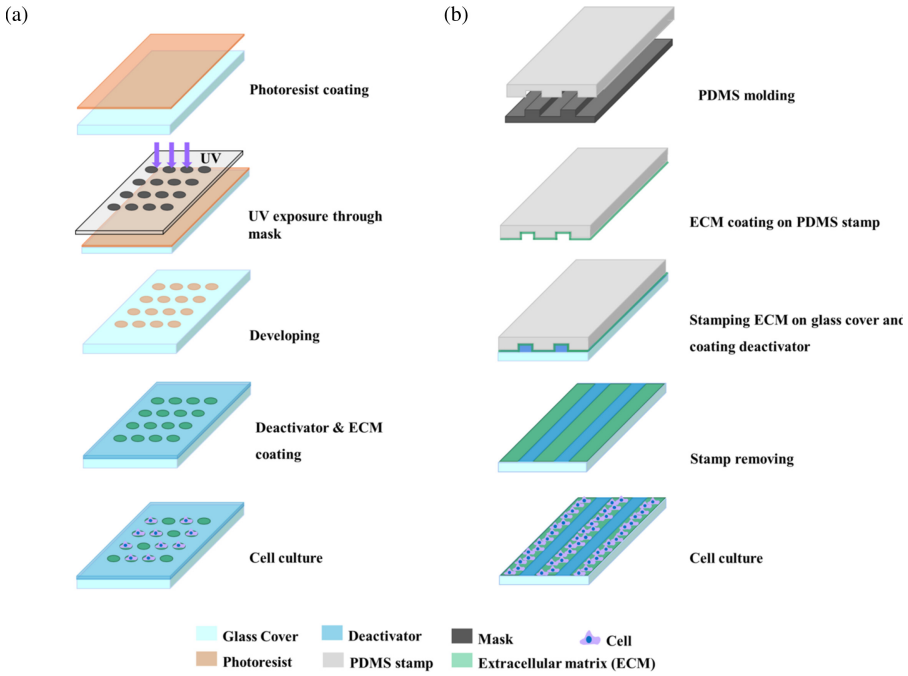

Fig. 1. Fabrication of micropatterns. (a) Schematic diagram of substrate preparation based on photolithographic micropatterning. (b) Schematic diagram of substrate preparation based on soft lithography micropatterning.

micropatterning, the key element of soft lithography is an elastomeric stamp with patterns as relief structures on its surface.[53] The stamp is typically fabricated by casting a liquid precursor, mostly using poly(dimethyl siloxane) (PDMS), against a master whose surface has been patterned with the complementary structures. This stamp is incubated with protein solution as the "ink", followed by transferring protein layer from PDMS to substrates through press. Regions without a protein layer should be coated with protein repelling regents.[54,55] Soft lithography is low-cost, experimentally convenient and has emerged as an applicable technology for multiple applications that include cell biology, microfluidics, lab-on-a-chip, microelectromechanical systems and flexible electronics/photonics.[56] At present, the master molds of soft lithography have been manufactured by combining traditional photolithography with two-photon polymerization, achieving high-resolution complex three-dimensional (3D) structures by convenient operation.[57] Furthermore, additive manufacturing (AM), also known as 3D printing, is used to produce lab-on-a-chip master molds, which are combined

Table 1. Advantages and disadvantages of the micropatterning technique.

	Advantages	Disadvantages
Photolithography	– Simple-established process. – Easy to change the pattern through photomask design. – High resolution and precision for making ultrastructure.	• Unfriendly to small batch production due to the high cost of the device and materials. • Slow manufacturing speed. • Cell manipulation is limited to 2D substrates. • Used photoresists and organic solvents might be cytotoxic.
Soft lithography	– Low cost and simple make. – Suitable for planar or non-planar 2D or 3D structures. – The chemical inertness of polymeric stamps makes them reusable. – The strong projection ability of polymeric stamp is conducive to optical inspections.	• The elasticity and thermal expansion of polymeric stamp limits pattern accuracy. • The softly of polymeric stamp limits its aspect ratio. • Polymeric stamp has shrinkage and deformation during curing.

with elastomeric stamps for soft lithography to simplify the process.[58]

3. Application of Micropatterning in Single Cell Control

Since the emergence of 2D micropatterning techniques, they have been widely used in biological research from tissue engineering to single-cell manipulation. The key point of its application in single cell-manipulation is the pattern designing according to target cellular components. The following content introduced some typical and elegant designs and applications of 2D micropatterning in single-cell manipulation.

3.1. *Micropatterning regulates cytoskeletons*

Intracellular cytoskeletons, including actin filaments, microtubules and intermedia filaments, play crucial roles in various cellular processes [59–63] Actin cytoskeleton, as the most highly-dynamic component, dominates the generation of intracellular force, cell-matrix/cell adhesion and cell locomotion, etc.[64–66] Microtubules, as the principal rigid construction in the cell, determine the maintenance of cell shape, intracellular transport and cell division.[67,68] Intermediate filaments, e.g., vimentin and keratin, that extend from the nuclear into the cytoplasm, not only support the rigidity of cells, but more importantly, are related to other cellular processes, such as cell differentiation and intracellular transport.[69,70] All these cytoskeleton components are popular targets of 2D micropatterning-mediated regulation.

