Cell Function

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Engineering Substrate Topography at the Micro- and Nanoscale to Control Cell Function

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The interaction of mammalian cells with nanoscale topography has proven to be an important signaling modality in controlling cell function. Naturally occurring nanotopographic structures within the extracellular matrix present surrounding cells with mechano-transductive cues that influence local migration, cell polarization, and other functions. Synthetically nanofabricated topography can also influence cell morphology, alignment, adhesion, migration, proliferation, and cytoskeleton organization. We review the use of in vitro synthetic cell–nanotopography interactions to control cell behavior and influence complex cellular processes, including stem-cell differentiation and tissue organization. Future challenges and opportunities in cell–nanotopography engineering are also discussed, including the elucidation of mechanisms and applications in tissue engineering.

1. Introduction to Cell–Nanotopography Interactions

1.1. Native Cell–Nanotopography Interactions

Extracellular matrix (ECM) proteins exhibit abundant nanometer-scale structures that are hypothesized to contribute to cell–matrix signaling. The basement membranes of many tissues exhibit rich nanotopographies that interact directly with adjacent cells.[1,2] Nanotopography is also present in individual ECM molecules, such as collagen molecules, which are approximately 300 nm long and 1.5 nm wide.[3] These molecules can form fibrils that extend for tens of micrometers in length and have diameters between 260 and 410 nm.[4] Cells interact with native topographical structures in many ways, often through a phenomenon known as contact guidance. Contact guidance is a leading example of a naturally occurring phenomenon that is characterized by the response of cells to structures on the micrometer and sub-micrometer scale. Contact guidance is an essential component in regulating cell migration, which is modulated by organized ECM proteins.[5] Migration can also be influenced by surrounding cells, as in the case of fibroblast migration in vivo[6] and coordinated epithelial cell migration on a collagen substrate in vitro.[7] T-cell migration is also known to be highly dependent upon cell–biomaterial interactions with native ECM proteins.[8] Contact guidance can also play an important role in the migration of individual cells or groups of cells or tissue.[9] The migration of nematocytes in hydra is guided bidirectionally by a fibrous mat produced from epithelial muscle processes,[10] despite the absence of any detectable chemotactic gradient. Contact guidance is also an important component in efficient organelle formation, such as axonal guidance and growth cone motility.[11]

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1.2. Cell–Nanotopography Responses on Synthetic Substrates

Recent developments in advanced micro- and nanofabrication techniques have enabled the fabrication of substrates that are able to recapitulate the structure and length scale of native topography in two-dimensional substrates. Cells respond to two-dimensional synthetic topographic substrates in a wide array of responses, which depend upon many factors, including cell type, feature size and geometry,[12] and the physical properties of the bulk substrate material, including substrate stiffness.[13] Of particular interest for this Minireview is the effect of synthetic substrates with features that exhibit

long-range order and have sizes between approximately 10 nm and 3 μm. Although these length scales include micro- and nanometer-sized structures, they will herein be categorized and referred to as nanotopography for simplicity. This selection criterion excludes a large body of work that has examined the collective effects of other systems that lack long-range order, including nanofibers,[14] electrospun fibrous mats, and substrates with nanoroughness.[15–17] Three basic nanotopographic geometries that will be discussed are nanogratings, nanopost arrays, and nanopit arrays (Figure 1). Nanotopography affects basic cell function in almost all types of mammalian cells (Tables 1 and 2). Cell–nanotopography interactions can induce different effects within a single cell type owing to the effect of nanotopography coupled with the physicochemical properties of the substrate. Cell–nanotopography interactions also vary across cell type, feature size, and feature geometry. Nevertheless, there are some general trends that can be extricated from the rapidly growing body of literature.

