HHS Public Access

Author manuscript

Curr Opin Solid State Mater Sci. Author manuscript; available in PMC 2022 February 01.

Published in final edited form as:

Curr Opin Solid State Mater Sci. 2021 February ; 25(1): . doi:10.1016/j.cossms.2020.100873.

New perspectives on the roles of nanoscale surface topography in modulating intracellular signaling

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Abstract

The physical properties of biomaterials, such as elasticity, stiffness, and surface nanotopography, are mechanical cues that regulate a broad spectrum of cell behaviors, including migration, differentiation, proliferation, and reprogramming. Among them, nanoscale surface topography, i.e. nanotopography, defines the nanoscale shape and spatial arrangement of surface elements, which directly interact with the cell membranes and stimulate changes in the cell signaling pathways. In biological systems, the effects of nanotopography are often entangled with those of other mechanical and biochemical factors. Precise engineering of 2D nanopatterns and 3D nanostructures with well-defined features has provided a powerful means to study the cellular responses to specific topographic features. In this Review, we discuss efforts in the last three years to understand how nanotopography affects membrane receptor activation, curvature-induced cell signaling, and stem cell differentiation.

Keywords

Surface topography; Nanotopography; Cell-material interface; 2D ligand patterning; 3D nanostructures; Extracellular matrix; Integrin; T-cell receptor; Membrane curvature; Cell differentiation

1. Introduction

Physical properties of biomaterials modulate the mechanotransduction of surrounding cells. Nanoscale surface topography is a physical property that has been shown to affect cellular and tissue responses, including adhesion, migration, growth, morphogenesis, and differentiation[1–5]. This suggests new possibilities in biomaterial development by utilizing

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Conflict of interest statement

Nothing declared.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

nanotopography to promote *in vivo* stability, biocompatibility, and biofunctionality. It is thus important to understand how cells sense and respond to the surface nanotopography of biomaterials. In the last two decades, advances in nanofabrication techniques have heralded a new research area utilizing precisely engineered two-dimensional (2D) nanoscale patterns and three-dimensional (3D) nanostructured surfaces to control cell signaling, morphology and stem cell fate for tissue engineering and regenerative therapy[6–8]. For example, 2D patterning has been employed to control ligand spatial arrangement and its effect on receptor signaling [9–11]. 3D nanostructures such as nanopillars, nanogrooves, nanoneedles, nanoholes, and nanoroughs, which constitute a wide variety of surface topographies, have been shown to activate specific intracellular signaling pathways [12–14]. It is worthwhile to note that many bio-sensing devices adopt similar 3D nanostructures [15–18]. These enormous bioengineering efforts have significantly broadened our understanding of the effects of nanotopography in controlling cell behaviors. Many of the works have been reviewed in previous articles [5,6,9,14,19]. Here, we focus on new studies and new understandings in the last three years.

Cells sense and respond to natural or artificial extracellular nanotopography by using their intrinsic signaling pathways. The use of well-designed nanostructures has allowed researchers to elucidate the signaling events induced by specific topographical features [20–22]. At the nanoscale, surface features are about the size of single proteins (a few to tens of nanometers) or protein complexes (tens to hundreds of nanometers). Therefore, protein activities can be directly affected by 2D or 3D topographical arrangements of the surface features. In this review, we will discuss recent works on how 2D ligand patterning and 3D nanostructures affect intracellular signaling pathways, including membrane receptor activation, endocytosis, actin polymerization, and mechanotransduction. We will also highlight recent studies regarding the gene expression and stem cell differentiation regulated by nanotopography, and provide current perspectives on the underlying mechanisms.

2. 2D Ligand nanotopography regulates the receptor-ligand interactions

Many intracellular signaling pathways begin with the ligand-induced activation of cell-surface receptors. The spatial properties of ligands (e.g., spacing and clustering) vary in extracellular environments, which modulate the assembly and the clustering of surface receptor-based protein complexes. The ligand/receptor clusters, as where receptors engage downstream signaling molecules, are critical to the signaling of a broad range of receptors, such as adhesion receptors-integrins[23], growth factor receptors (e.g., FGFR, TGFRs, and VEGFR)[24–26], and cell-cell contact receptors, such as Notch, Eph, and T-cell receptors[27–29]. For example, compared with their monovalent alternatives, the biomimetic multivalent ligand nanoclusters that can simultaneously activate and assemble multiple surface receptors, have been shown to stimulate more physiologically relevant cell signaling[30–33]. In this section, we will review recent studies that use 2D nanotopographic patterning of ligands to understand the effects of ligand distribution on the receptor signaling, using integrins and T-cell receptors as the paradigmatic models.

2.1 Nanotopographic patterning of ligands regulates the activation of integrin receptors

Integrins are a family of cell-surface receptors that mediate the assembly of adhesion complexes between the extracellular matrix (ECM) and the cytoskeleton. Many adhesion molecules engaged by integrins (e.g., talin and vinculin) are structurally and functionally mechanosensitive[34,35]. Therefore, the size and composition of integrin-mediated adhesions are modulated not only by the biochemical property of ligands but also by the forces in the fibrous structures of ECM and cytoskeleton. Integrin-mediated adhesions are central to the mechanotransduction machinery in physiological processes such as cancer metastasis, wound healing, and embryonic development[36].

At the nanoscale, most ligands for integrin receptors present in the ECM are not distributed continuously or uniformly. For example, the average span of one fibronectin molecule (a dimer of two polynucleotides) in fibronectin fibrils is ~92 nm (Fig. 1a)[37], while each polynucleotide has only one Arg-Gly-Asp (RGD) motif as an integrin-binding ligand[38]. This showcases that the integrin binding sites are a network of periodic features along the basic units of ECM fibrils (Fig. 1b, 1c). Previous studies have shown apparent differences between cell adhesions on the uniformly coated 2D substrates and their counterparts in the 3D ECM [39–41]. The underlying mechanisms are not fully understood, likely involving differences in both the substrate rigidity and the spatial distribution of ligands.

