Biomaterial nanotopography-mediated cell responses: experiment and modeling
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The rapid development of fabrication and processing technologies in the past two decades has enabled researchers to introduce nanoscale features into materials which, interestingly, have been shown to greatly regulate the behavior and fate of biological cells. In particular, important cell responses (such as adhesion, proliferation, differentiation, migration, and filopodial growth) have all been correlated with material nanotopography. Given its great potential, intensive efforts have been made, both experimentally and theoretically, to understand why and how cells respond to nanoscale surface features, and this article reviews recent progress in this field. Specifically, a brief overview on the fabrication and modification techniques to create nanotopography on different materials is given first. After that, a summary of important experimental findings on the mediation of nanoscale surface topography on the behavior of various cells, as well as the underlying mechanism, is provided. Finally, both classical and recently developed approaches for modeling nanotopography-mediated cell adhesion and filopodial growth are reviewed.

Keywords: biomaterials; topography; cell–material interaction; experiment; modeling

1. Introduction

The emergence of nanotechnology has demonstrated extraordinary capacity of nanoscale structures in affecting activity and fate of biological systems, leading to the births of many new research areas such as nanobiomaterials, nanomedicine, and nanobiotechnology \cite{1}. Up to date, a myriad of studies in these areas have unveiled the important role of nanomaterials in modulating functions and responses of cells and tissues, paving the way for delicate control or mediation of biological events through specifically designed and engineered biomaterials including molecular assemblies, polymers, ceramics, glasses, and metals. Influences of the aforementioned nanomaterials on the responses of different cells and tissues such as bone, neural, vascular, skin, and so on have all been reported \cite{1–3}, where the regulation is commonly believed to be achieved through the nanoscale properties on the material surface, including surface chemistry, topology, hydrophilicity, etc. Among these factors, nanotopography has received increasing attention in the past decade due to the rapid development of fabrication technologies.

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By definition, surface topography includes the gradual undulations, sharp spikes, and even pores present on the material surface [4]. In this regard, nanotopography indicates that the size of these surface features are in the nanometer range (typically 1–100 nm, sometimes up to a few hundreds nanometers). Note that, a landscape at this scale leads to a dramatically increased surface area and, eventually, results in high surface activity as well as the formation of large amount of surface substructures like grain boundaries [5]. More importantly, given that the size of surface features in this case is comparable to those of proteins and cell organelles, extensive interactions between the material and corresponding biological entities are expected to take place. Therefore, exploring the interplay between material nanotopography and cell behavior has become an important topic in biotechnology and biomedical engineering, attracting researchers from both experimental and theoretical fields. In terms of publication numbers, reports on biomaterial nanotopography-mediated cell responses have increased rapidly since the late 1990s. Advances in this field have also brought new insights and tools to medicine, biology, and clinical studies, and led to a large number of applications.

The purpose of this article is to review recent progress in experimental and theoretical studies in this area, with particular emphasis on how material nanotopography regulates cell adhesion, proliferation, differentiation, morphology, migration, and filopodial dynamics as well as on modeling cell–substrate interactions and filopodial growth under the influence of surface roughness. However, to put everything into context, a brief overview on fabrication techniques that allow the introduction of well-defined nanoscale surface features is presented first. It is also worthy to point out that although issues like cytotoxicity and apoptosis are important in studying the response of cells to nanomaterials, we do not deal with these aspects here since the depth and complexity of such topics are beyond the scope and space limitations of this article.

2. Brief overview on fabrication of nanotopography

In general, strategies to create nanotopography can be categorized into two groups: top-down and bottom-up. Nanotechnology has been considered as a meeting point of these two approaches, whose effectiveness in fabricating desired topographies has been widely demonstrated (with representative examples illustrated in Figure 1). Roughly speaking, the top-down strategy often refers to techniques such as machining, etching, milling, or successive cutting large-sized bulk materials, while the bottom-up strategy builds a material from units, usually via self-organization or self-assembly of atoms and molecules, at much smaller scales. Several important and frequently used fabrication techniques for generating nanotopography are introduced as follows:

**Nanolithography.** Nanolithography uses lights, charged ions, or electron beams to transfer the geometric pattern from a pre-made photomask to a photosensitive layer coated on the target material, and then relies on a series of posttreatments to chemically engrave the transferred pattern into the material or allow the deposition of new compounds along the pattern [6]. It has been demonstrated that, using different types of radiations, sub-10-nm features can be created via nanolithography [7].

**Nanoimprint.** This technique generates nanoscale patterns by physically deforming the solid and thus can be used for direct imprint. Usually, the imprinted material will be used as photoresist for pattern transfer in conventional lithography. Nanoimprint has many advantages over radiation-based patterning techniques, including high resolution of sub-3 nm and capabilities for large-area and 3D patterning.
Nanoprinting. Nanoprinting uses a stamp with surface patterns to transfer a liquid ink onto a surface [8]. The feature resolution obtained from such technique is poorer than nanoimprint, because of the difficulties in controlling the flow and amount of the ink [7]. Improved nanoprinting technique uses solid inks that can detach from the stamp, on which they are originally imprinted/deposited, after bonding to the target surface [9].

Deposition techniques. The most widely known deposition approaches include physical vapor deposition (PVD), chemical vapor deposition (CVD), and atomic layer deposition (ALD), which are all commonly used in fabricating nanoscale thin films, fibers, whiskers, and tubular or rod-like structures. PVD is a coating method that transports physically vaporized materials from a source onto a substrate in a vacuum chamber where condensation of vapors will form a thin film with atomic- to nanoscale roughness on the surface. In comparison, CVD, as indicated by its name, is a deposition method via chemical reaction of vapors or gas phases. The CVD technique is very versatile for creating nanomaterials with multiple dimensions (from 0D to 3D), highly ordered topographies (from dots, wires to scaffolds), and complex compositions. ALD employs sequential use of a gas-phase chemical process with atomic-level precisions at low temperatures (<100°C) or room temperature [10]. The precise control over the thickness of the deposited layer at low temperatures renders ALD advantageous in handling bioactive materials as well as in fabricating nanotopography with extremely small roughness.

Figure 1. Top-down and bottom-up approaches for fabricating nanotopographies. Examples shown (clockwise from top) are (i) an electron microscopy image of a nanomechanical electrometer obtained by electron-beam lithography; (ii) patterned films of carbon nanotubes obtained by microcontact printing and catalytic growth; (iii) a single carbon nanotube connecting two electrodes; (iv) a regular metal-organic nanoporous network integrating iron atoms and functional molecules; and (v) seven carbon monoxide molecules forming the letter ‘C’ positioned at the tip of a STM (reprinted from [11] with permission).
Self-assembly. Self-assembly techniques are bottom-up in nature where, driven by local energy minimization, micro- or nanostructures are formed spontaneously in the material [7]. Among such approaches, unguided self-assembly refers to that we allow the process to take place without exerting any control which, naturally, will lead to material system with sub-domains randomly oriented/structured. However, within each domain, the components or building blocks are orderly arranged. To overcome this issue, templates can be used to guide the self-assembly from which desirable orientation of nanostructures within sub-domains of the material can be created. Such technique is called template-assisted self-assembly.