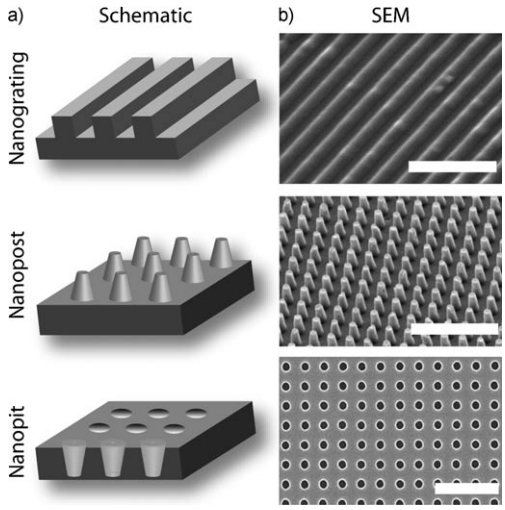

Figure 1. Schematic depictions (a) and SEM images (b) of representative nanotopography geometries. Three basic nanotopography geometries include nanograting (45° tilt, scale bar 5 μm), nanopost array (15° tilt, scale bar 5 μm), and nanopit array (0° tilt, scale bar 1 μm). Schematics not drawn to scale.

1.2.1. Morphology

Perhaps the most palpable effect of nanotopography on cell function is the impact upon cell geometry. Many cell types typically respond to nanogratings by simultaneously aligning and elongating in the direction of the grating axis (Table 1). This response has been observed across numerous species and in various cell types, including fibroblasts, endothelial cells, stem cells, smooth muscle cells, epithelial cells (Figure 2), and Schwann cells.[44] Neurites extending from neuroblastoma cells (PC12) cultured on nanogratings have also been shown to exhibit enhanced alignment and extension when cultured in the presence of nerve growth factor (NGF). The morphological response is seen in cells cultured on substrates with feature widths as small as 100 nm and depths as small as 75 nm.[45] Stronger responses have been observed across decreased feature pitch and increased depth, the latter generally producing the stronger effect. Other studies have demonstrated that some nanograting feature sizes induced alignment of cells both parallel and orthogonal to the nanograting axis.[46] There are several examples of cell types that do not respond to nanogratings, including human-derived leukocytes, keratinocytes, and monocytes.[47] Hence, even widespread morphological effects of cell–nanotopography interactions are not universally observed across all cell types. Substrates with nanopost and nanopit features elicit a more subtle effect on cellular morphology. Many studies have demonstrated the reduction of spreading on nanoposts and nanopits, although the overall effect of these structures on cell area is unclear (Table 2). Other studies have observed either increased[39] or constant filopodia formation[37] and reduction in adhesion complex formation.[33]

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Table 1: Generalized cell responses to nanograting topography.[a]

Cell type[b]	Feature size	Substrate material[c]	Elongation, alignment	Cell area	Attachment, adhesion	Proliferation	Biased migration	Other	Ref.
h endothelial cells	600 nm	PDMS	++	−−		−−	++	organized into cellular superstructures	[18]
b endothelial cells	2 μm	PGS	++						[19]
r endothelial cells	750 nm–10 μm	Ti			+				[20]
h embryonic stem cells	600 nm	PDMS	++		−−	−−		cytoskeleton disrupting agents impact response	[21]
h mesenchymal stem cells	350 nm–10 μm	PDMS	++	−−		−−		differentiation into neuronal lineage	[22]
rC6 glioma	266 nm	PS	++				++		[23]
hEKCs (HEK-293)	200–430	PS	++			++			[24]
b smooth muscle cells	350 nm	PDMS, PMMA	++			−−		polarized MTOC[d]	[25]
h fibroblasts	50–600 nm	Si	++	−		−			[26]
fibroblasts	3–5 μm	Ti	++			0		increase in fibronectin mRNA, incorporation	[27]
h corneal ECs	70–2100 nm	Si	++	−−	++			biased lamellipodia extension	[28, 29]
h corneal ECs	2–20 μm	PS	++	−−			++		[30]
h corneal ECs	1–4 μm	quartz					++	study of coupled topography, electric field, and soluble factors	[31]
PC12	70–1900 nm	Si	++					cooperative neurite extension	[32]

[a] No entry: data not available; ++: increase under all conditions; +: increase under most conditions; 0: no detectable change; −: decrease under most conditions; −−: decrease under all conditions. [b] h, b, and r indicate human, bovine, and rat cells, respectively. [c] PDMS = poly(dimethylsiloxane), PS = polystyrene, PGS = poly(glycerol sebacate), PMMA = poly(methyl methacrylate). [d] MTOC = microtubule organization center.