To study the effects of ligand spacing on integrin-mediated adhesion, researchers have used 2D nanopatterning to control the spatial distribution of ligands. For example, nanofabricated ligand nanodot arrays (ordered or disordered, locally clustered or extended) have a well-defined spacing that can change from 0 to 500 nanometers (Fig. 1d, 1e). The ligands on each nanodot (≤10 nm in diameter) can only bind to one integrin molecule (~8-12 nm in width) due to the steric effect (Fig. 1f)[42]. It was found that the spacing between nanodots drastically affects the formation of focal adhesions with an upper threshold usually in tens of nanometers, beyond which focal adhesions can not form. This is attributed the fact that adhesion proteins (e.g., talin and α-actinin) cannot bridge integrins that are too far away[42,43]. The upper threshold of the ligand spacing was found to be larger on a disordered ligand array than on an ordered one [44]. These studies also revealed that at least four RGD-binding integrins arranged as polygonal clusters are required for the formation of mature adhesion[45].

Unlike the polygon arrays of nanodots used in earlier studies, most ligands are arranged in a network of nanofibrils in the real ECM. Recently, the Sheetz group manufactured 10-nm RGD-peptide nanolines using electron-beam lithography, to mimic the thinnest ECM fibrils[46]. Because of the steric hindrance between integrins, the nanolines are considered as linear arrays of ligands. The authors found that, in order to support focal adhesion and promote cell spreading, the maximum spacing between two nanolines is 70 nm (Fig. 2a). It is consistent with the upper threshold of ligand spacing determined using nanodots[42]. More interestingly, this study shows that a pair of 10-nm nanolines with 70-nm internal gaps are more effective in inducing focal adhesions than single 40-nm wide nanolines (Fig. 2a, two columns on the right). The authors demonstrate that the advantage of paired nanolines is due to the unliganded integrins aggregating and bridging RGD-binding integrins across the gap to form large adhesion patches (Fig. 2b). They also showed the crossed nanolines, which

more resembles the ECM network, can also enhance cell adhesions owing to the small gaps around intersections (Fig. 2a). This study provides a new angle to understand the effects of ligand spacing in the interconnected fibrous ECM.

Around the same time as the Sheetz study, Oria *et al.* found that substrate stiffness can regulate how ligand nanotopography affects the cell adhesion[47]. They manufactured nanodot arrays embedded in polyacrylamide-based hydrogels, using block copolymer micelle nanolithography. In this system, the rigidity of the gels and the spacing between ligands are both tunable. The authors found that, unlike rigid substrates that only have an upper threshold for ligand spacing, there is an optimal ligand spacing, i.e. both an upper threshold and a lower threshold, on soft substrates. The optimal ligand spacing promotes the largest focal adhesions. The authors found that the optimal ligand spacing increases as the substrate stiffness decreases. For example, for fibroblasts, the upper threshold of RGD ligand spacing on the glass surface (Young's modulus, 50-90 GPa) is between 30 and 50 nm, whereas the optimal ligand spacing is 50 nm on the firm (150 kPa) hydrogel, 100 nm on the medium-soft (10-30 kPa) hydrogels, and 200 nm on the soft (1.5-5 kPa) hydrogels. Force loading is known to positively correlate with the substrate rigidity. For soft substrates, small spacings are enough to generate sufficient forces to activate adhesion molecules while large spacings will not be able to recruit enough liganded integrins, there is an optimal ligand spacing that supports the growth/maturation of cell adhesion on soft substrates. These results suggest that focal adhesion formation integrates the effects of ligand spacing and substrate force loading.

2.2 Nanopatterning of ligands regulates T-cell receptor activation

The T cell receptor (TCR) plays a crucial role in the T lymphocyte-mediated immune responses. Upon recognizing the agonist peptide-bound major histocompatibility complex (pMHC), TCR triggers T cell activation through a series of signal transductions[48]. The first step of TCR triggering is to form a massive (~200 nm) signaling protein complex known as the TCR signalosome[29,49][49], which mainly involves a tyrosine kinase cascade consisting of kinases LCK and ZAP70, immunoreceptor tyrosine-based activation motif (ITAM), and adaptor protein LAT (Fig. 3a,3b).

According to the most prominent kinetic segregation model[50], at the resting state, transmembrane tyrosine phosphatase CD45 constantly dephosphorylates LCK, preventing it from activation (Fig. 3a). When TCR-ligand complexes bring T cell into proximity (~15 nm) to its target[51], the close membrane apposition excludes CD45 because of the large size (~21 nm in height) of CD45's carbohydrate-rich extracellular domain[52], unleashing the tyrosine phosphorylation of LAT to recruit hundreds of proteins building up the TCR signalosome (Fig. 3b). The CD45-kinase segregation model has well explained most previous observations, except very few outliers.

TCR signaling is extremely sensitive: only a few (~1-4) agonist pMHCs are required to trigger the activation of T cells[53–55]. However, aggregations of tens or even hundreds of TCR receptors are present in signalosomes[56]. To interrogate how ligand spatial arrangement contributes to the sensitivity of TCR triggering, Cai *et al.* used a gold-nanoparticle array platform where a single ligand is bound to each gold nanoparticle

embedded in a flat supported lipid bilayer[57]. In this platform, the spacing, density and height (distance to the target cell-mimicking supported lipid bilayer) of pMHC ligands are well-controlled. When the ligands are on the flat surface, the steric exclusion of CD45 occurs and TCR signaling shows no preference for different ligand spacings, confirming the segregation model (Fig. 3c, e). However, when the ligand is lifted by 10 nm, the distance between membranes cannot hinder the diffusion of CD45 into the contact region (Fig. 3d). In this case, robust TCR signaling can only occur on surfaces with small lateral ligand spacing (≤50-nm), which can artificially create TCR aggregation (Fig. 3f). The study suggests an aggregation model which proposes closely-packed ligands resulting in TCR clustering to trigger TCR activation even when CD45 cannot be segregated [58].