Phase separation technique. This technique relies on the simultaneous phase separation due to miscibility between different compounds like block copolymers. Such block copolymers with temperature-dependent miscibilities are initially connected and mixed well with each other under certain temperature. When the mixture is heated to another temperature, phase separation occurs as different block copolymers become non-mixable. Since the block copolymers are connected at their ends, internal stress induced by phase separation can force the spatial segregation among them and, eventually, result in ordered nanopatterns.

3. Experimental studies on nanotopography-mediated cell responses
This section overviews recent progress in the experimental studies on nanotopography-mediated cell responses from three aspects: cell functions on various nanotopographies, cell locomotion on nanostructured surfaces, and the current understanding of possible mechanisms behind. Here, the term ‘cell function’ includes cell adhesion, proliferation, differentiation, etc. How the cell functions are influenced by a wide spectrum of nanomaterials will be discussed. On cell locomotion, the focus is on the growth and movement of filopodia on substrates with nanotopography. At last, possible mechanisms behind the regulation of biomaterial nanotopography on cell activities are briefly summarized.

3.1. Cell behavior on nanotopography of different materials
3.1.1. Polymers and macromolecules
Polymer and macromolecule are among the earliest reported nanomaterials whose topography can alter cell responses [12,13]. Owing to the wide use of polymers and macromolecules in biomedical applications, their abilities to regulate cell activities have drawn great attention. It is important to emphasize that many natural tissues are essentially composed of nanoscale biopolymers or biomacromolecules (with a hierarchical architecture). Therefore, by mimicking their natural counterparts, synthetic polymers and macromolecules might be able to enhance/regulate the functions of specific cells or tissues. This principle has been demonstrated through the wide application of synthetic polymers and macromolecules (like poly(lactic-co-glycolic) acid (PLGA), poly(dimethylsiloxane) (PDMS), poly(ε-caprolactone) (PCL), polyurethane (PU), peptide amphiphile, etc.) in clinical practice (for example, PLGA is approved by the FDA) as well as in laboratory research.

In particular, the capabilities of polymeric surfaces with ordered nanofeatures in regulating the behavior, including adhesion, growth, alignment and elongation, of vascular cells have been convincingly demonstrated. For instance, it was found that PLGA surfaces with 200-nm spherical topography promote the adhesion of endothelial and smooth muscle cells, compared to smooth PLGA substrates or surfaces with 500-
100-nm features. Interestingly, such enhanced adhesion is correlated with a higher fibronectin adsorption on the PLGA substrate with spherical 200-nm topography [12,14]. Similarly, in vivo studies revealed that cells (and tissue) respond quite differently to PCL with nanocylindrical topography (cylinders of 160 nm in height and 100 nm in diameter) and natural polyester with nanopit topography (pits of 100 nm in depth and 120 nm in diameter) [15]. In vivo tests also showed that, compared to PCL with nanopits topography or flat PCL substrates, nanocylindrical PCL leads to a significantly higher vascular cell density (in terms of the number of microvessels per unit area) along with a decreased inflammation. The effect of alternating nano and micron roughness on cellular response has been examined as well. Specifically, PDMS films consisting of periodic arrays of nanogrooves (500 nm) with spacings ranging from 22 to 80 μm were fabricated, and it was found that the adhesion and spreading of rat aortic endothelial cells (RAECs) were greatly enhanced on the patterned PDMS surface with the largest spacing where cell elongation was almost twice of that on non-patterned surfaces [16]. These results clearly demonstrate that different polymer nanotopographies indeed lead to distinct responses of vascular cells. Particularly, enhanced endothelial responses are usually observed on continuous 2D nanotopographies (grooves, strips, and cylinders).

Besides vascular cells, the behavior of bladder smooth muscle cells (BSMC) is also sensitive to nanotopography of polymeric materials. For example, both long-term growth and proliferation of BSMC (up to 5 days) were greatly enhanced on PLGA and PU films (chemically etched) with random nanometer surface topographies (feature dimensions 50–100 nm) compared to those with surface feature dimensions larger than 10,000–15,000 nm [17]. Similar nanotopography-mediated cell responses are also reported in 3D PLGA or PU nanoscaffolds, where BSMC adhesion, proliferation, and production of extracellular matrix (ECM) proteins such as elastin and collagen were all enhanced [18]. These studies attributed the enhanced cell functions to the nanometer surface roughness of polymers which is comparable to those exhibited by ECM components in native bladder tissue, forming a favorable microenvironment for BSMC attachment and growth. This hypothesis was further tested and validated by examining the assembly of BSMC cytoskeleton on polymer nanotopographies, given that anchorage-dependent cell growth should be strongly influenced by the spreading and reshaping of the cytoskeleton [19]. For example, Baker et al. fabricated aligned polystyrene (PS) nanofibers of 200 nm in diameter by electrospinning and found that such nanotopography could guide the organization of actin filaments in BSMCs in a way similar to that in native bladder tissue [20].

For bone cells like osteoblasts (bone forming cells), the enhancing effect of polymer nanotopography with feature sizes ranging from 10 to 100 nm on various cell responses has been well established by a great number of studies [1,21–23]. Here, the extensive examples of osteoblast are not provided due to space limitation. Nevertheless, it is worth mentioning that both topographical (e.g., feature geometry, periodicity, etc.) and non-topographical (chemistry, surface energy, charge, etc.) factors will affect the activities of osteoblast. For instance, pits-patterned PS and islands-patterned poly(L-lactide) (PLLA) surfaces, with similar surface roughness (both having a feature size of ~450 nm), were used for examining osteoblast–substrate interactions, and it was found that cell adhesion is much more prominent on PLLA island surface than on PS pit nanotopography. The study also revealed that both nano- and micron-patterned PLLA and PS surfaces could enhance cell adhesion compared to smooth PLLA and PS substrates, while cell proliferation was statistically similar on all topographies (i.e., nano- and micron-patterned and smooth ones) [24]. Based on these results, caution should be exercised when defining an optimal
nanotopography for cell responses, and categorizing material topography solely by roughness value should be avoided.

It is not difficult to infer that different types of cells will probably respond distinctly to the same polymer nanotopography. This kind of cell type-specific response suggests that polymer nanotopographies might be able to selectively mediate the functions and activities of various cells. Indeed, as reported by Smith et al. [25], the presence of nanometer scale roughness on PLGA surfaces, prepared by NaOH-etching, inhibits the adhesion of fibroblast (cell forms connective tissue) while, interestingly, promotes osteoblast adhesion. Subsequent studies have further demonstrated that a decreased fibroblast adhesion on PLGA, PU, and PCL substrates will be induced only if the surface roughness is in the range of 50 to 100 nm [26]. This selective mediation on different cell types renders the possibility of suppressing the activities of undesired cells while simultaneously promoting the response of target cells. For example, it is conceivable that one can utilize polymer nanotopographies to decrease cancer cell functions but maintain or even enhance the responses of normal cells. Following this logic, Zhang et al. examined adhesion, proliferation, apoptosis, vascular endothelial growth factor (VEGF) secretion of breast epithelial adenocarcinoma (BEA) cells and lung epithelial carcinoma (LEC) cells on PLGA nanotopographies with spherical surface features (i.e., nanosmooth or with feature diameters of 23, 300 and 400 nm, Figure 2). Interestingly, compared to all other PLGA topographies mentioned above, significantly decreased functions of BEAs (including decreased proliferation rate, increased apoptosis, and decreased VEGF synthesis) were observed on 23-nm featured PLGA surfaces while, at the same time, the proliferation rate of healthy breast epithelial cells was found to increase by ~24% [27]. For LEC cells, the lowest growth rate was found on PLGA nanotopography with a feature diameter of 400 nm while the least VEGF synthesis was observed on the 23-nm featured surface [28].