Actin enriches at most near-edge structures of the cell,[71] and choreographs a highly dynamic interplay with the matrix.[72,73] Extensive researches focused on the effect of spatial confinement on actin cytoskeleton and revealed various underlying mechanisms based on 2D micropatterning. A recently-found intriguing phenomenon is that on isotropous circular pattern, single cell actin cytoskeleton formed a chiral left–right asymmetry pattern, which arises from α-actinin1-dependent self-organization of the actin cytoskeleton on the pattern (Fig. 2(a)).[74] Further, this single-cell left–right asymmetry was generalized to the multicellular system in which a group of actin assembly regulators on chirality of individual cells and collective cells were revealed, including mDia1, profilin1, CapZβ and $\alpha-$actinin1.[75] This actin-driven cell chirality may underlie tissue and organ asymmetry in development. Asymmetrical patterns induced asymmetrical actin dynamic. For instance, Xing *et al.* found a "secondary" ring of actin cytoskeleton distributed along the periphery of the circular pattern, which was induced by the actin retrograde flow and spatial confinement (Fig. 2(b)).[76] Besides isotropous patterns, asymmetrical patterns were also applied in the investigations of actin cytoskeleton characteristics. Elena *et al.* monitored the dynamics of the stress fiber network during cell spreading by seeding U2OS cells on rectangular micropatterns with distinctive opening positions, revealing that the geometry of the ECM and the initial adhesion position determine the process of cell spreading and stress fiber network "memory" (Fig. 2(c)).[77] Weißenbruch *et al.* observed distinct phenotypes of NM IIA-KO and NM IIB-KO cells on asymmetrical cross-shaped micropatterns (Fig. 2(d)).[78] As the principal components of the cytoskeleton, actin distributes at the most-edge regions of the cell.[79] They can directly sense the spatial confinement and activate subsequent signaling pathways, establishing actin as the most popular target for 2D micropatterning.

For microtubules, most micropatterning-related researches chose centrosome, the microtubule organizing center (MTOC) and the geometrical center of the cell, to regulate microtubules. Using various patterns including triangular and circle, Ana *et al.* revealed that the centrosome is located at the subcellular zone center determined by the pulling forces from the actomyosin network (Fig. 2(e)).[79] Similar observations were obtained from two-cell systems on complex patterns, such as "H" and bowtie patterns (Fig. 2(f)).[80] Micropatterning techniques are also applied to investigate intermediate filament, especially vimentin, and its interaction with other cytoskeleton components. In mouse embryonic fibroblasts (MEFs), both square and circle-shaped cells have similar vimentin distributions, while asymmetric and polarized-shaped cells have distinct vimentin distributions that avoid the sharp edges of the cell (Fig. 2(g)).[81] Experiments with vimentin-null MEFs adherent to polarized (teardrop) and unpolarized (dumbbell) patterns show that the absence of vimentin alters microtubule organization and perturbs cell polarity.[81] Using "crossbow"