However, *in vivo*, the agonist pMHC ligands rarely pre-cluster before binding to TCRs. They are often sparsely distributed among large amounts of other membrane proteins on the surface of target cells. Therefore, the high sensitivity and selectivity of TCR triggering are crucial[59]. Recent works suggest that the nanotopography of TCR-mediated contacts may contribute to the sensitivity. Specifically, the surface of T cells is covered with microvilli, narrow and short nanoscale plasma membrane protrusions where TCRs are enriched[60]. The microvilli are highly dynamic and can be stabilized by local TCR-ligand interactions to form long-lasting physical contact with the target cell (Fig. 3b)[61]. The presence of microvilli nanotopography was found to enhance CD45-kinase segregation and subsequent TCR signaling[62,63]. Fernandes *et al.* proposed that microvilli nanotopography imposes a constraint on the contact size (Fig. 3b), which is critical for the ligand discrimination (selectivity) of TCR[64].

3. 3D nanotopography modulating intracellular processes and the curvature hypothesis

Membrane curvature quantitatively measures the physical bending of cellular lipid bilayers. The generation of membrane curvature is actively modulated by cellular processes, such as endocytosis, exocytosis, and migration, by the local enrichment of specialized proteins and lipids or by the forces from the cytoskeleton[65]. 3D nanotopography of extracellular environments can also physically impose curvatures on the cell membrane [22,66,67]. Previous works have shown that imposed membrane curvatures can recruit and activate curvature-sensitive proteins, e.g., BAR-domain proteins[68–71]. In this section, we will review the recent studies in support of a membrane curvature hypothesis, which proposes the 3D nanotopography modulates protein activities and intracellular signaling by inducing local membrane curvatures.

3.1 Nanotopography-imposed membrane curvatures enhance endocytosis.

Membrane receptors, ligands, and lipids are internalized continuously through endocytosis. It is a key process for a cell to reorganize its membrane system, regulate signaling, and adapt to its environment. Because endocytosis involves membrane bending and curvature generation, it is not surprising that endocytosis is sensitive to curvatures of the cell membrane. To understand how plasma membrane curvatures affect endocytosis, it requires precise control on the location and the value of membrane curvatures.

Recently, Zhao *et. al.* revealed that 3D nanotopography enhances clathrin-mediated endocytosis by inducing membrane curvature on the plasma membrane [72]. The authors used electron beam lithography to fabricate nanostructures with well-defined nanoscale curvatures, e.g. nanobars (Fig. 4a), nanopillars, and CUI patterns(Fig. 4b)[72,73]. They found that clathrin and dynamin strongly prefer the ends of vertical nanobars that have high curvature values as compared to the flat side walls, while another membrane-associated protein GFP-CAAX is distributed uniformly along the nanobar (Fig. 4c). Many endocytic proteins are known to be curvature-sensitive such as Fcho1, Epsin1, and dynamin2, which all show clear preference to nanotopography-induced membrane curvatures (Fig. 4c). Other endocytic proteins that are not known to be curvature sensitive such as AP2, clathrin, intersectin, and the cargo transferrin receptor also accumulate at high curvature locations, likely due to their interactions with curvature-sensing proteins. Furthermore, dynamic measurements show that nanotopography-induced membrane curvatures reduce the time for clathrin-coated pits assembly, which suggests that nanoscale curvatures lower the membrane bending energy barrier for endocytosis.

In a related study, Gopal *et al.* cultured cells on vertical nanoneedles and demonstrated that membrane curvatures-induced by nanoneedles enhanced all three major endocytosis pathways including clathrin-mediated endocytosis and caveolin-dependent endocytosis [74]. However, Zhao *et al.* showed that caveolin1 does not show strong preference for membrane curvatures of 150 nm as clathrin does. Thus, the specific geometrical thresholds for the promotion of different endocytic pathways are yet to be determined.

3.2 Nanotopography-imposed membrane curvatures induce actin polymerization.

Contact guidance refers to a phenomenon in which the substrate physical structures regulate the shape and movement of cells [75]. It has appeared in the literature for more than one century[76]. The most obvious effect of contact guidance is that cells organize their actin cytoskeleton based on the nanoscale patterns presented by the ECM, e.g., the ridges of the collagen fiber bundles[1,77]. To explore the causes and consequences of the effect, researchers have probed actin and actin-associated signaling pathways in numerous types of cells on manufactured surfaces with well-defined nanostructures, such as nanogrooves, nanolines, and vertical nanopillars[78–87]. There are two typical observations: the alignment of actin cytoskeleton orientation with that of nanogrooves and nanolines[78–82]; and the loss of actin stress fibers in the company with local accumulation of actin filaments around the nanostructures[83–87]. In both cases, the integrin-mediated adhesions are redistributed or reduced to accommodate the changes in the actin cytoskeleton. However, current evidence suggests the altered cell adhesions are not the origin of the nanotopography-dependent actin reorganization. Notably, some types of cells, such as T cells, migrate parallel to nanogrooves and orient their actin cytoskeleton accordingly without the participation of integrins[88].

The consensus of results from a large number of studies across many different cell types suggest that there are universal underlying mechanisms for actin reorganization in response to nanotopographical features. Recently, Lou and colleagues explored this possibility by looking at how nanotopography-induced membrane curvature, as a sole variable, would affect actin reorganization[89]. Using nanobars to locally impose both high- and zero-

curvatures in the plasma membrane, they showed that actin filaments accumulate preferentially around highly curved (≤400 nm in diameter) membranes (Fig. 5a). They demonstrated that, upstream to actin filaments formation, a membrane curvature-sensing protein FBP17 is activated by nanotopography and subsequently activates the actin nucleator Arp2/3 complex. They also showed the assemble and disassemble of curvature-dependent actin filaments occur spontaneously within several minutes. In another study, Martino et. al. developed 3D nanostructures made of light-responsive azopolymer that dynamically induced membrane curvature in situ[90]. They demonstrated that the actin filaments assembled within minutes of curvature formation on the membrane. These studies demonstrated that the formation of plasma membrane curvatures is sufficient to drastically disrupt actin stress fibers and focal adhesions in the entire cell (Fig. 5b,5c).