Table 2: Generalized cell responses to nanopost and nanopit topography.[a]

Cell type	Feature size	Substrate material[b]	Spreading	Attachment, adhesion	Proliferation	Other	Ref.
h osteoblasts	300 nm (pit)	PC		−−		reduced area of adhesion complexes	[33]
r cardiomyocytes	150 nm	PEG		++			[34]
mP19EC stem cells	300–500 nm	PEG		++			[35]
h mesenchymal stem cells	300 nm (pit)	PMMA				osteogenic differentiation	[36]
h bone marrow cells	300 nm (pit)	PC	−−			constant filopodia formation	[37]
h fibroblasts	400–700 nm (post)	PLA		++	−−		
h fibroblasts	50–600 (post)	Si	−		0		[26]
h fibroblasts	80 nm (pit)	Si	−−			gene array analysis	[38]
h fibroblasts	35–120 nm (pit)	PCL	−			increased filopodia	[39]
h fibroblasts	35–120 nm (pit)	PCL, PMMA		−−		biased orientation	[40]
m fibroblasts	750–1500 nm (post)	PDMS with fibronectin	0			constant traction forces across geometry	[41]
r fibroblasts	60–150 nm (pit, post)	PCL		−		increased adhesion on random nanoposts	[42]
HeLA	160–1000 nm (post)	PS	−−		0		[43]

[a] No entry: data not available; ++: increase under all conditions; +: increase under most conditions; 0: no detectable change or trend in data set; −: decrease under most conditions; −−: decrease under all conditions. [b] PC = polycarbonate, PEG = poly(ethylene glycol), PLA = poly(L-lactic acid), PCL = poly(ε-caprolactone).

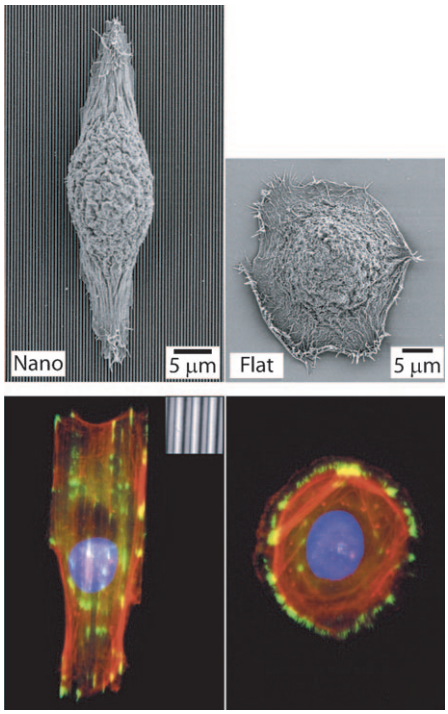

Figure 2. Cell nanograting response in epithelial cells. Epithelial cells respond to nanograting through alignment and elongation along the grating axis, as evident from fluorescent (bottom) and SEM (top) micrographs. Other cell types exhibit similar morphological responses when cultured on nanograting substrates (Table 1). Reproduced with permission of the Company of Biologists.

1.2.2. Attachment and Adhesion

The length scale of synthetic nanotopography can be designed to mimic that of extracellular matrix proteins,[2] including collagen,[48] which may substantiate the hypothesis that nanotopography can enhance attachment and adhesion of mammalian cells. Nanogratings generally appear to enhance the adhesion in various cell–biomaterial geometry combinations, while nanoposts and nanopits generally reduce initial cell attachment (Tables 1 and 2). Further studies must be aimed at elucidating the apparent dependence on feature size and geometry for differential adhesion.

1.2.3. Proliferation

Nanotopography has also been shown to affect the proliferation profiles of various cell types. In general, cells cultured on nanogratings exhibit lower proliferation rates than cells cultured on planar substrates (Table 1). The effect of nanopost or nanopit substrates on proliferation is more ambiguous, as some combinations of geometry, length scale, substrate material, and cell types promote more rapid proliferation while others reduce proliferation rate (Table 2). There appear to be no obvious trends to predict the effect of nanopost or nanopit geometries on proliferation. Furthermore, there are currently no widely accepted hypotheses regarding the mechanism for the effect of cell–nanotopography interactions on cell proliferation.