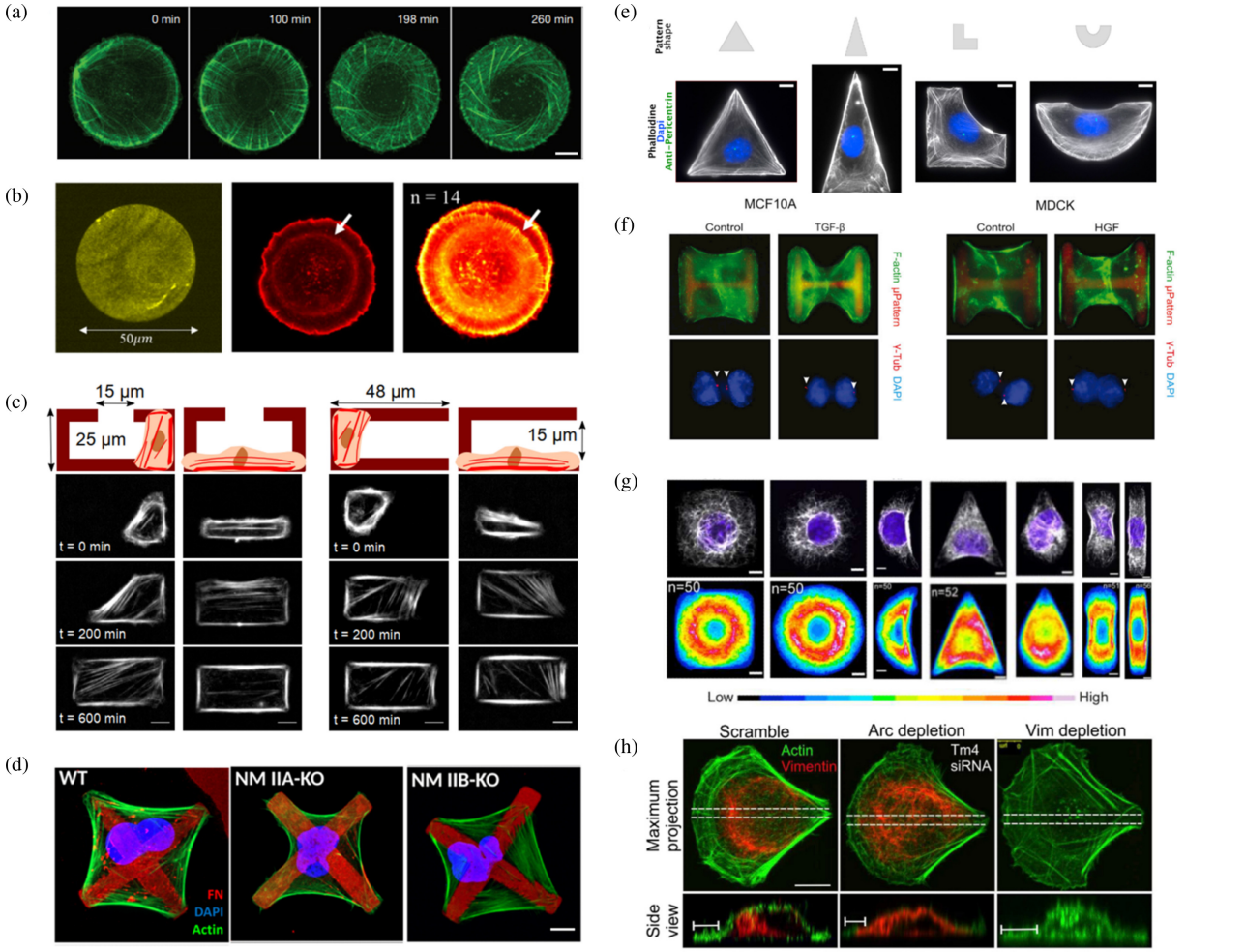

Fig. 2. Micropatterning regulates cytoskeleton. (a) Single cell actin cytoskeleton formed a chiral left–right asymmetry distribution on isotropous circular pattern.[74] Scale bar, 10 μm. (b) A "secondary" ring of actin cytoskeleton distributed along the periphery of the circular pattern.[76] (c) On rectangular micropatterns with distinctive opening positions, the geometry of ECM and the initial adhesion position determined the process of cell spreading and stress fiber network.[77] Scale bar, 10 μm. (d) Myosin-KO cells exhibited distinct actin phenotypes on asymmetric cross patterns.[78] Scale bar, 10 μm. (e) Centrosome located at the subcellular zone center determined by the pulling forces from actomyosin network.[79] Scale bar, 10 μm. (f) Two-cell systems on complex patterns of H and bowtie patterns, centrosome located at the subcellular zone center.[80] (g) Both square and circle-shaped MEF cells had similar vimentin distributions. On the contrary, in asymmetric and polarized-shaped cells vimentin avoids the sharp edges.[81] Scale bar, 5 μm. (h) On "crossbow"-shaped micropatterns, vimentin network reciprocally restricted the retrograde movement of arcs.[82] Scale bar, 10 μm.

shaped micropatterns to induce actomyosin arc, Jiu *et al.* revealed that the vimentin network reciprocally restricts retrograde movement of arcs and hence controls the width of flat lamella at the leading edge of the cell (Fig. 2(h)).[82] Unlike the wide distribution of actin cytoskeleton, microtubules and intermediate filaments are located around the nucleus. Since limited effects on the microtubules and intermediate filaments were observed upon spatial confinement at present. Hence, further investigations may gain more insight into the regulatory mechanism of these two cytoskeleton components via 2D micropatterning techniques.

The designed micropatterns should be closely associated with the target cytoskeleton which acts as the first and principal sensors of extracellular geometry. For instance, in actin-related investigations, the pattern could contain some fine structures, e.g., hollow patterns,[77,78] due to high dynamic and mechanical functions. Differently, the designs should focus on the size and outlines of the pattern for relatively rigid components including microtubule and intermediate filament.