Though these studies have not provided a comprehensive explanation to nanotopography-dependent actin reorganization, it is a significant step forward, supporting a membrane curvature-based hypothesis. It is worth noting that the hypothesis agrees with the previous observation that actin preferentially polymerizes around the nanoridges[80,81]. Moreover, the dynamic actin-waves along nanoridges were shown to determine cell morphology and direct cell migration in the absence of integrins[80].

4. Nanotopography alters gene expression and stem cell differentiation

Cells sense nanotopography and stimulate intracellular signals through their receptors, membranes, and cytoskeletal reorganization. The signals further induce phenotypic changes in cells, most notably cell differentiation. In the past decade, surface nanotopography has been repeatedly demonstrated to affect stem cell osteogenesis[91], adipogenesis[92], chondrogenesis[93], neurogenesis[94], and myogenesis[92]. Phenomenological observations have been reported by many studies and reviewed elsewhere [6]. Here we focus on recent studies of molecular and cellular mechanisms underlying these observations. Understanding how nanotopography-induced mechanical signals are transmitted toward gene regulation will benefit the fields of tissue engineering and regenerative medicine.

Notch receptors, which play important roles in mechano-induced epithelial stem cell differentiation [95], have recently been shown to be involved in nanotopography mediated cell differentiation. Kang *et al.* showed that 800 nm PDMS nano-grooves coated with silk films enhance the human corneal limbal epithelial cell differentiation. Both their RNA sequencing results and Q-PCR results revealed that nanotopography increased the expression level of Notch and components of Notch signaling pathway in stem cells [96]. However, why nanogrooves enhance Notch signaling is yet to be fully understood.

For topographic sensing, the signals initiated at the cell membrane propagate to the intracellular domain and ultimately to the nucleus to regulate gene expression. The downstream pathways including Rho/ROCK, PI3K/Akt[97], ERK/MAPK[98], Wnt/β-catenin[99–101] and YAP/TAZ[102] have been reported to play roles in nanotopography-mediated stem cell differentiation. The most widely studied is the YAP/TAZ pathway. YAP is a mechanosensitive transcription activator and its activity, indicated by cytosol-to-nucleus translocation, is correlated with high actomyosin contractility and osteogenesis. Early

studies show that nanotopography affects YAP activity, but some report increased YAP activity [103]while others report the opposite effect [104–106]. This discrepancy could be due to differences in the features of nanotopography used in these studies (Table 1). Recently, Seong *et al.* used size-tunable vertical nanoneedles to systematically measure stem cell behavior using imaging based linear discriminant analysis[107]. Different needle diameters were shown to impact stem cell morphology, gene expression, and nuclear membrane curvature in different degrees, and sometimes in opposite directions. For example, they showed that under that same circumstance, vertical nanoneedles of 700 nm tip width lead to higher number of focal adhesions while nanoneedles of 50 nm lead to lower number when compared with the planar surface[107]. In this study, they found that nanotopography of all geometries reduces YAP activity and YAP-target genes. Besides changing intracellular signaling pathways, studies show that nanotopography can directly influence the expression of proteins, such as integrins and nuclear envelope protein lamins. The level of these proteins determines the mechanical properties of the whole cell [106]. Furthermore, 3D nanotopography has been shown to change epigenetics in stem cells [108,109]. Comparisons between stem cells on flat and nanotube surfaces confirm that changes in cell adhesion and actin cytoskeleton are accompanied by down regulation of histone deacetylases and changes in microRNAs levels. Though great progress has been made in understanding the contribution of mechanical cues in directing stem cell differentiation, how these mechanical cues assist stem cells to pass the proliferation checkpoint and differentiation checkpoint and commit certain cell fate are yet to be fully elucidated.

5. Conclusions and emerging questions

Cell culture dishes have simplified the cell attachment surface into a homogeneous and flat two-dimensional concept. However, the extracellular environment is far from homogeneous and a living cell interacts with its environment in a 3D manner. Nanoscale surface topography has been demonstrated by many studies to affect cell behavior, but the underlying mechanisms of how cells sense nanotopography are yet to be fully understood.

One of the major challenges is that surface topography is a high-dimensional space of features such as domain size, height, steepness, shape, distance between peaks, valley/peak ratio, orientation, symmetry, pattern, and more. Differences among these features may explain the different and sometimes opposite effects of nanotopography on cell behavior reported in previous studies [96,110]. Cells sense different topographic features using distinct mechanisms. As discussed earlier, cells sense the size/shape of 3D nanotopography by imprinted membrane curvature and sense the 2D ligand patterns by receptor clustering. Therefore, to understand the molecular mechanism, one needs to assess the nanotopographical features quantitatively and systematically, by varying one parameter at a time via precision engineering.

Another major challenge is that the interface between the cell membrane and the nanotopography is in the range of a few to tens of nanometers, which is beyond the spatial resolution of optical microscopy. Transmission and scanning electron microscopies have been employed to resolve the interface [66,111], but electron microscopy methods are

challenging to implement, cannot be applied to live cells, and does not provide chemical information. An reliable and easily-accessible method such as super-resolution microscopy to visualize the interface would greatly assist our understanding of how cells interface with different nanotopography. Finally, a thorough proteomic analysis of the interface and comparisons of the protein components at interfaces with different nanotopography will further elucidate the molecular mechanisms of how cells sense nanotopography.

Using nanotopography to control cell behavior offers unique advantages for tissue engineering and regenerative medicine. Nanotopography is stable, easy to control, and affects local tissues as compared with peptide or drug-based approaches. However, the large potential set of topographic parameters and the lack of the mechanistic understanding of how cells interact with topographic features, has resulted in a primarily phenomenological approach. Resolving this grand challenge will ultimately lead to rational design of surface topography to induce desired cell functions and behaviors.

Acknowledgments

Financial support

This work was financially supported by National Institutes of Health 1R01GM128142 and 1R01GM125737.

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* of special interest

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Highlights:

- A brief overview of cellular responses to the surface nanotopography of biomaterials is presented.
- Recent advances in understanding the influences of 2D ligand nanotopography on cell-surface receptor signaling are highlighted.
- The 3D surface nanotopography regulates important cell behaviors by inducing cell membrane curvatures.
- Current perspectives on the nanotopography-induced gene expression and cell differentiation are summarized.
- The future challenges and directions to study the nanotopographical effects on cellular processes are discussed.