1.2.4. Migration

The effect of nanotopography on migration is typically observed in cells cultured on nanogratings. Many cell types have exhibited biased migration in the direction of the grating axis and increased overall migration velocities. Cells exhibiting such behavior include endothelial cells,[18] epithelial cells,[30,31,49] osteoblasts,[50] and C6 glioma cells[23] (Table 1). Nanotopography also biases markers for directional migration, as shown in work by Yim et al. in which microtubule organization centers (MTOCs) were observed to be polarized as a direct consequence of the nanograting.[25] Furthermore, the polarization of MTOCs was observed to supersede directional migration cues from wound healing.[25] Enhanced migration is a response that is typically coupled with elongated morphology and alignment of the cell body with the nanograting axis.[18,23,50] There have been limited studies regarding cell migration on nanopit or nanopost arrays compared to nanogratings. One study by Tzvetkova-Chevolleau et al. studied the migration of normal (3T3) and malignant (SaI/N) mouse fibroblasts on poly(dimethylsiloxane) (PDMS) substrates with nanograting and nanopost arrays.[51] Nanogratings biased the migration vector of both 3T3 and SaI/N cells along the grating axis. Rectangular arrays of nanoposts appeared to bias the direction of migration of 3T3 cells but not of SaI/N cells. Although there was no discernable effect of nanotopography on the migration velocities of 3T3 cells, there was a significant impact of nanoposts on SaI/N cells. SaI/N cells cultured on nanoposts exhibited a wide range of migration velocities, including a high percentage of cells that attained high speeds. Additional studies must be conducted to further examine the potential impact of nanopost and nanopit geometries on migration profiles.

2. Cell–Nanotopography Interactions for Controlling Complex Function

2.1. Genotypic Alteration

Nanotopography is known to alter the gene expression profiles of various cell types. These genetic profiles have been analyzed first through analysis of individual genes and subsequently by comprehensive gene analysis studies. A study by Chou et al. suggested that the mRNA levels and stability in human fibroblasts were influenced by nanogratings.[27] More specifically, fibroblasts cultured on titanium nanogratings expressed higher levels of fibronectin mRNA with increased stability. Furthermore, nanogratings induced higher levels of fibronectin incorporation into cell-matrix proteins. More recent work has utilized gene array techniques to probe the effects of nanotopography on genome-wide expression in fibroblasts[52] and mesenchymal stem cells.[22,36] Fibroblasts cultured on nanopits exhibited widespread downregulation of many genes, including those associated with apoptotic initiation, DNA repair, and transcription regulation.[38] Other genes are upregulated in this instance, including

TGF-βr2 and those involved in regulating G-protein signaling.

2.2. Differentiation

The concomitant impact of topography on both basic cell function and gene expression in many cells types suggests that nanotopography could potentially be utilized as a signaling modality for directing differentiation. There has been significant progress in this direction, despite the fact that coordinated work in this specific application of nanotopography has only recently been explored. Work by Yim et al. suggests that human mesenchymal stem cells (hMSCs) cultured on nanogratings can be preferentially differentiated into neuronal lineages as determined by the presence of synaptophysin, tuj1, and nestin markers as well as by the upregulation of MAP2.[22] The enhanced differentiation of hMSCs has also been explored using nanopit arrays. In work by Dalby et al., osteoprogenitor cells and hMSCs were cultured long-term on PMMA nanopit arrays of varying order.[36] The symmetry and order of the nanopits was found to significantly affect the expression of osteopontin and osteocalcin, two bone-specific ECM proteins, in both cell types (Figure 3). While hMSCs cultured on completely ordered or completely random nanopits did not lead to expression of these two proteins, hMSCs cultured on slightly irregular substrates did exhibit significant amounts of these proteins of interest. Increased bone nodule formation was also evident in hMSCs cultured on these substrates relative to substrates with either completely ordered or completely random features. Human MSCs were also cultured on three specific substrates: 1) nanopits, 2) planar substrates in the presence of dexamethasone (DEX), a soluble factor that can induce bone formation (positive control), and 3) planar substrates without DEX (negative control). Human MSCs cultured on nanopits expressed a similar level of many osteoblast-specific genes when compared to hMSCs cultured on flat substrates in the presence of DEX. Furthermore, some genes were specifically upregulated in hMSCs cultured on nanopits compared to hMSCs cultured with DEX alone. The results from these two studies demonstrate the potential of nanotopography to direct cell fate. Furthermore, the complementary findings of hMSCs cultured on nanogratings and ordered-disordered nanopits suggest the potential for selective, controllable differentiation based solely on the geometry of the nanotopographic substrate.