Along with the growing interest of utilizing stem cells in regenerative medicine and cell therapy, mediation of stem cell fate by biomaterial nanotopography becomes an important research topic. For example, Dalby et al. reported the control of the differentiation of human mesenchymal stem cell (MSC) via nanoscale symmetry and disorder on polymethylmethacrylate (PMMA) substrates [29] where symmetric or disordered nanopits of 120 nm in diameter and 100 nm in depth were introduced on the surfaces. The results showed that highly ordered or symmetric nanotopographies produce very low or negligible MSC adhesion and osteoblastic differentiation, while MSCs on random nanotopography exhibit a more osteoblastic morphology and undergo osteospecific differentiation more. Another recent study from the same group reported the capability of PCL nanotopography to maintain MSC phenotype and multipotency for a long period of time up to 8 weeks [30]. With regard to material composition, nanotopography of both natural and synthetic polymers and macromolecules has been used for supporting MSC differentiation and self-renewal [31]. Figure 3 shows representative natural and synthetic nanopatterns that are successfully fabricated to offer template for MSC proliferation and differentiation.

Besides nanotopography, the rigidity of material is also reported to influence the focal adhesion formation (will discuss later), cytoskeletal organization, and mechanical properties of human MSC [32]. Specifically, MSCs cultured on 350-nm gratings of PS and PDMS showed decreased expression of several integrin subunits, compared to that on the non-patterned substrates, and assumed an elongated morphology accompanied by the alignment of actin cytoskeleton. Furthermore, MSCs on gratings surface of stiffer PS appear to have a lower elastic modulus and apparent viscosity. However, the
effects of nanotopography become insignificant when very soft PDMS substrates are used. This result again demonstrates that non-topographical factors like material rigidity have a significant impact on stem cell functions and such influence may surpass the effect of nanotopography. Hence, a variety of considerations, including topography, must be taken into account when designing high-performance nanobiomaterials.

It is important to point out that polymeric materials are generally soft (and can have large variations in stiffness) and therefore are more likely to change their topographies under stress compared to other types of materials. In addition, it has been well documented that the behavior of cells is strongly influenced by the compliance of their microenvironment [33]. Therefore, cell responses on stressed polymers, a common scenario for biological system in vivo, are probably affected by complex interactions between the nanotopography and deformability of the material. Since how topography is affected by stress filed within the material is still not well understood, experiments alone could sometimes be insufficient (or even impossible) and, hence, the introduction of theoretical and modeling approaches to this filed become absolutely necessary.
3.1.2. Metals and alloys

Due to their prevalent use in orthopedic and dental implants, metals and alloys (including Ti and its alloys, stainless steels, CoCrMo alloys, and Ta) are always among the key materials in nanotechnology research for biomedical applications. Cell responses (mostly bone cell responses) on metal nanotopography have been extensively investigated, and some research outcomes have already been employed in the design of new medical implants [3,34]. For instance, it has been shown that osteoblast adhesion, proliferation and differentiation can all be significantly increased on nanophased Ti, Ti6Al4V, CoCrMo, and stainless steel with nanoscale topographies compared to those with micron-rough or nanosmooth topographies [35,36]. Study by Tambasco de Oliverira et al. also demonstrated that nanotextured Ti, with ~10-nm honeycomb-like pits on the surface, enhanced metabolic activities of osteogenic cells (specifically, the upregulations of bone sialoprotein and osteopontin) [37]. In general, there is a rich literature on promoting bone cell functions by metal surface nanotopographies and readers can refer to such work if interested. Besides bone cells, the responses of fibroblast, endothelial and smooth muscle cells can be also controlled (i.e., promoted or impeded) by Ti and Ti alloy
nanotopographies [38,39]. For example, Khang and Lu et al. examined the role of Ti nanotopographies on vascular and bone cell adhesion using flat (surface fluctuation <1 nm), nano (with feature dimensions of 30–40 nm and 2–6 nm in lateral and vertical directions), and nanosubmicron (with feature dimensions of 100–250 nm and 20–40 nm in lateral and vertical directions, respectively) surfaces [39]. Both cell types show increased adhesion response on nanosubmicron and nanotopographies, and the trend of adhesion enhancement is the same as that for surface energy, that is nanosubmicron (69 mJ/m²) > nano (62 mJ/m²) > flat (54 mJ/m²). Comparing to the flat surface, although the nanosubmicron surface had the highest surface energy and the greatest cell adhesion densities, the nanosurface was found to be more efficient in increasing both surface energy and cell adhesion when considering the relatively smaller changes in surface area and surface roughness.

In comparison with polymers, metal is sometimes a better choice for investigating/controlling cell mobility or morphology due to its high stiffness, high stability, and ordered atomic structure. Figure 4 shows the migration of RAECs on Ti surfaces with flat (F-Ti, root mean square (RMS) roughness of 0.38 nm), nanorough (N-Ti, RMS roughness of 1.15 nm), and submicron rough (S-Ti, RMS roughness of 13.77 nm) topographies within 24 hr, indicating that surface undulations may actually help cell locomotion [40]. Control of cell morphology and alignment can also be achieved by proper design of surface topography on metallic materials. For example, it has been

![Figure 4](image_url)

Figure 4. Fluorescence microscopy images showing the migration of rat aortic endothelial cell (RAEC) on flat (F-Ti), nanorough (N-Ti) and submicron rough (S-Ti) topographies. Cells were stained with calcein AM. Scale bars = 200 mm. Dash lines indicate starting points at time zero (adapted with permission from [40]).
shown that nanorough and micron-patterned Ti surfaces can direct the morphology and adhesion of osteoblast [41]. Specifically, alternating stripes with different roughness values (RMS roughness of 20 and 57 nm, respectively) were created on the Ti surface, and it was found that osteoblasts tended to align along the patterned stripes as well as adhered more to the smoother parts (i.e., regions with a RMS roughness of 20 nm). More interestingly, decreasing the width of smoother stripes from 80 to 20 μm led to a drop in the density of osteoblasts adhering to these areas as well as a change in the osteoblast morphology from an elongated shape to a more rounded one.

Although not universally observed, there are a lot of studies suggesting that the positive role of material nanotopography on promoting various cell functions is associated with the higher adsorption of adhesion proteins (e.g., fibronectin, vitronectin, etc.) on nanofeatured surfaces, compared to conventional, micron topographies. Such increased protein adsorption is believed to be responsible for the enhanced osteoblast adhesion on Ti, Ti6Al4V, and CoCrMo nanotopographies [35]. But again, whether this is really the case remains unclear. Some studies reported that the increased fibronectin and/or vitronectin adsorption is due to the elevated surface energy when the surface features change from micron to nanometer scales. However, the actual situation may be much more complicated than this as other investigations have attributed the higher protein adsorption to the increased surface area and/or confined space when topography changes.