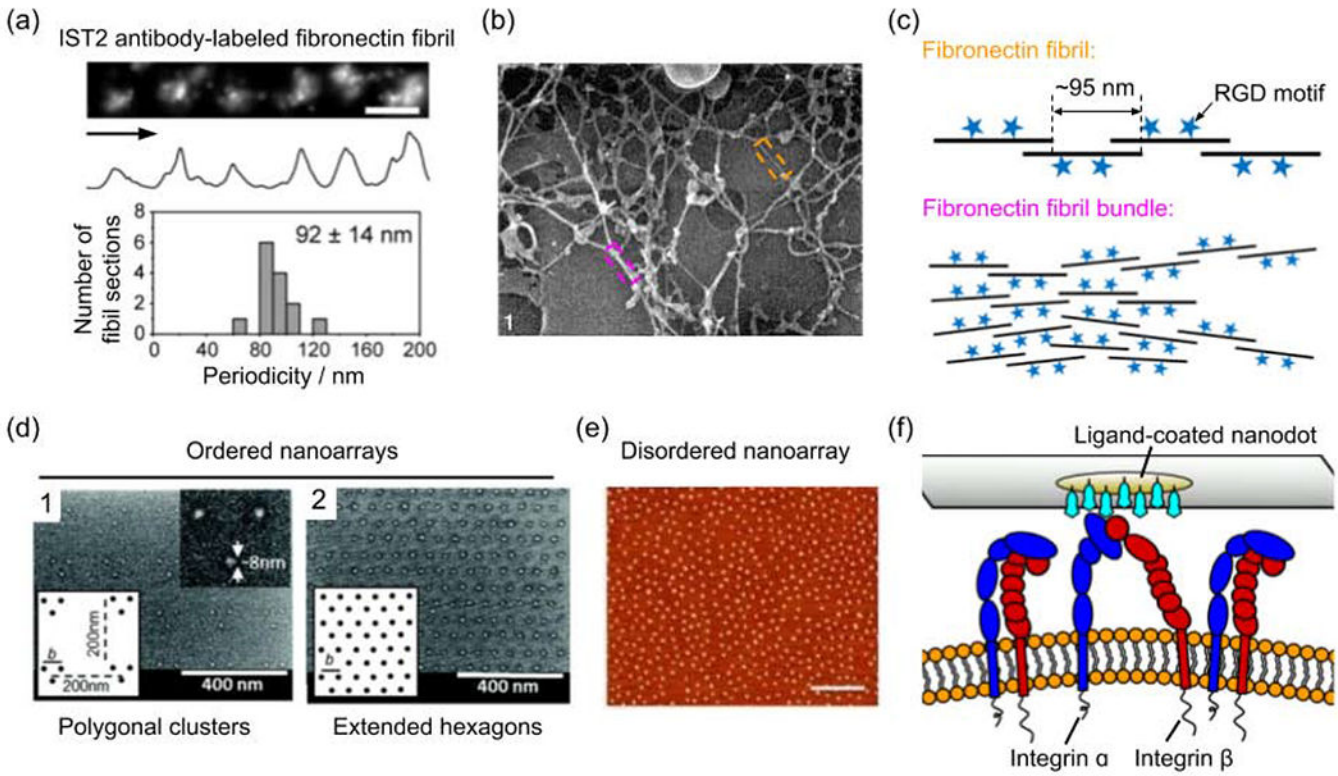

Figure 1.

The nanotopography of integrin-binding motifs. **(a)** The fibronectin molecules in the fibril are distributed periodically as shown by antibody IST2 immunostaining. **(b)** High-resolution cryo-scanning electron microscopy (SEM) image of fibronectin fibril network formed in human skin fibroblast cultures. Horizontal field width = 1.6 μm[112]. **(c)** Schematic presentation showing the spatial distribution of RGD motifs in fibronectin-based ECM. **(d)** SEM images of ordered ligand nanodot arrays that are arranged as polygonal (triangle shown in the representative image 1) clusters and extended hexagons (image 2)[45]. **(e)** Atomic force microscopy (AFM) image of disordered ligand nanodot array with an average interparticle distance of ~92 nm. Scale bars: 400 nm[44]. (f) Schematic presentation showing the steric hindrance between integrins for binding the ligands coated on nanodots. (a) is adapted with permission from [37], Springer Nature. (b) is adapted with permission from [112], Wiley. (d) is adapted with permission from [45], American Chemical Society. (e) is adapted with permission from [44], American Chemical Society.