2.3. Cell Superstructure

Modulating cell–nanotopography interactions also has the potential to influence cell–cell interactions and to generate complex multicellular structures. One such example is the culture of human endothelial progenitor cells (EPCs) on

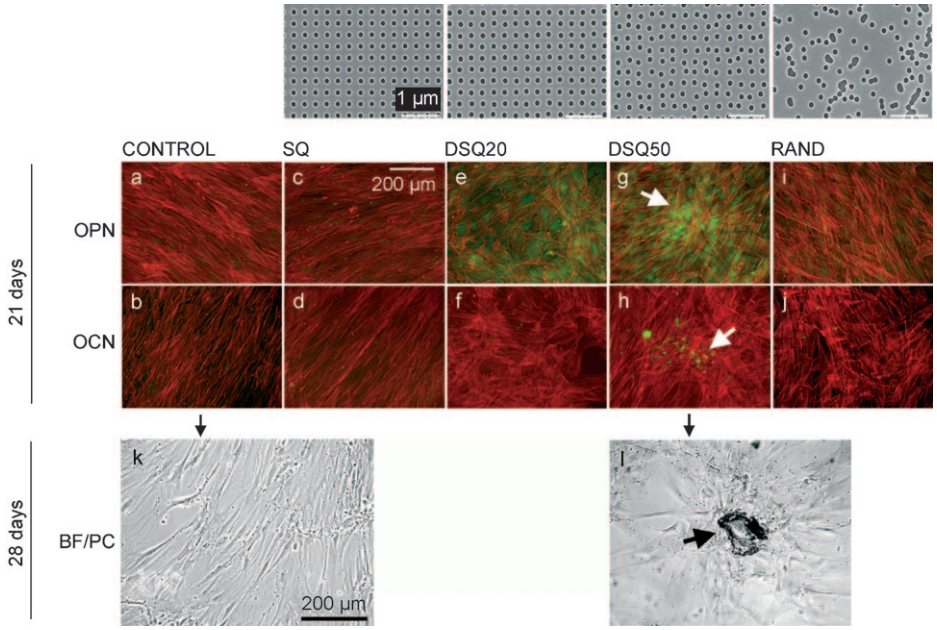

Figure 3. Directed differentiation of human mesenchymal stem cells to osteoblast lineage using nanopit substrates. The top row shows images of nanopit arrays fabricated by electron-beam lithography (scale bars all 1 μm). All have pits of 120 nm diameter (100 nm deep, absolute or average 300 nm center-to-center spacing) with square (SQ), displaced-square 20 (±20 nm from true center, DSQ20), displaced-square 50 (±50 nm from true center, DSQ50), and random placements (RAND). a, b) Human MSCs cultured on a planar control substrate, note the fibroblastic appearance and no osteopontin (OPN)- or osteocalcin (OCN)-positive cells; c, d) on the SQ array, note the fibroblastic appearance and no OPN- or OCN-positive cells; e, f) on the DSQ20 array, note OPN-positive cells; g, h) on the DSQ50 array, note OPN- and OCN-positive cells and nodule formation (arrows); i, j) on the RAND array, note the osteoblast morphology, but no OPN- or OCN-positive cells. Parts (a–j) have the same scale. k, l) Bright-field/phase-contrast (BF/PC) images showing that hMSCs cultured on the control (k) had a fibroblastic morphology after 28 days, whereas hMSCs cultured on DSQ50 arrays (l) exhibited mature bone nodules containing mineral. Parts (k) and (l) have the same scale. Reprinted by permission from Macmillan Publishers Ltd: Nature Materials.[36]

PDMS nanogratings.[18] EPCs responded to alterations in substrate topology with reduced proliferation and enhanced migration in this study. The protein-level expression of endothelial markers in EPCs cultured on both nanogratings and planar substrates was similar. However, EPCs cultured on nanogratings for up to 6 days formed segregated multicellular band structures (Figure 4) that were approximately 100 μm wide and spanned hundreds of micrometers in length. The morphology of EPCs organized into superstructures on nanogratings contrasted significantly with EPCs cultured on planar substrates, which formed confluent monolayers. The band structures found in EPCs cultured on nanogratings formed well-defined and organized capillary tubes in an in vitro matrigel assay. Confluent layers of EPCs formed during culture on planar substrates did not form distinct capillary tubes. This preferential formation found in EPCs cultured on nanotopographically modified substrates is hypothesized to occur for several reasons. First, alignment, elongation, and increased migration velocities influenced morphology and cell–cell interactions. This biased contact produced band structures, which served as capillary-tube precursors. Second, reduced proliferation of EPCs cultured on nanogratings prevented the formation of confluent monolayers. This reduced cell density could serve to enhance cell mobility during the induction of capillary tube formation with matrigel. Regardless of the possible mechanism, this study demonstrates the expanded potential for nanotopography to control the formation of multicellular structures.