3.2. *Micropatterning regulates cell migration*

ECM geometry cues have long been recognized as crucial factors in cell migration.[83,84] Micropatterning creates certain tracks, e.g., straight stripes, for single migrating cells. This kind of one-dimensional confinement provides both a certain orientation and spatial confinement from vertical directions of migration. Using stripes of different widths to provide physical confinement like those encountered physiologically by cells, *Mohammed et al.* identified substrate adhesion region confinement as a key determinant of speed in cell migration, i.e., stronger confinement often leads to higher migrating speed (Fig. 3(a)).[85] Similarly, the migration of NIH3T3 cells and Hela cells were found to be significantly affected by the widths and arc radiuses of the guided micro-stripes that they migrated fastest on the straight micro-stripes with width of about 20 μm (Fig. 3(b)).[86] Using micro-lanes with fields of alternating fibronectin densities, Schreiber *et al.* found a biphasic behavior with maximal velocity for intermediate fibronectin densities, i.e., the velocity of cells first increased and then decreased as the rising of fibronectin densities (Fig. 3(c)).[87]

Other patterns, such as dumbbell shapes, were used to provide limited confinement to direct migration. In such two-state micropatterns, cells exhibit intricate nonlinear migratory dynamics for a cancerous (MDA-MB-231) and a non-cancerous (MCF10A) cell line (Fig. 3(d)).[88] Besides, Xing *et al* applied dumbbell-shaped patterns to control the separation of two daughter cells during cell division, and further induce long and regular intercellular bridges between two daughter cells (Fig. 3(e)).[89]

Micropatterning can also manipulate the initial state of cell migration. Jiang *et al.* restricted the initial shape of individual cells to asymmetrical geometry through micropatterns, e.g., teardrop shapes, in which cells have polarized morphology, proving that the polarization direction of the cell will determine the direction of its movement (Fig. 3(f)).[90] It was also found that pre-confinement by teardrop shape caused the polarized distribution of MTOC and nucleus-Golgi complex, and this polarity was weakened when the cells were released from the confinement.[91]

Interestingly, some noncontinuity patterns were applied in the mechanism investigation of cell migration. For instance, *Vecchio et al.* designed triangular and teardrops array adhesive patterns and found that cell directionality is determined by the coupled dynamics of the focal adhesion collective behavior and the cellular environment (Fig. 3(g)).[92] Using ladder-type micropatterns, Garbett *et al.* demonstrated that T-plastin, an actin-crosslinking protein, synergizes with focal adhesions to strengthen the protrusive actin network at the leading edge, thus promoting membrane protrusions through ECM gap (Fig. 3(h)).[93] Using alternate stripe and circular patterns, a novel migratory phenomenon was observed that macrophages can migrate on alternate adhesive-nonadhesive substrates in a classical mesenchymal mode, due to highly-enriched podosomes formed on nonadhesive regions (Fig. 3(i)).[94] Notably, 2D micropatterning has also been widely applied in the manipulation and quantitative study of collective cell migration,[95–97] showing its powerful ability in cell migration investigations.

Cells cannot fulfill their functions without migration. In living organisms, the highly complex microenvironment forces cells to evolve unexpected mobility to squeeze through tissue space. Micropatterning can help quantize environmental parameters, and therefore paving a way for better insight into the intrinsic properties of cell migration. In the future, more ingenious patterns should be designed considering cell types and specific biological questions.

3.3. *Micropatterning regulate nucleus and gene expression*

2D spatial confinement shapes the nucleus through the force transferred from cytoskeleton.[98–100] It has long been known that altering the shape and size of cells by single-cell micropatterning could determine the growth and death of individual cells, indicating that spatial confinement via 2D micropatterning can regulate cellular gene expression and cell fate.[101] This top-down regulation was also found in macrophages that elongated macrophage shape leading to higher pro-healing (M2) expression and reduced pro-inflammatory (M1) secretion, which was associated with the modulation of the cytoskeleton (Fig. 4(a)).[102] Similarly, spatial confinement imposed by micropatterning suppressed actin polymerization, and thereby reduced lipopolysaccharide-activated transcriptional programs (biomarkers IL-6, CXCL9, IL-1β, and iNOS) by