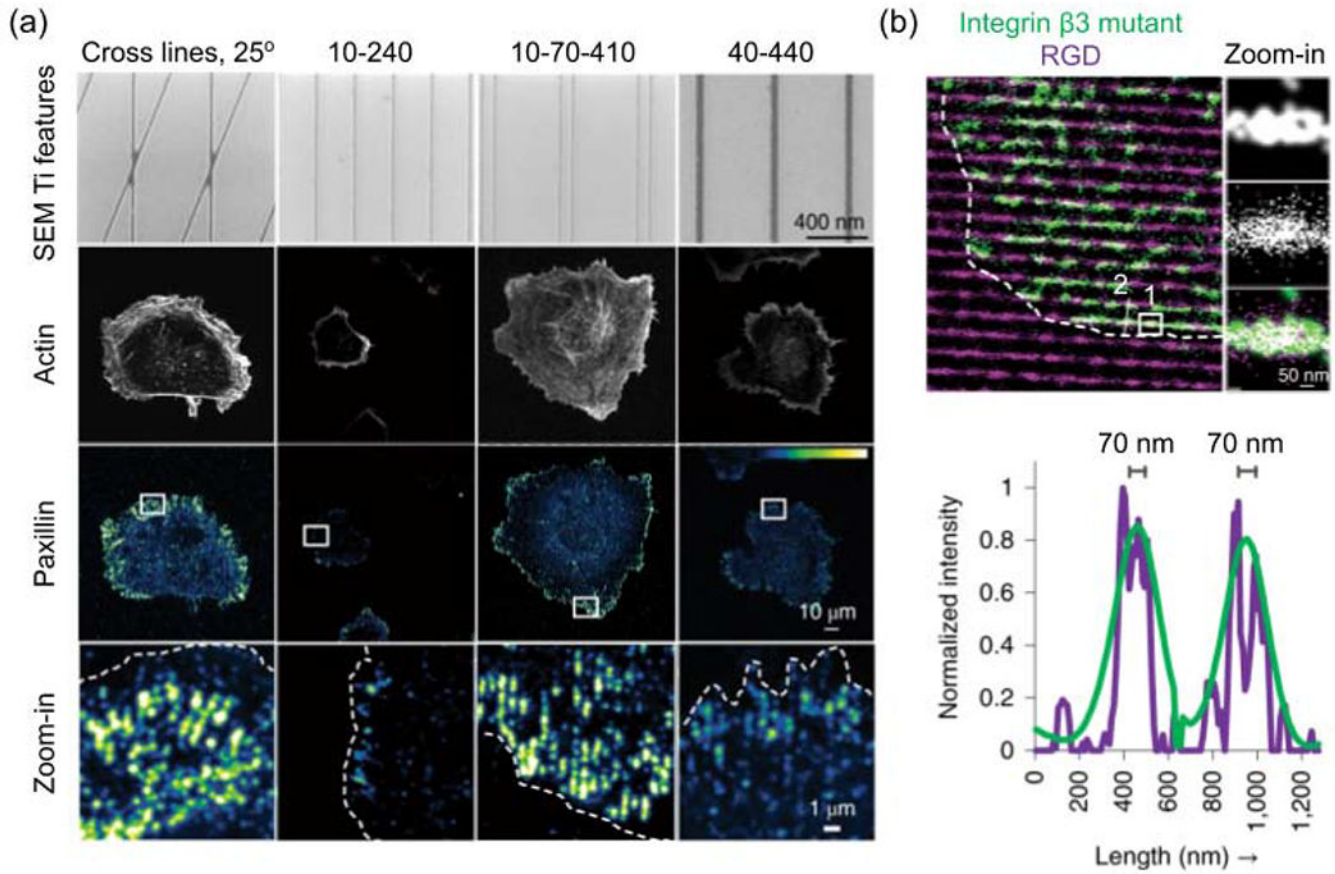

Figure 2.

Cell adhesion on nanoline arrays. **(a)** Representative images of cell adhesion and spreading on different nanoline patterns, which are systematically named by the geometric parameters of features. For line pairs, the first number is line width, the second is internal edge-to-edge spacing between the two lines in a pair, and the third is edge-to-edge spacing between adjacent line pairs, in a unit of nm. Fluorescent protein (mApple)-tagged paxillin indicates the cell adhesion. **(b)** Integrin beta3 binds to nanolines coated with the RGD ligand. The figure is adapted with permission from [42], Springer Nature.

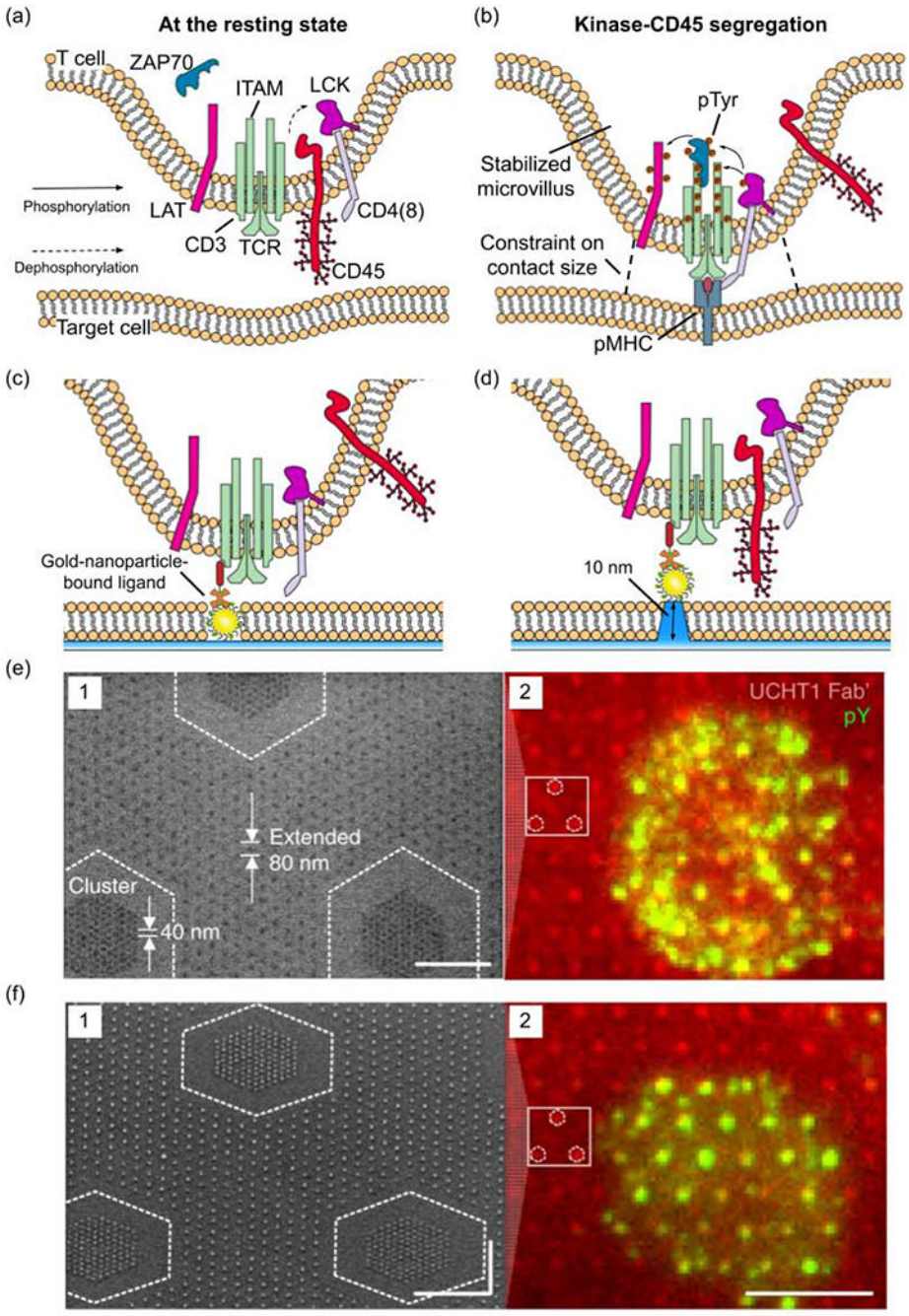

Figure 3.