3.1.3. Ceramics and non-metals

Besides polymers and metals, ceramics and other non-metallic materials such as Si and carbon nanostructures also exhibit strong nanotopographical effects on cell functions. For example, alumina (or Al2O3) and titania (or TiO2), native oxides of Al and Ti, respectively, have been widely used in orthopedic applications. A study showed that nanoporous alumina membranes with a pore diameter of 20 nm increase the growth and spreading of platelet as well as the expression of P-selectin (a cell adhesion molecule on the surface of activated endothelial cells), while the 200-nm membrane showed little effect [42]. In addition, nanograined alumina and titania surfaces with nanometer roughness are well known for enhancing osteoblast adhesion, proliferation, and differentiation (including mineral deposition and collagen synthesis) compared to conventional, micron rough oxides [43,44]. Similarly, it has also been demonstrated that surface topographies of nanotubular titania, fabricated by anodizing Ti, can enhance bone cell functions [45,46]. More interestingly, recent study has found that, without osteogenic inducing media, altering the size of such nanotubular features will lead to either augmented human MSC adhesion or their direct differentiation into osteoblasts [47]. Specifically, small (30 nm in diameter) nanotubular topography promotes MSC adhesion without noticeable differentiation, whereas stem cells on larger (70 to 100 nm diameter) nanotubular topographies undergo dramatic elongation (10-fold increase), which is believed to induce cytoskeletal stress and subsequently trigger differentiation of MSCs into osteoblast-like cells.

A relatively less-known orthopedic coating material, nanocrystalline diamond (NCD), has also been used recently to regulate the behavior of bone cells [48–50]. In particular, it was found that rationally designed NCD topography and nanoscale roughness (RMS roughness typically <25 nm) could enhance both short-term (adhesion, proliferation) and long-term functions (differentiation and bone synthesis) of osteoblast compared to micron or submicron crystalline diamond (MCD) [51–53]. Figure 5 shows images of osteoblast adhered on NCD or MCD substrates, where significant difference in cell
morphology can be seen. Basically, compared to MCD, stronger adhesion between osteoblasts and NCD surface is formed leading to the occurrence of greater cell spreading, enhanced cell proliferation and differentiation. Such enhancement in cell responses could result in faster tissue regeneration in vivo, and this hypothesis has been partially verified by a recent animal study. In the study, diamond rods were implanted in rat femoral diaphysis and, as expected, faster growth of connective tissue was observed on NCD than MCD after 4 and 8 weeks [54].

Cells also respond to carbon nanotubes (CNTs) and carbon nanofibers (CNFs). For example, linear CNF patterns (30 μm in width) on polycarbonate urethane (PCU) have been used to control the spatial attachment of osteoblasts [55]. Specifically, selective adhesion and alignment of osteoblasts on CNF patterns as well as greater attraction forces between fibronectin and CNF patterns were all observed (compared to pure PCU substrate). More importantly, the study showed that the aligned osteoblast adhesion on CNF patterns was coupled with an enhanced calcium phosphate mineral deposition along CNFs, indicating the capability of CNF nanotopography in regulating osteoblast functions (such as migration and bone formation). In addition, CNT nanotopography has been reported to guide stem cell differentiation as well. For instance, it was reported that poly(acrylic acid) (PAA)-grafted CNT thin films could selectively differentiate human embryonic stem cells into neurons while maintaining excellent cell viability. In addition, the neuron differentiation efficiency of PAA-grafted CNT thin films was two times and one time greater than that on PAA and poly-l-ornithine (commonly used for neuron culture) substrates, respectively [56]. Because CNT and CNF are frequently used as reinforcement or conductive component in biomaterials to improve their mechanical or electrical properties, the ability of CNT and CNF to regulate cell functions will certainly add new dimensions to the development of composite biomaterials in the future.

Figure 5. Atomic force microscopy (AFM) images of (a) nanocrystalline diamond (NCD) and (b) submicron crystalline diamond (MCD) surfaces. Scanning electron microscopy images of osteoblasts adhering on (c) NCD and (d) MCD substrates after 48-hr culture, revealing distinct cell morphologies (modified with permission from [51]).
In the mechanistic study of nanotopography-mediated cell responses, Si has attracted great attention due to its mature usage in microfabrication and lithography. In particular, various nanotopographies can be easily fabricated on Si surfaces, allowing researchers to systematically investigate how surface topography affects the behavior of cells. Using interference lithography and deep reactive ion etching, Choi et al. created altered 3D features on Si surfaces and examined the response of fibroblast to such topographies [57]. Specifically, Si nanoposts and nanogrates with varying three-dimensionalities (50–600 nm in nanostructure height) but the same pattern periodicity (230 nm in pitch) and tip shape (needle- or blade-like sharp tips) resulted in distinct fibroblast responses: fibroblasts exhibited significantly smaller size and lower proliferation rate on needle-like nanoposts, but enhanced elongation and alignment on blade-like nanogrates. These phenomena became more pronounced as the nanotopographical three-dimensionality (structural height) increased. In a comprehensive review by Biggs et al., the effect of Si nanogroove topography on cell adhesion is summarized [58]. Generally, nanotopographies with a groove pitch <35 nm and a depth <70 nm do not initiate contact guidance in most types of cells, while reduced adhesion and oblique adhesion (i.e., reduced alignment with orientation) are more likely to appear when the groove depth is much larger than 300 nm. For more details, readers can refer to this review article. At last, there have been a good number of studies on biomaterial-based topographical controls of cell behavior and functions [59–62], which is also highly related but will not be covered here due to space limit.

3.2. Cell filopodial dynamics on biomaterial nanotopography

Recently, examining the behavior of individual cells in response to nanotopography is becoming an increasingly popular research area, presumably because direct evidence and more insights on cell–nanomaterial interactions can be provided by such studies. Particularly, cell locomotion on nanomaterial surface has attracted great attention due to its pivotal role in processes like wound healing and neural regeneration. For triggering cell locomotion on a biomaterial surface, accumulating evidence has shown that cell filopodia play an important role in the initiation of cell locomotion by sensing and transducing signals (chemical as well as mechanical) from outside into the cell [63]. Filopodium is the finger-like protrusion composed of parallel bundles of actin filaments wrapped around by cell membrane, as illustrated in Figure 6. The role of filopodia in serving as sensors for cells to seek microenvironment and sites for adhesion and migration has been well documented [64]. Therefore, understanding filopodial dynamics on biomaterial nanotopography will be critical in analyzing the response of cells to topographical cues.