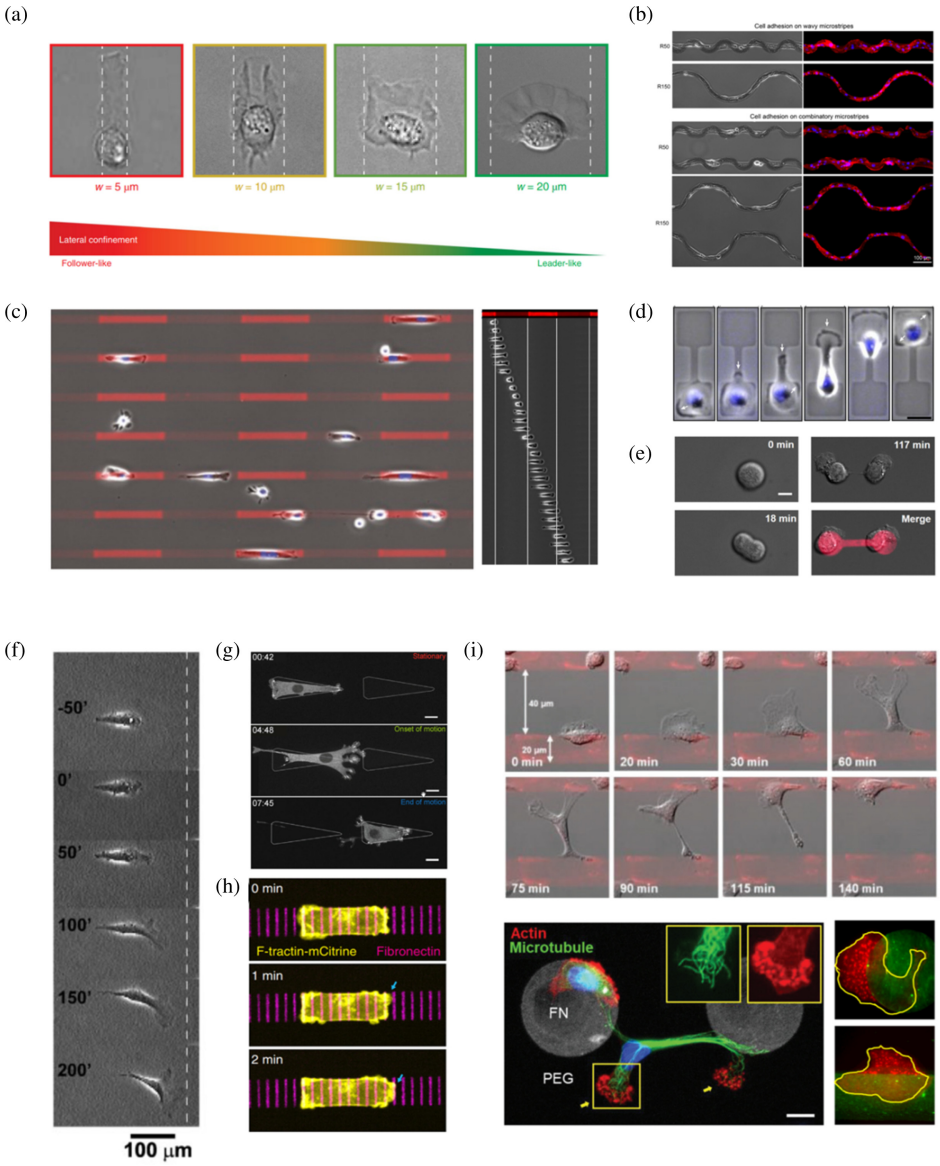

Fig. 3. Micropatterning regulates cell migration. (a) Substrate adhesion region was identified as a key determinant of cell migration speed using stripes of different widths to simulate the physiological limitation.[85] (b) NIH3T3 and Hela cells migrated fastest on the straight micro-stripes with width of 20 μm.[86] (c) Cell migration at different speeds on micro-lanes with alternating fibronectin densities.[87] (d) MDA-MB-231 and MCF10A cell lines exhibit intricate nonlinear migratory dynamics in dumbbell-shaped micropatterns.[88] Scale bar, 25 μm. (e) Dumbbell-shaped patterns controlled the separation of two daughter cells and induced long intercellular bridges between two daughter cells.[89] Scale bar, 25 μm. (f) The polarization direction of the tear-drop-shaped cell will determine the direction of its movement.[90] (g) On triangular and teardrops array adhesive patterns, direction of cell migration was determined by the coupled dynamics of the focal adhesion collective behavior and the cellular environment.[92] Scale bar, 15 μm. (h) T-plastin synergized with focal contacts to strengthen the protrusive actin network at the leading edge, promoting membrane protrusions through ECM gap on ladder-type micropatterns.[93] (i) Macrophages migrated across alternate adhesive-nonadhesive substrates in mesenchymal mode with enriched podosomes on nonadhesive regions.[94] Scale bar, 10 μm.

mechano-modulating chromatin compaction and epigenetic alterations (HDAC3 levels and H3K36-dimethylation) (Fig. 4(b)).[103]

Micropatterning creates a specific extracellular environment, e.g., physical confinement, cell-cell contact, to induce cell differentiation.[104,105] Joo *et al.* designed reproducible laminin (LN)-polylysine cell culture substrates and induced the differentiation of neurons and astrocytes (Fig. 4(c)).[106] On similar stripe patterns, human mesenchymal stem cells (hMSCs) had an up-regulation of several hallmark markers associated to neurogenesis and