The effects of ligand nanotopography on TCR triggering. **(a)** Schematic presentation showing the T cell at its resting state before TCR binding to the pMHC ligand. **(b)** Schematic presentation showing T cell activation and kinase-CD45 segregation from the contact site. **(c,d)** Schematic presentations showing the TCR triggering by nanofabricated patterns of anti-CD3 antibody-bond gold nanoparticles. The nanoparticles are embedded in a flat lipid bilayer surface (c) or lifted by 10-nm high glass pedestals (d). **(e,f)** SEM images of anti-CD3 antibody-bond gold nanoparticle arrays (image 1 of each figure, scale bars: 500

nm) on the flat surface (e) or lifted by 10 nm on glass pedestals (f). The nanoparticles are distributed as 40 nm clusters embedded in an 80 nm extended array. The image 2 of each figure shows the TCR triggering indicated by the immunofluorescence signal of phosphorylated tyrosine (green) on anti-CD3 antibody (red)-bond gold nanoparticle arrays, scale bar, 5 μm. (c-f) are adapted with permission from [57], Springer Nature.

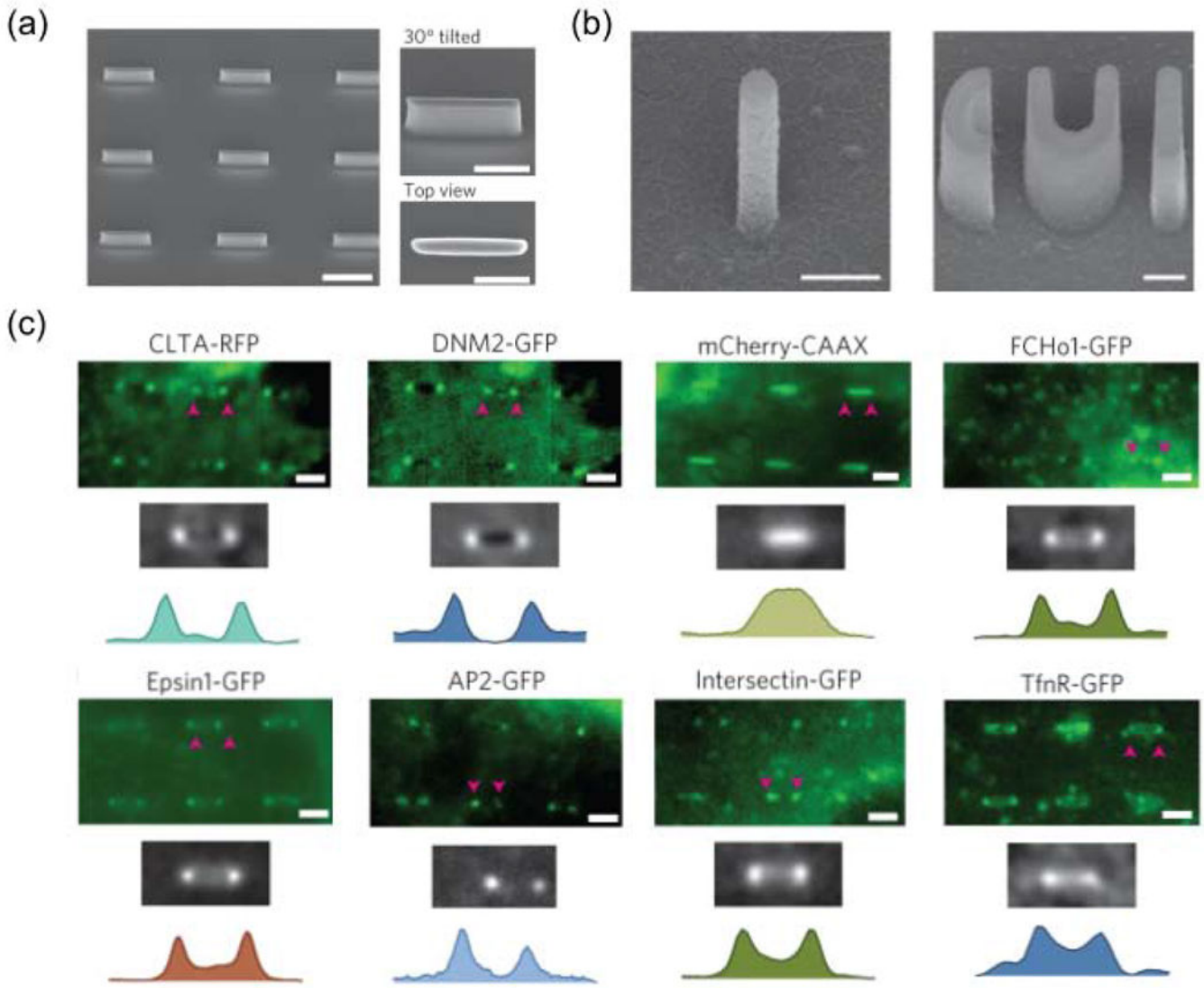

Figure 4.