There are already some studies focusing on the interplay between filopodia and substrates with different rigidities. For example, recent experimental and modeling investigations indicated that filopodia can respond to the stiffness of the microenvironment via a myosin-directed retrograde flow mechanism [65]. As a result, periodic deformation will occur in soft substrates (elastic modulus <1 kPa) due to the slow actin retrograde flow and high traction force generated at the cell–substrate interface. In contrast, little deformation will be induced in stiff substrates (with moduli as high as about 100 kPa) because the filopodial traction force in this case is much smaller. However, in light of all the studies mentioned in previous sections, this knowledge on filopodium–substrate interactions is clearly inadequate or even inapplicable for most biomaterials whose stiffness is typically in the range of MPa to GPa (such as metals, ceramics, and many polymers). As a result, the traction force generated can hardly deform these stiff materials and a filopodium may
have to follow exactly the surface profile during its growth. Therefore, topography of these biomaterials should become a critical factor affecting filopodial dynamics and other related processes such as cell adhesion and spreading. Following this logic, a number of studies have been performed and found that, indeed, the growth dynamics of filopodia is regulated by the nanotopography of stiff materials. For example, filopodia formation of human osteoblast on MCD and NCD substrates, with distinct topographies but similar surface chemistry and hydrophobicity (surface energy), has been examined [53]. Interestingly, filopodia of osteoblasts cultured on MCD films tend to converge to specific sites on the diamond surface, while radial and parallel filopodia are formed on the NCD substrate. In addition, a recent study also reveals that, compared to MCD films, filopodial extension velocity of osteoblast is much faster on the NCD substrate, supporting the idea that filopodial locomotion is also influenced by the surface nanotopography [67]. It was reported that fibroblasts growing on well-defined 3D sharp-tip Si substrate form only short filopodia along the sharp tips, when the height of nanoposts is around 500–600 nm. In comparison, long filopodia, starting from the tip all the way down to the valley between nanoposts, can be developed if the height decreases to 50–100 nm (the spacing between nanoposts is fixed at 230 nm) [57]. Another study using the similar Si substrate found that fibroblast filopodia could reach to the bottom surface when the feature height of sharp-tip nanotopographies (nanoposts or nanogratings with a periodicity of 230 nm) is between 50 and 100 nm and, consequently, the cell was well spread. However, when the feature height increases to the range of 200–600 nm, most filopodia failed to reach to the bottom and the cell was unable to conform to the surface nanotopography (instead only contacted with the sharp tips) [68]. Similarly, Dalby et al. reported that fibroblast filopodia can sense the topography of PMMA substrates, with nanocolumn arrays up to a feature height of ~160 nm, while the topographical sensitivity of filopodia on PCL nanopit arrays can be as low as 35 nm in pit diameter [69,70].

Despite these preliminary results, important questions on how filopodia interact with stiff topography, how the deformation of a filopodium occurs near the surface features, and how the ‘walking style’ of filopodia relates to cell spread on different topographies have not been answered. These issues will be further discussed in Section 4 when computational and modeling efforts in this area are presented.
3.3. Mechanisms of nanotopography-mediated cell responses

The studies summarized above have clearly supported the idea that biomaterial nanotopography plays a crucial role in mediating cell responses/functions including adhesion, spreading, proliferation, differentiation, and migration. However, the underlying mechanisms as well as what exactly happens at the cell–material interface are still not well understood to date. Undoubtedly, these questions are critical for the rational and effective design and fabrication of nanobiomaterials in the future. Currently, several possible answers have been suggested.

3.3.1. Selective protein adsorption

There are a great number of studies focusing on whether and how the material nanotopography can enhance the adsorption of a list of cell adhesion proteins, especially fibronectin and vitronectin. It is well documented that increase in fibronectin or vitronectin adsorption on material surfaces can promote short-term cell adhesion and, over a prolonged period, proliferation [71,72]. It is also commonly believed that only nanotopography with feature dimension similar to those of surface-bound proteins (~10 nm) affects their activities [73]. For example, Gonzalez-Garcia et al. studied fibronectin adsorption on well-defined nanotopographies of 14-, 29- and 45-nm-deep pits on PLLA/PS membrane [74]. Fibronectin adsorption quantified by radiolabelling revealed that the amount of protein was higher on the 14-nm-deep pit nanotopography than on the other two surfaces. More interestingly, distribution of fibronectin <10 μg/ml on 14-nm-deep pit topography tends to segregate in valleys (Figure 7), while fibronectin of high concentrations or distributed on other nanotopographies appears to be more evenly coated on the surface. These results clearly demonstrate the effect of nanotopography with feature scale around 10 nm on the amount and distribution of fibronectin absorbed on PLLA/PS surface.

![Figure 7. Selective adsorption of fibronectin on 14-nm-deep pit nanotopography of PLLA/PS. Height (first row) and phase (second row) AFM images for the nanotopography after fibronectin adsorption from solutions of concentrations 2, 5, 10, and 20 μg/ml. The height image allows one to identify the peaks and valleys in the surface while the phase image provides direct observation of fibronectin distribution. Reprinted with permission from [74].](image)
However, whether the material nanotopography alone is enough to increase the adsorption of specific proteins still remains unclear. Some studies reported a significant increase in fibronectin or vitronectin adsorption on surface topographies in the nanometer regime, which is probably due to the increased surface area or altered hydrophilicity. An investigation found that increasing the roughness of titania surface (from 15 to 30 nm) also leads to an increase in adsorbed proteins (including bovine serum albumin, fibrinogen, and streptavidin) and, furthermore, the increase in protein adsorption is actually much higher than that in the surface area. This interesting phenomenon is probably caused by elevated protein nucleation on the surface which is promoted by surface nanoscale pores [75]. In contrast, our recent studies revealed that the amounts of fibronectin adsorbed per unit true surface area were not statistically different for MCD and NCD films with similar hydrophobicity, but osteoblast and fibroblast adhesion and proliferation were significantly enhanced on NCD surface [2,76]. Cai et al. also showed that the nanotopography on Ti has little influence on the adsorption of albumin and fibrinogen [77]. Similarly, a study on the adsorption of hen egg lysozyme on poly(ether sulfone) (PES) surfaces clearly demonstrated that neither the adsorption density nor the structural stability of lysozyme was influenced by variations in the nanometer scale PES roughness (average roughness values ranging from 5 to 60 nm) [78].

In fact, a recent study on ultrahigh molecular weight polyethylene surfaces suggests that nanotopography, chemistry, crystallinity, and molecular chain anisotropy could affect protein and assembly at the same time [79]. It is possible, as demonstrated by the study, that nanotopography loses its effectiveness on mediating protein adsorption when surface chemistry and hydrophobicity are changed. Therefore, although quite some studies point toward that selective adsorption of adhesion proteins on material nanotopography might be the key for its influence on cell functions [14,72,80], more investigations are needed to uncover how nanotopography interacts with other material properties and collectively influences protein adsorption and assembly [71]. Unfortunately, existing techniques still have limitations in probing real-time, ultra-small-scale phenomena such as protein behaviors and hence may not be sufficient to answer questions of this kind. To this end, modeling and computational approaches have been vigorously developed and employed to understand and predict cell–nanotopography interactions, as will be covered in Section 4.

3.3.2. Focal adhesion

Another way to probe the underlying mechanisms of nanotopography-mediated cell responses takes a molecular biology approach and focuses on the so-called focal adhesions, where close contact between the cell and ECM is established. The formation of focal adhesion requires the assembly of various proteins which then physically link the cell cytoskeleton (F-actin) to the ECM, as illustrated in Figure 8. Focal adhesions also serve as signaling hubs where information from outside (like mechanical or chemical stimuli) can be transmitted into the cell and, subsequently, affect its behavior. As such, it is conceivable that focal adhesions may play a key role in the observed nanotopography-mediated cell responses. Many studies have been conducted along this line, and readers are suggested to refer to the thorough review by Biggs et al. for more details [58].