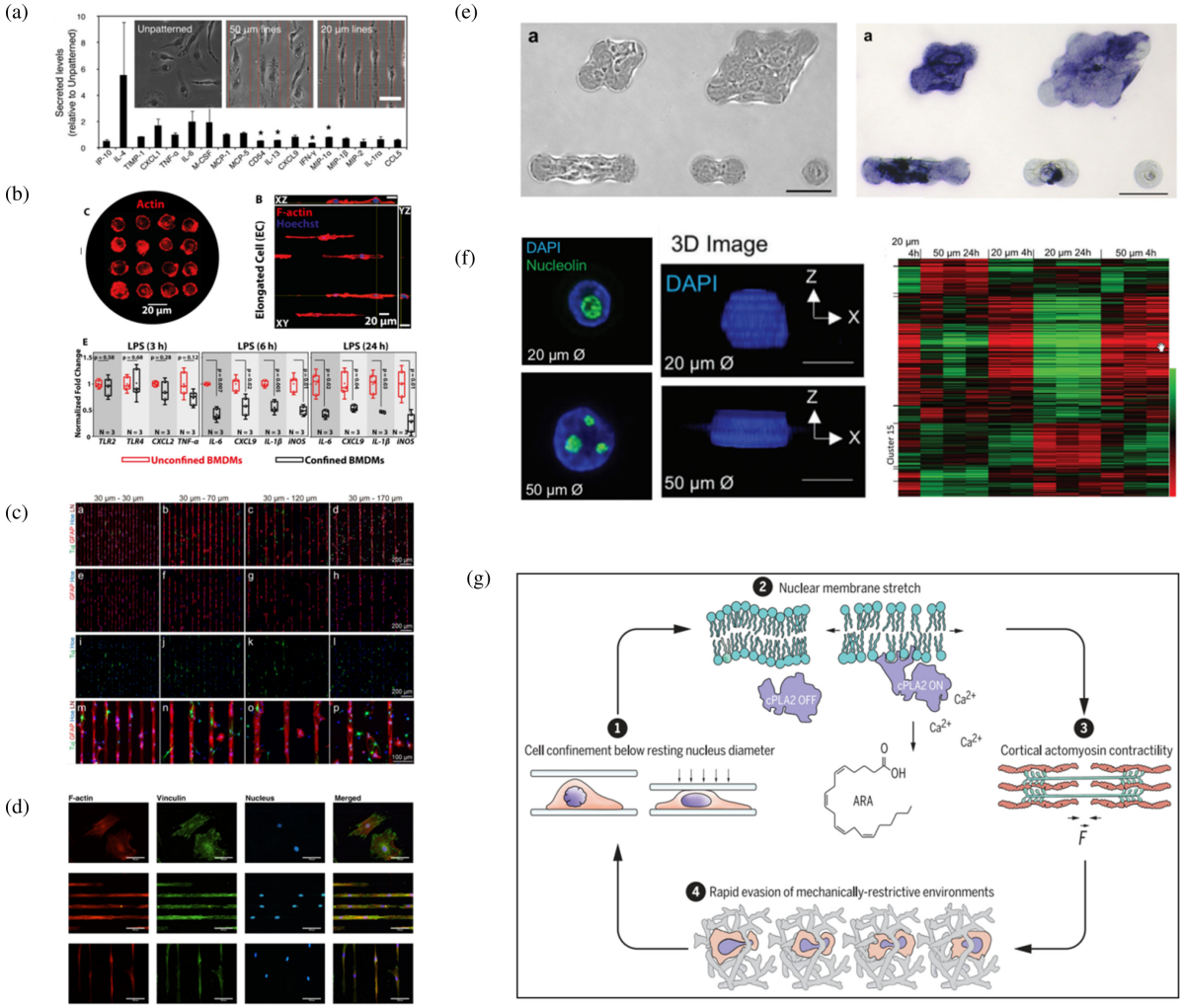

Fig. 4. Micropatterning regulated nucleus and gene expression. (a) Elongation of macrophage shape leads to higher pro-healing (M2) expression and reduced pro-inflammatory (M1) secretion.[102] Scale bar, 50 μm. (b) Spatial confinement imposed by micropatterning suppressed actin polymerization, and reduced lipopolysaccharide-activated transcriptional programs by mechanomodulating chromatin compaction and epigenetic alterations.[103] (c) The alignment and differentiation of neurons and astrocytes were controlled on reproducible laminin-polylysine substrates.[106] (d) On stripe patterns, human mesenchymal stem cells (hMSCs) had an up-regulation of several hallmark markers associated to neurogenesis and myogenesis. Osteogenic markers were specifically down-regulated or remained at its nominal level.[107] Scale bar, 100 μm. (e) On micropatterns with different numbers of microdomains, rat mesenchymal stem cells on single-cell patterns exhibited less significant differentiation than paired or aggregated cells.[108] Scale bar, 50 μm. (f) Limited adhesion on small micropatterns promoted fusion of nucleoli, alongside a reduction in nuclear volume and condensation of heterochromatin.[109] Scale bar, 10 μm. (g) Nuclear deformation leads to inner nuclear membrane unfolding, allowing for the deformation-dependent activation of cPLA2 signaling, and thus regulating myosin II activity to facilitate.[111]

myogenesis while osteogenic markers were specifically down-regulated or remained at their normal level (Fig. 4(d)).[107] Another study using micropatterns with different numbers of microdomains revealed that rat mesenchymal stem cells on single-cell patterns exhibited less significant differentiation than paired or aggregated cells. For those stem cells in contact, the extent of differentiation was fairly linearly related to the extent of contact characterized by coordination number (Fig. 4(e)).[108]