Nanotopography enhances clathrin-mediated endocytosis in a curvature-dependent manner. (a) SEM images of a quartz nanobar array showing individual nanobars of 150 nm width, 2 μm length, 1 μm height and 5 μm pitch. Scale bar, 2 μm. Nanobar structure locally induces high curvature at the two ends and zero curvature in the middle. **(b)** SEM images of quartz nanopillar and nanoCUI structures. Scale bars, 500 nm. [73] **(c)** High-magnification fluorescence images, scale bars, 2 μm. They show the bar-end/high-curvature distributions of clathrin(CLTA), dynamin2 (DYM2), mCherry-CAAX, FCHo1, Epsin1, AP2, Intersection and TfnR on six nanobars. Other endocytic proteins involved in different stages of endocytosis were also shown in the work. (a,c) are adapted with permission from [72], Springer Nature. (b) is adapted with permission from [73], Springer Nature.

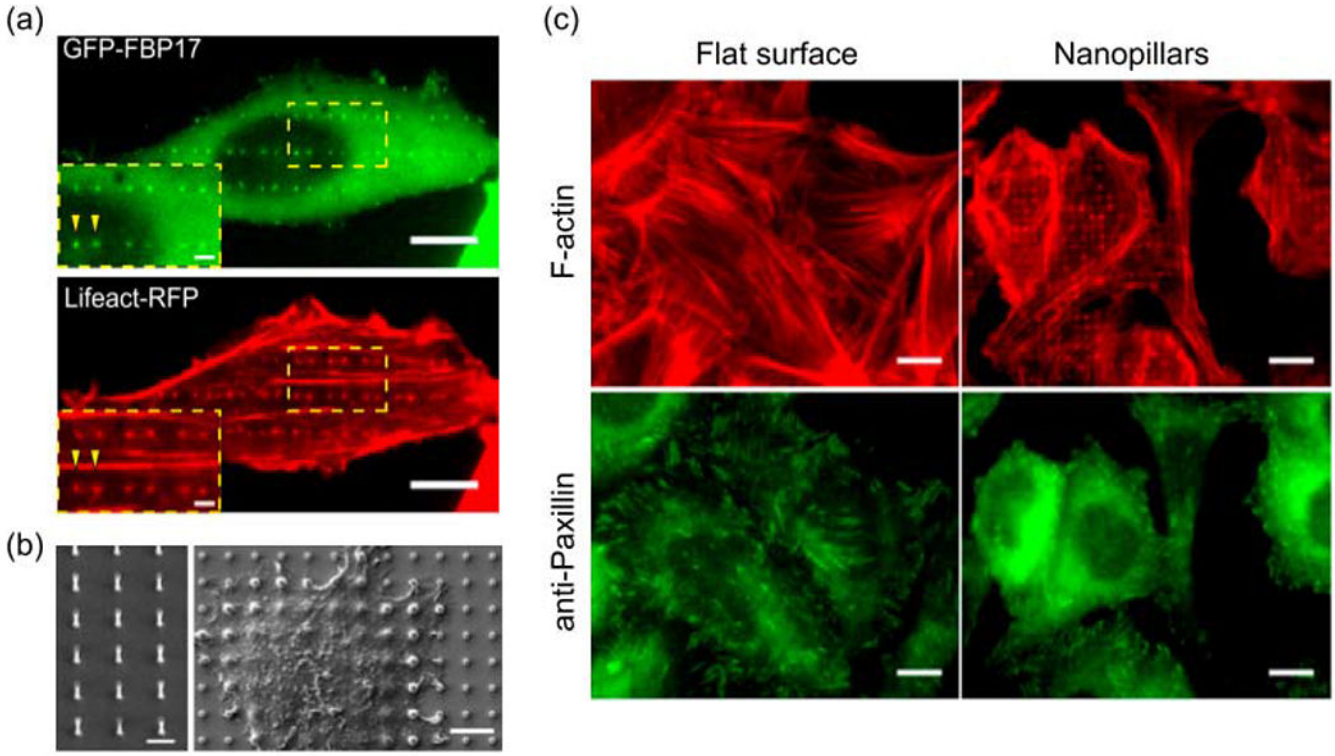

Figure 5.

Nanotopography induces F-actin polymerization in a curvature-dependent manner. **(a)** F-actin, probed by Lifeact-RFP, and FBP17 preferentially accumulate to the ends of nanobars, which represent high curvature locations. Scale bars, 10 μm. **(b)** SEM images of a vertical quartz nanopillar array used in this research and cells growing on nanopillars. Left: scale bar 2 μm. Right: scale bar 5 μm. **(c)** Anti-paxillin staining of focal adhesion and phalloidin staining of F-actin in U-2 OS cells growing on the flat surface and nanopillars. Scale bars, 10 μm. (a-c) The figures are adapted with permission from [89]. Copyright 2019, PNAS.

Table 1.

Summary of the impact of nanotopographical features on YAP translocation and stem differentiation*

Cell type	Topography	Diameter	Height	Pitch	Material	Culture condition	Effect on differentiation	Yap translocation	ref
hMSCs**	Rough surface	200nm	Not mentioned	Not mentioned	glass	conditioned osteogenic media, Day 3 on topography	Enhancement of hMSC Osteogenesis	(TAZ) Cytosol to nucleus	[103]
iPSC***	Nanopillar,nanograting	500nm	560nm	500nm-1μm	PDMS	neurogenic media, Day 6 in culture	Promote neuronal differentiation	Nucleus to cytosol	[104]
MSC	Nanorod	200nm	1.8-2.5μm	dense	bulk metallic glass	Media with adipogenic and osteogenic factors mix, 24 h on nanorod	Promote adipogenesis	Nucleus to cytosol	[105]
HUVEC**** and hMSC	Nanoneedle	50nm-700nm at the tip	3-4μm	Around 2μm	silicon	Basal media with no induction factor, 6h/24h post seeding	Promote adipogenesis in adipogenic media. Not 700nm but 50nm nanoneedles inhibit osteogenesis in osteogenic media. No effect in basal media.	Nucleus to cytosol	[106, 107]

* Studies of Tissue/Embryo differentiation is not included

** hMSC: human mesenchymal stem cell

*** iPSC: Induced pluripotent stem cells

**** HUVEC: Human umbilical vein endothelial cells