As pointed out earlier, the formation of focal adhesion is a highly complex process. Roughly speaking, specific binding between α- and β-chain transmembrane proteins (i.e., integrins) and corresponding motifs in ECM molecules (e.g., the RGD motif in fibronectin and vitronectin) must take place first. After that, integrins are laterally reinforced by the
assembly of various adhesion proteins like vinculin and talin (Figure 8). With increased integrin recruitment, large and matured focal adhesion plaques will emerge which can then be connected to the cytoskeleton/cell nucleus through F-actin bundles (often referred to as stress fibers) [58]. Many studies have been conducted to examine how material nanotopography influences focal adhesion-related kinases, including focal adhesion kinase (FAK) which regulates integrin-dependent signaling pathways, and extracellular signal-regulated kinases (ERK) 1 and 2 that are activated by FAK and mediate cell differentiation and survival. For example, it has been shown that the enhanced integrin–material interaction and cell spreading, induced by nanotopography, can upregulate the expressions of FAK and ERK1/ERK2 in osteoprogenitor cells [81,82]. Other studies reported that nanotopographical features can influence or even disrupt focal adhesion formation through the perturbation of integrin activation and clustering. For example, by restricting possible cell–substrate adhesion to discrete functionalized dots where only one integrin molecule can attach to, Arnold et al. [83] have, for the first time, revealed that there is a critical spacing (~70nm) between individual dots for stable cell–ECM adhesion. It was found that tight adhesion patches can be formed if dots are spaced more closely together than this distance. On the other hand, no apparent adhesion was observed for spacings larger than this critical value. Evidently, although there are lots of unknowns, existing evidence supports that biomaterial nanotopography has significant impact on the formation and functioning of focal adhesions and hence could possibly regulate cell behavior through integrin-specific signaling pathways.

4. Modeling on biomaterial nanotopography-mediated cell responses

4.1. Surface roughness/pattern-mediated cell adhesion

To perform functions like differentiation and migration, living cells need to establish stable attachment with ECM. Cell–ECM or cell–cell adhesion can be achieved via nonspecific attractions, such as van der Waals interaction, between two opposing surfaces or via the formation of non-covalent bonds between the so-called ligand and receptor molecules. Different theoretical models have been developed to capture the essential features of specific as well as nonspecific adhesions.
4.1.1. Modeling non-specific adhesion

Roughly speaking, there are two major approaches, surface energy based and interaction based, for studying nonspecific adhesions. In the first approach, the effect of possible surface attachment is described by a single parameter $\gamma$ (adhesion energy) representing the energy reduction per unit area when two surfaces are brought together. Under such description, the adhesive contact between an elastic sphere with radius $R$ and a flat surface (Figure 9a) can be analyzed in a simple manner by the JKR model [87] where relationships among the pulling force (i.e., the force trying to separate the sphere from the surface), the radius of the contact area, and the displacement of the point of action of the force can be extracted by examining the work done by the pulling force and the change in the energy stored in the system, in the form of either elastic strain energy or energy of adhesion in the contact zone. In particular, the maximum pulling force $F_{cr}$, beyond which the adhesion becomes unstable and sudden detachment is expected to take place, may be expressed as

$$F_{cr} = 3\pi\gamma R. \quad (1)$$

Actually, Equation (1) is valid for relatively large and softer particles; however, for small and stiff spheres, often referred to as the DMT limit, this relation should be modified to [88]

$$F_{cr} = 4\pi\gamma R. \quad (2)$$

Recent observations have suggested that, indeed, JKR theory can be used to describe real cell–ECM adhesion [89]. Interestingly, it has also been found that the critical force needed to detach a thin-walled vesicle adhering to a substrate assumes the same form as the prediction of the JKR or DMT model, albeit with a different numerical coefficient [90].

The second approach for describing nonspecific adhesion starts with the assumption that the attraction between two surfaces originates purely from van der Waals interaction. Under such circumstance, the critical force for detaching a sphere adhering to a flat substrate can be expressed as [91]
where $A$ is the so-called Hamaker constant \[92\] and $H$ is the separation distance between the particle and the surface, often taken to be $\sim 0.3 - 0.4\text{nm}$ for ideal surfaces in adhesion. It must be pointed out that unlike in the JKR model, for example, no elasticity has been considered here. On the other hand, such approach provides an actual description of adhesion mechanism, recalling that the surface energy $\gamma$ is a phenomenological description of the adhesion process.

4.1.2. Non-specific adhesion on wavy/rough surfaces

Once the surface becomes non-flat, the behavior of adhesion will, evidently, deviate from those predicted by simple theories mentioned above. Johnson \[93\] appears to be the first to examine the mechanics of adhesive contact involving wavy surfaces. In particular, Johnson found that the sinusoidal wavy surface of an elastic half space (in contact with a rigid flat substrate) can be fully flattened if the surface energy is large, whereas weak adhesion only leads to partial attachment between two surfaces. Based on a similar approach, the enforced separation between a sphere and an elastic half space with a single wavelength cosine surface (Figure 9b) was considered by Guduru \[84\]. Interestingly, it was found that such detachment process is not smooth but decorated with the separation of interface in alternating stable and unstable segments, resulting in an increase in the work of separation compared to a flat surface. Furthermore, according to this work, surface waviness will, counterintuitively, lead to an elevated maximum pulling force.

It must be pointed out that studies mentioned above all assume that surface asperities have the same height, a simplification that might be too ideal. It is conceivable that in real rough surface contact, the adhesion behavior may actually be dominated by several big asperities. Following the interaction-based approach, a more realistic configuration was adopted by Rabinovich et al. \[85,86\] where the spherical particle is assumed to be in adhesion with a rough surface described as the superposition of two profiles both consisting of close-packed hemispherical asperity caps and troughs but with different peak-to-peak distances ($\lambda_1$ and $\lambda_2$, respectively), as illustrated in Figure 9c. It was then suggested that, for surfaces with nanoscale roughness, the critical force for detachment can be expressed as:

$$F_{cr} = \frac{AR}{6H^2} \left[ \frac{1}{1 + 58.14R \cdot \text{rms}/\lambda_1^2} + \frac{1}{(1 + 1.82R \cdot \text{rms}/H)^2} \right]$$ (4)

where rms stands for the root mean square roughness of the surface. Note that Equation (4) can be regarded as a modification to the relation shown in Equation (3), with the first term in the bracket accounting for the contact interaction of the sphere with an asperity and the second term representing the non-contact attraction of the particle with an effective surface plane.

Another consequence of nanoscale contact (i.e., the size of the asperity or the particle itself is of the order of nanometers) is that both the elastic and adhesion energies involved will become comparable to the thermal energy $k_B T$. As such, the attachment/detachment in this case should be interpreted in probabilistic rather than deterministic terms as predicted by Equations (1–4). For example, by treating the separation process as a
thermally activated escape from an energy well \cite{95}, the average time for the forced detachment, between a nanosized spherical particle and a soft substrate in adhesive contact (Figure 10a), to take place is shown in Figure 10b. Evidently, in direct contrast to that from deterministic considerations, detachment can occur even if the pulling force is well below the critical pull-off level. Furthermore, when the radius of the particle is down to \( \sim 5 \) nm, the particle will spontaneously separate from the substrate within seconds even without any disruptive force (Figure 10b), a finding likely to have broader implications in understanding how cells interact with nanoparticles and/or surfaces with nanoscale roughness.