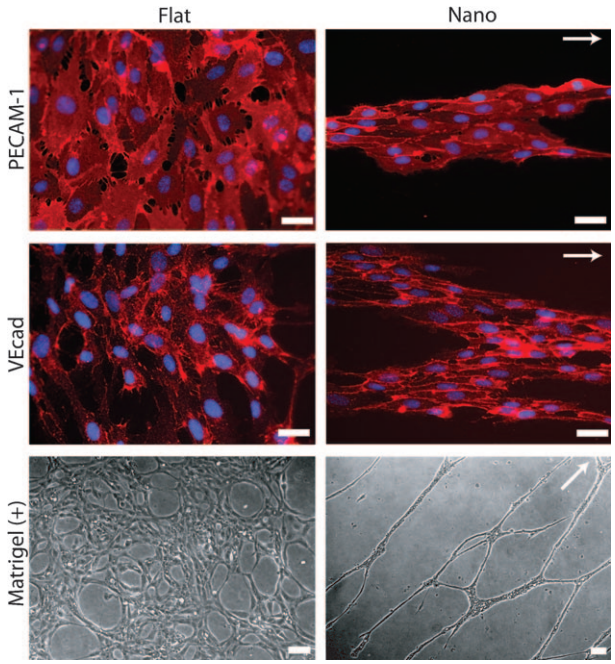

Figure 4. Nanograting substrates promote organized multicellular structures and enhance in vitro capillary tube formation. Protein-level expression of platelet/endothelial cell adhesion molecule-1 (PECAM-1) and vascular endothelial cadherin (VEcad), two endothelial cell markers, was constant in endothelial cells cultured on both planar and nanograting substrates. However, endothelial cells cultured on planar substrates formed confluent monolayers, while endothelial cells cultured on nanogratings were organized into multicellular band structures. These aligned band structures formed aligned capillary tubes in an in vitro matrigel assay (Matrigel (+)). The grating axis is indicated by the white arrows. Scale bars 50 μm. Adapted from reference [18]

3. Engineering Synthetic Nanotopographic Substrates for Tissue Engineering

3.1. Fabrication

There are a variety of advanced nanofabrication methods available for creating nanotopographic substrates with short- and long-range order, which have been discussed in detail elsewhere.[53] The effect of order and symmetry of features has been demonstrated in numerous studies.[36,40,42] These findings suggests that the reaction of cells to substrates with feature roughness of a characteristic length scale should not be assumed to be equivalent to those cells cultured on ordered nanotopography of similar feature size. The nanofabrication of substrates with long-range order across a wide range of feature sizes and geometries has been pursued using a variety of methods, including traditional photolithography, electron-beam photolithography, and interference lithography. Although suitable for creating ordered arrays of features, these processes are expensive and time-consuming, and they require access to intricate equipment. Alternative approaches have been pursued to fabricate polymeric substrates containing structures with long-range order, including the use of diblock copolymers[54] for nanograting lamellae[55] and nanosphere lithography[56] for nanopits.[57] Advanced fabrication techniques and compatible materials must eventually be synergized as a means to integrate cell–nanotopography interactions into advanced tissue-engineering scaffolds. There are several potential fabrication strategies for integrating nanotopographic cues into three-dimensional structures, including advanced two-photon polymerization[58,59] and microscale origami.[60,61] The integration of nanotopographic cues into three-dimensional scaffold design and fabrication remains a challenging pursuit.