Limited adhesion on small micropatterns also promotes the fusion of nucleoli, alongside a reduction in nuclear volume and condensation of heterochromatin. These changes in nucleolar architecture are mediated by altered chromatin biomechanics and depend on the integration of the nucleus with the actin cytoskeleton. Functionally,

nucleolar remodeling regulates ribogenesis and protein synthesis, demonstrating that nucleolus is another key intracellular mechano-sensing element (Fig. 4(f)).[109] Since nucleus morphology is affected by 2D micropatterning, it is easy to figure out that 3D spatial confinement could also regulate nucleus and gene expression. For instance, it has been found to facilitate cell migration and mesenchymal-to-amoeboid transition through variation in actomyosin activity.[110] Recently, it was revealed by two independent research groups that the nucleus can act as an elastic deformation gauge allowing the cell to measure the deformation of cell shape by spatially 3D confinement of the physical microenvironment.[111,112] Specifically, nuclear shape deformation leads to inner nuclear membrane unfolding, allowing for the deformation-dependent activation of cPLA2 signaling, and thus regulates myosin II activity to facilitate cell migration (Fig. 4(g)).[111] Therefore, the nucleus can act as a sensor that adapts to the extracellular environment and regulates cell phenotype. Spatial confinement

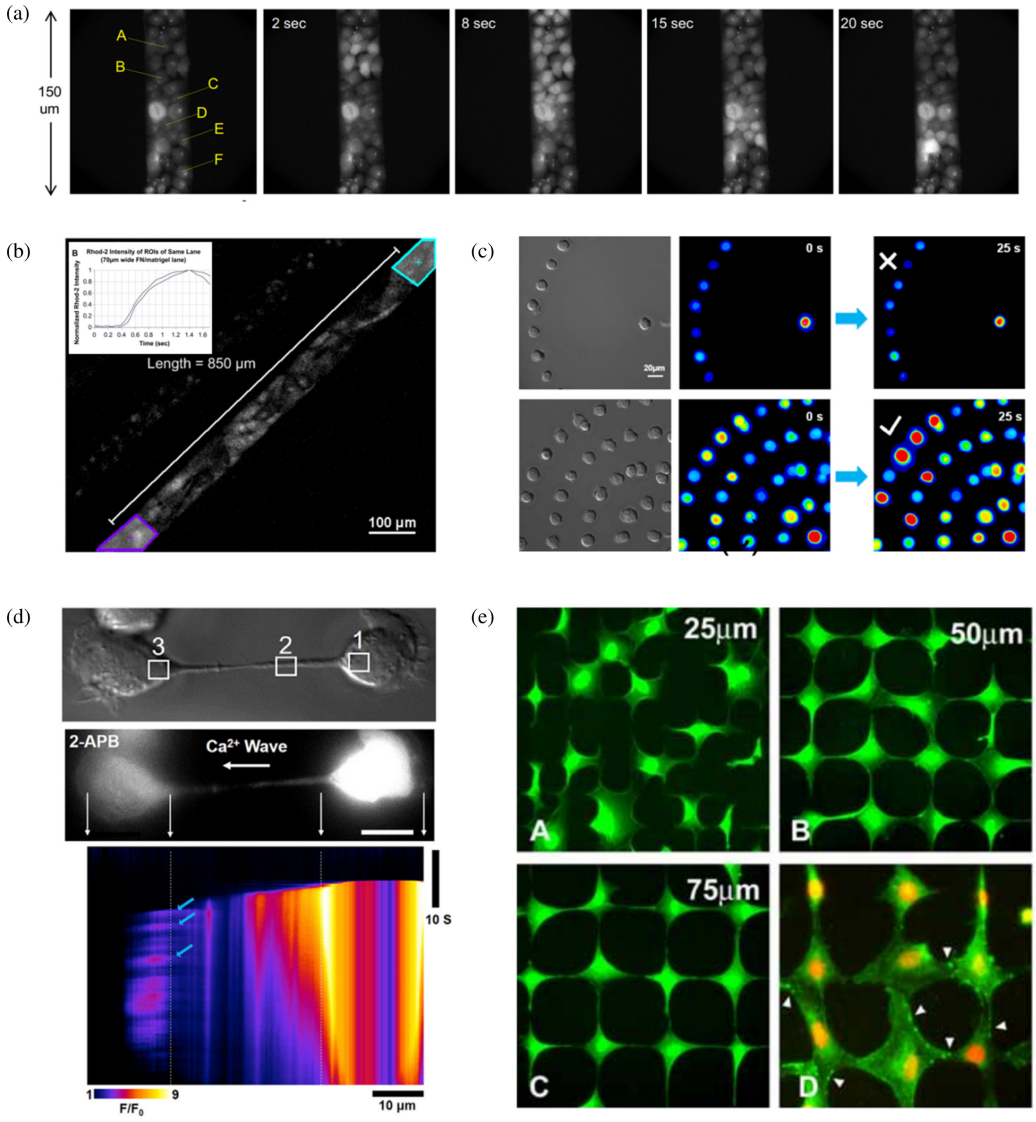

Fig. 5. Micropatterning-based intercellular communication assay. (a) Intercellular Ca^{2+} waves in HeLa cells expressing Cx43gap junction channels.[129] (b) Intercellular Ca^{2+} waves propagate in the lane-shaped collective human ESC-derived cardiomyocytes cells.[130] (c) In microglial cells arranged in concentric circles, ICWs propagated on account of the regenerative transmitter release from the relay station cells located in the propagating path.[131] (d) On dumbbell-shaped micropatterns, Ca^{2+} signal transferred over the intercellular bridges mediated by both passive Ca^{2+} diffusion and IP_3-mediated endogenous Ca^{2+} response.[89] Scale bar, 10 μm. (e) Ca^{2+} response in micropatterned osteoblastic networks mediated by gap junctions.[132]

combining 2D and 3D micropatterning may be a more effective approach in the regulation of nucleus morphology, gene expression, as well as cell fate. Considering cells residing on the surface of mucosa or lumen interior, 2D spatial confinement may simulate the *in vivo* environment for them. For those types of cells tightly in contact with other cells from all directions, 3D spatial confinement should be the proper approach. Furthermore, it still remains to clarify whether there is an underlying difference between 2D and 3D confinement on nucleus and gene expression.

3.4. *Micropatterning-based intercellular communication assay*

Intercellular communication allows cells to share information and facilitate various functions in multicellular organisms.[113,114] *In vivo*, some cells contact tightly with other cells (e.g., epithelial cells), while other cells are distributed discretely in space (e.g., macrophages and neutrophils), resulting in a diversity of intercellular communication, such as paracrine,[115–117] gap junction,[118–120] tunneling nanotubes.[121–123] On traditional substrates, cells spread and migrate randomly and disorderly, which brings inconvenience to intercellular communication assay. Therefore, micropatterning technique, which is capable of arranging cells as needed, provides a unique opportunity to investigate intercellular communication in a controllable and quantitative manner.