\subsection*{4.1.3. Modeling specific adhesion}

For adhesion mediated by specific interface interactions, the formation and breakage of ligand–receptor bonds takes on central importance in the detachment process. One important feature of receptor–ligand pairs is that their association/dissociation is generally stochastic in nature, that is, every bond will separate eventually if one waits long enough. On the other hand, any broken bond can reform if proximity is maintained. In a pioneering work \cite{96}, Bell proposed that the dissociation rate \( k_{\text{off}} \) of a single ligand–receptor bond should depend on the force \( f \) acting on the bond as

\begin{equation}
\dot{k}_{\text{off}} = k_0 \cdot \exp \left( \frac{fa}{k_B T} \right)
\end{equation}

where \( a (\sim 0.1 \text{nm}) \) represents the separation distance for bond breakage to take place and \( k_0 \) is a constant. Physically, Equation (5) means that the tendency for a bond to break increases exponentially with respect to the magnitude of the stretching force. A direct consequence of adopting such description is that the maximum force a single bond \cite{97} or a cluster of bonds \cite{98} can sustain will depend strongly on the rate at which load is
applied, a feature that has been demonstrated by numerous experiments. In addition, because of the stochasticity of bond dissociation, it was concluded that a cluster must possess certain amount of bonds in order to achieve a lifetime (i.e., the average time one needs to wait before all bonds within the cluster break) that is of biological significance \[99\].

### 4.1.4. Specific adhesion on nanopatterned surfaces

Recent advances in processing and fabrication technologies have enabled researchers to create material surfaces that allow live cells to adhere to specific regions. The size of such functionalized zones can be of the order of micrometers, allowing the formation of bond clusters, or down to \(~\)10nm which is around the diameter of a single adhesion molecule and hence, at most, only one bond can be formed there.

Theoretical attempt has been made to analyze the strength of a bonded cell–substrate interface where adhesion is the result of clusters of discrete bonds distributed along the interface \[100\]. It was found that those bonds closest to the edges of clusters will be subjected to disproportionately large forces in transmitting loads across the interface, in analogy with well-known behavior in elastic crack mechanics. More interestingly, it was demonstrated that there is an optimum cluster size for maximum interface strength. This size arises from the competition between the non-uniform force distribution among bonds (as mentioned above), which prefers smaller clusters, and stochastic nature of bond breakage, which tends to promote larger clusters. In addition to size, the influence of factors like loading direction \[101\] and material anisotropy \[102\] on the response of adhesion clusters has also been carefully examined.

As pointed out earlier, Arnold et al. \[83\] have revealed that the spacing between individual bonds cannot exceed a critical value (\(\sim\)70nm) for stable cell–ECM adhesion. A theoretical explanation for this remarkable finding has been proposed recently \[103,104\] where the basic hypothesis is that entropy alone can disrupt any adhesion if bonds are spaced far apart. Specifically, the interplay between thermal undulations of a soft elastic membrane immersed in a thermal bath and its ‘binding’ with the substrate at discrete points was examined within the framework of classical statistical mechanics. Treating the effect of adhesion as the confinement of membrane within energy well at each binding site, it has been demonstrated that the standard deviation in membrane fluctuations will be less than the width of the potential well on those points if their spacing is relatively short. So, statistically speaking, cell–substrate adhesion is achieved. On the other hand, if the spacing is relatively large, the standard deviation in membrane fluctuations on bonding points can far exceed the width of the potential well and, hence, in this case, the membrane is not actually adhered to the substrate. Interestingly, estimate of the critical value of spacing obtained from this idealized model is in reasonable agreement with actual experimental observations mentioned above.

### 4.2. Surface topography-mediated filopodia growth

Serving as antennae for the cell to probe its microenvironment, filopodia have been found to play important roles in processes like cell spreading, wound healing, and angiogenesis, as mentioned above. For this reason, intense theoretical and simulation efforts have been devoted to dissect how such finger-like structure is formed (driven by the polymerization of tightly bundled actin filaments inside), grows on and interacts with the ECM.
4.2.1. Force generation by actin polymerization

Evidently, the first step for modeling filopodia growth is to understand how propelling forces are generated by polymerizing actin filaments, pushing against cell membrane (or more generally a load surface), and eventually leading to the growth of these spiky protrusions. Actually, this is also the key issue in analyzing many other actin-driven phenomena such as the movement of some bacteria in host cells or the formation of lamellipodia at the leading edge of migrating cells. The famous Brownian ratchet model was first proposed by Peskin et al. around two decades ago [105] to answer this question where the central idea is that the Brownian motion of the load itself is large enough to temporarily create a gap, bigger than the size of an actin monomer, between the load surface and the actin filament. So if polymerization is fast enough, then a monomer can be added to the filament tip which, effectively, keeps pushing the load forward. It was later suggested by Mogilner and Oster [106] that thermal excitations-induced bending of actin filaments could be more important (compared to the aforementioned random movement of the load) in creating the tip-surface separations allowing continuous polymerization to take place. Recently, a generalized formulation of this process was also developed where fluctuation of filament tips is treated as the Brownian motion of particles in a potential field whose profile represents the elastic deformation of filament itself, as well as possible interactions between the tip and load surface [107].

An important conclusion from these theoretical investigations is that, macroscopically, the growth speed $V$ of a polymerizing filament will decrease exponentially with respect to the resisting force $f$ acting on its tip, that is

$$V \approx V_0 \cdot \exp \left[ \frac{-f\delta}{k_BT} \right]$$

where $\delta$ represents the projected size of a single actin molecule and $V_0 = k_{pol} \cdot c \cdot \delta$ is the free growth speed a polymerizing filament can achieve, with $k_{pol}$ and $c$ being the assembly rate and concentration of actin monomers at the tip respectively. Hence, a large resisting force will effectively shut down the elongation of filaments as expected. Following this approach, the complex trajectories of Listeria, a pathogen that can hijack the actin polymerization machinery of host cells to propel itself, have been explained by realizing that the polymerization as well as the orientation of filaments may not be spatially uniform on the bacterial surface [108,109].

4.2.2. Modeling filopodia growth on a flat surface

Based on the force–velocity relationship, that is Equation (6), obtained for a single filament, Mogilner and Rubinstein [110] examined the mechanics involved in the formation of filopodia on a flat substrate by considering the diffusion of G-actin, as well as the polymerizing dynamics, inside these finger-like protrusions. In their elegant model, a group of actin filaments was assumed to be bundled together in the filopodium to share the resisting force from the membrane (Figure 11a). Furthermore, to sustain the continuous polymerization, it was proposed that actin monomers must be transported by diffusion from the cell body to the protrusion tip, serving as a limiting factor for the growth speed of filopodia. So, effectively, it is a diffusion-reaction type of model with mechanical information incorporated in the polymerization kinetics of F-actin. Interestingly, it was estimated that a minimum of ~10 filaments are needed to overcome
the resistance of membrane. In addition, the maximum length a filopodium can achieve was found to be limited by buckling if the number of filaments inside is relatively small and by G-actin diffusion for strong actin bundles.