3.2. Utilization of Cell–Nanotopography Cues

Cell–nanotopography interactions have the ability to control stem-cell differentiation and cellular superstructure, both of which have obvious implications for use in tissue engineering. However, the use of nanotopography as a cue to modulate basic cell function has potential use in scaffold design as well. For example, the influence of nanogratings on morphology can be used to form aligned populations of cells, which are important for the structure and function of smooth muscle cells and endothelial cells. Vascular tissue-engineering scaffolds are of particular interest because of the correlation between alignment and cell function for multiple cell types in close proximity to one another. Recent work has led to the fabrication of a tubular scaffold with multiple nanograting surfaces.[61] The influence of nanotopography on adhesion could also serve as a method to create patterned arrays of

cells without the need for direct patterning of proteins. Incorporating nanotopographic cues directly into a three-dimensional scaffold may therefore overcome the intrinsic planar limitations of microcontact printing[62–64] and related methods.[65,66] Observed enhanced migration on nanogratings also has potential implications for the design of guidance channels for peripheral nerve regeneration. For example, tubular conduits modified with nanogratings could enhance the migration of Schwann cells into the injury site to promote axonal regeneration.[67] These structures could also potentially promote the rapid migration of neurites across the nerve gap.

3.3. Material Selection

There has been substantial progress in the design and fabrication of nanotopography in a wide spectrum of materials. These techniques are typically designed to be used in combination with materials that are directly adapted from or closely related to the semiconductor industry. Bulk materials processing and nanofabrication strategies for many nanotopographic surfaces are typically fine-tuned for silicon, silicon oxide, polycrystalline silicon, and other inorganic material systems such as titanium. These substrates can be used directly or serve as masters for replica-molding of organic polymers such as PDMS, polystyrene (PS), poly(methyl methacrylate) (PMMA), polycarbonate (PC), and poly(ethylene glycol) (PEG) for in vitro applications or biodegradable polymers such as poly(ε-caprolactone) (PCL), poly(L-lactic acid) (PLA), poly(glycolic acid) (PGA), and poly(L-lactic-*co*-glycolic acid) (PLGA) for potential use in vivo. Although the aforementioned biodegradable candidate materials are ubiquitous in biomedical applications, they have significant drawbacks, including bulk degradation and rigid mechanical properties for PLA, PGA, and PLGA. Noncompliant materials can result in localized inflammation within the dynamic in vivo mechanical environment.[68–70] Novel material selection is of critical importance as cell–nanotopography interactions continue to be utilized in tissue-engineering applications. Synthetic and natural materials must not only be selected on the basis of cell–biomaterial interactions but also on the basis of compatibility with nanofabrication processes. Natural proteins exhibit many advantages, including favorable cell–biomaterial interactions. However, difficulty in processing and the potential for immune response[71,72] may limit widespread adoption. Synthetic biodegradable elastomers[68,69,73,74] offer advantages such as ease of processing, variety of physical and mechanical properties, favorable tissue response and biodegradation kinetics,[75,76] and compatibility with nanofabrication techniques.[19,77,78]

4. Mechanisms for Nanotopographic Sensing and Response

Studies demonstrating significant influence of nanoscale topographic features have yet to elucidate well-defined mechanisms of cell–nanotopography interaction. In particular, several characteristics of this field of study confound the pursuit of precise interaction mechanisms. There are a virtually infinite number of potential combinations of cell types, biomaterial compositions, and topographic feature arrangements. Cell–nanotopography interactions are also transient, which increases the difficulty in extricating a mechanistic view of the contact-guidance response.[52] For example, cells in long-term culture can secrete additional extracellular matrix proteins, which can lead to convoluted topographic signaling.[79] The large potential set of experiments and cell-specific outputs has resulted in a primarily phenomenological approach to studying cell–nanotopography interactions. Despite this large body of work, little is known about the origin or underlying mechanism of the effect of topographical cues on cell function. Various theories have been proposed to explain such phenomena as the alignment and elongation of cells along the grating axis in nanograting substrates. Recent work has begun to interrogate cell–topography interactions with a focus on elucidating the mechanism, including identifying relevant signal transduction pathways and the role of organelles (including the cytoskeleton). Nevertheless, there is a significant opportunity to further explore cell–nanotopography interactions, which could lead to refinement and more comprehensive predictive models of cell–nanotopography interactions.[80]