Intercellular calcium waves (ICWs), as a principal form of intercellular communication, are mediated by multiple pathways.[124–128] For tightly-contacted multicellular system, lane patterns are widely used to quantify contact-dependent ICW. Specifically, ICWs were observed in HeLa cells expressing Cx43gap junction channels (Fig. 5(a)).[129] Besides, using a large number of lanes with widths ranging from 20 mm to 110 mm, there is no clear link between pattern width and calcium propagation rate for the human ESC-derived cardiomyocytes cells (Fig. 5(b)).[130]

Applying spatial-discrete patterns, e.g., concentric circles, Xing *et al.* revealed that in microglial cells, ICWs propagated on account of the regenerative transmitter release from the relay station cells located in the propagating path (Fig. 5(c)).[131] Further on, they designed dumbbell-shaped micropatterns to resolve spatiotemporal characteristics of ICW signal transfer over the intercellular bridges, a plasma continuity formed between the two daughter cells.[89] It was revealed that both passive Ca^{2+} diffusion and IP_3-mediated endogenous Ca^{2+} response contribute to the ICW propagation between intercellular-bridge-connected cells (Fig. 5(d)).[89] Similarly, using micropatterned assemblies, osteocytic networks showed more sensitive and dynamic than osteoblastic networks based on their Ca^{2+} response mediated by gap junctions, especially under low-level physiological-related fluid shear stress stimulations (Fig. 5(e)).[132,133] Taken together, different micropatterns, e.g., discrete patterns and interconnected micropatterns, would work for distinct types of intercellular communication.

4. Outlook

Since the birth of micropatterning, this advanced technique has presented significant contributions to biological and biomedical researches, especially in the field of cell biology. Here, we summarized current mainstream methods, i.e., photolithographic micropatterning and soft lithography micropatterning techniques, for single-cell level manipulation, as well as their applications in cell biology research, including cytoskeleton, cell migration, nucleus and gene expression, and intercellular communication. Besides, 2D micropatterning offers a platform to manipulate the behavior of collective cells. However, challenges still exist in developing easy-to-use and hypotoxicity craft. More imaginative and diverse micro-patterns need to be designed for complex cell biology problems due to the imperfect simulation of the physiological environment. We hope that, with the progress of various micropatterning techniques, more mysteries of life can be observed and resolved.

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Conflicts of Interest

The authors declare that there are no conflicts of interest relevant to this article.

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