It must be pointed out that the role of membrane in [110] was simplified as merely providing a constant resisting force which is then equally distributed to all filaments in the bundle. However, a later study by Atilgan et al. [111] suggested that the growth of filopodia can be greatly enhanced by thermal undulations of the membrane. In particular, by taking into account the elastic energy associated with membrane stretching and bending, it was found that an F-actin bundle encased in a flexible membrane protrudes much faster than that pushing against a rigid surface. The distortions of membrane also provide a configurational force on individual filopodia trying to merge them into larger ones. Interestingly, a later study showed that membrane elasticity can even guide branched F-actin networks into bundled filament protrusions [112]. Recently, Papoian’s research group has conducted extensive theoretical and computational investigations in this area. For example, their stochastic simulations showed that the distribution of filament lengths in the filopodium should be pretty narrow [113], a phenomenon can be understood by realizing that tips of longer filaments will be subjected to higher membrane loads, as well as surrounded by less G-actins, and hence will polymerize slower. In addition, the rate of the retrograde flow, which is the molecular motor-driven movement of actin filaments toward the cell center, has been found to severely diminish filopodial extension.

4.2.3. Filopodial growth on a rough surface

Experimental evidence has shown that the growth speed of filopodia can be significantly reduced by surface roughness [53]. However, up to date, very few theoretical attempts have been made to examine this issue. Recently, based on the classical work by Mogilner and Rubinstein [110], a simple model [67] has been proposed where surface topography is assumed to influence a filopodium in two ways: (1) the surface can exert attractions, via specific or non-specific manners as discussed earlier, on the filopodium and cause its deformation when two surfaces are in close proximity; and (2) the tip of the filopodium can be blocked by a surface asperity resulting in a delay in its growth (Figure 11b). Specifically, treating the actin bundle as an elastic beam, the influence of surface
roughness on the growing speed of filopodium was captured by expressing the resisting force $f$, appeared in Equation (6), as

$$f = f_m + f_h$$

where $f_m$ stands for the membrane resistance, that is the constant force a filopodium must overcome when growing on a flat substrate, and $f_h$ represents the horizon component of the contact force between the tip and asperity, with magnitude depending on factors like the local geometry of the surface (that is a steeper asperity results in a larger $f_h$ and eventually blocks the filopodial growth more severely), the strength of filopodium–substrate interactions (i.e., a stronger cell–substrate adhesion will bend the filopodium further toward the rough surface and hence impede its elongation more), the nature of the contact (frictional or frictionless), etc.

Simulations conducted on realistic rough surface profiles suggested that (1) in general, roughness tends to slow down the growth of filopodia and (2) the process will be dominated by a few big surface asperities that the protrusion must ‘climb’ over while contributions from smaller ones are negligible, leading to sudden jumps in the tip position vs. time curve [61]. Predictions from this model are consistent with experimental observations conducted on diamond surfaces with nano- to submicron-scale roughness.

It is necessary to point out that several important factors, such as the roles of different regulatory proteins and substrate rigidity, have not been taken into account in the aforementioned study. For example, Zhuravlev and Papoian have shown that the appearance of capping proteins, which stop the polymerization of a filament when attaching to its tip, completely changes the growing dynamics of filopodia [114]. Specifically, it was found the molecular noise caused by capping protein binding and unbinding results in big filopodial length fluctuations, compared with minuscule variations in the actin-only system. Most recently, it was also found that molecular motors, capable of transporting G-actin by walking along the actin bundle, will be jammed close to the filopodial base which then induces a local pumping of actin monomers, due to the release of G-actin by the motor when detaching from the filament, and eventually leads to a non-monotonic distribution of G-actin in the filopodium [115]. In addition, study has also shown that the retrograde flow of actin in filopodium is tightly regulated by the substrate compliance [65]. In particular, it was found that stiff substrate leads to fast retrograde flow in the filopodia coupled with low traction forces generated at the cell–substrate interface. In comparison, slower (and often oscillatory) retrograde flows and relatively high tractions will be generated on soft substrates. Unfortunately, how to combine these features with surface topography in describing filopodial growth remains unclear at this moment.

5. Summary and future directions

Both experimental and modeling studies have clearly demonstrated the strong effects of biomaterial nanotopography on mediating cell responses, including adhesion, spreading, proliferation, differentiation, migration, and filopodial growth. Such influence is ubiquitous in many types of tissue cells as well as in different types of materials. However, there is not a general trend on how nanotopography could modulate cell behavior because the nanotopographical effects are material and cell type specific. Furthermore, the mechanisms behind the regulation of material nanotopography on cell activities remain largely unclear which has called for the development of new techniques and interdisciplinary approaches. To this end, computational and modeling approaches may open new avenues...
for addressing these critical challenges, given their capabilities in ‘visualizing’ subcellular and molecular events at the cell–material interface. Next, we discuss possible future research directions in this area.

For experimental study, newly developed nanofabrication, bio-detection, and imaging techniques can further advance our understanding on the biomaterial nanotopography-mediated cell responses. This includes better controls of material nanotopography and more accurate real-time observation techniques allowing us to closely monitor various events at the cell–material interface. In addition, direct and in situ observation of adhesion molecules, likely playing key roles in cell–nanotopography interactions, are becoming possible, which could definitely provide new insights into this important phenomenon.

Once a more clear relationship between surface adherent proteins/molecules and nanotopography is established, presumably based on the observation techniques mentioned above, mechanistic studies are expected to reach a new level that dissecting important signaling transduction and pathways involved in nanotopography-mediated cell responses becomes possible. Investigations of this kind will likely, and inevitably, also involve the use of molecular biology methods like genetic modification and knockout.

On the modeling side, although different theories have been developed to explain the influence of surface roughness on the adhesion between two solids, most of them (if not all) focus on non-specific interactions. As pointed out earlier, a unique feature involved in cell adhesion is the appearance of specific protein–protein bonds. Moreover, these transmembrane adhesion molecules tend to aggregate into small clusters, often referred to as focal adhesions, where strong cell–ECM attachments are achieved. However, the question of how surface topography modulates the formation and evolution of these dynamic adhesion patches remains unanswered.

Regarding filopodia growth, the only existing model incorporating surface topography appears to be the one proposed in [67] where the problem was considered in a 2D setting. In reality, filopodia can also bend sideways to pass asperities blocking their growth as indicated from experimental observations. As such, how to take into account the 3D nature of the problem is certainly something warrants further investigations. In addition, whether and how (if indeed) surface roughness interacts with other factors like the retrograde flow or the molecular motor-driven active transport of actin during this process are all important issues need to be elucidated.

Last but not least, it is worthwhile to note that most available theoretical investigations focus on explaining/predicting the short-term cellular response based on physical arguments. However, very little progress has been made in modeling the influence of surface topography/patterning on the long-term functioning and fate of cells presumably due to: (1) the biological reaction of cells usually involve complicated (and sometimes alternative) signaling and activation pathways, some of which may not have been identified or fully understood yet; and (2) even all the molecular processes and key players involved are known, how to quantitatively describe their progressions as well as interactions across different time and length scales, even phenomenologically, is not easy. Clearly, bridging this gap will be a major challenge in the future.

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