4.1. Current Theories

We suggest that the morphological response serves as both an indicator of relevant cell–nanotopography interactions and a basis for second-order effects. The elongation and alignment of the nucleus is presumably another source for alteration of the gene profile as cells respond to substrate nanotopography.[52,81] The generalized consensus regarding the mechanism for the morphological response is that it arises from the generation of anisotropic stresses. However, the precise origin and specific role of the anisotropic stresses is still under debate. Theories for the basis of cell–nanotopography interactions will be discussed in the context of nanogratings. Contact-guidance kinetics of fibroblasts on titanium nanogratings suggest that microtubules align within 20 min after attachment and that their alignment preceded alignment of the overall cell.[82] This cluster of events is followed by the alignment of microfilament bundles at 40–60 min and focal adhesion contacts after 3 h. From this study it is clear that there are numerous organelles that are responsible for initializing and transmitting the effect of surface topography throughout the cell to influence overall cell functions, such as stress fiber formation, lamellipodia, and filopodia. One critical organelle that is thought to play an instrumental role in the contact-guidance response is filopodia,[39] which could be modulated through Cdc42 activation.[28] While this model can explain the mechanism of detection and transmission of cell–nanotopography interactions, several theories have been proposed to explain the origin of this response.

4.1.1. Intrinsic Protein Patterning through Substrate Discontinuities

This idea suggests that discontinuities in features lead to preferential protein absorption and subsequent protein patterning. Patterned protein deposition owing to topography could induce preferential alignment, just as micropatterned protein substrates can lead to preferential confinement of cells.[83] Micropatterned proteins of various feature sizes can induce dramatic changes in cell behavior, including morphology, proliferation, differentiation, and apoptosis.[84–86] However, this theory appears less likely in light of more recent studies, including observed contact-guidance phenomena in smooth, continuous features that are much larger than the length scale of proteins.[19,87]

4.1.2. Spatial Biasing of Focal Adhesion Formation

Nanotopography can induce the overall alignment and elongation of cells by first inducing the alignment of focal adhesions. The initial alignment of focal adhesions could result from asymmetric probability of focal adhesion formation owing to feature geometry or geometrically restricted focal adhesion morphology. The alignment of focal adhesions could then lead to an overall response in the cell morphology through the aforementioned intimate signaling connection between focal adhesions and cytoskeleton proteins. Although this theory may explain the connection between aligned focal adhesions and the aligned, elongated gross morphology, it does not sufficiently address the initial alignment of focal adhesions.

4.1.3. Preferential Actin Polymerization

Actin polymerization dynamics involved in cytoskeleton rearrangement are essential for cell attachment[88] and serve as a driving force for directional migration and morphological alterations.[89–91] Filopodia are highly motile organelles involved in many cellular processes, including migration[92] and sensing of local topography.[93] Filopodia formation perpendicular to the ridge–groove features is hypothesized to occur less frequently owing to unfavorable stress formation. Conversely, the formation of filopodia parallel to the ridge–groove features is more frequent, which leads to biased propagation, cytoskeleton rearrangement, polarization of the cell body, and ultimately a gross morphological effect of alignment and elongation. Highly dynamic filopodia serve as topographical sensors, which are able to detect the immediate surrounding environment. This theory is most consistent with the body of work on this topic.[19]

4.2. Potential Role of Small GTPases

The Rho family of GTPases has been shown to control the formation and organization of filaments that compose the actin cytoskeleton.[94] This activation of Rho, Rac, and Cdc42 GTPases controls a wide spectrum of cell functions, including cytoskeleton formation and remodeling, alterations in gene expression, cell-cycle progression, cell morphogenesis, and cell migration in many cell types.[95,96] Hence, we argue that these molecular switches likely play a vital role in the concerted response of cells to substrate nanotopography. Recent studies have only begun to explore the role of these signaling pathways in the context of cell–nanotopography interactions.[21,97] One key function that could directly connect nanotopographic signaling to cell responses is spatially biased focal adhesion formation[98] through Rho activation, which could have dramatic downstream effects on cell migration and signaling.[99] Focal adhesions influence cell morphogenesis[80,100] and have been shown to be sensitive to mechanical forces. [101] Future work must be conducted to further elucidate the dynamics between nanotopographic signaling and modulation of cell function.

4.3. Advanced Biological Techniques

The impact of nanotopography on gene expression is a somewhat intuitive extension of the effect on basic cell function. Recent quantification of the precise impact of nanotopography on genome-wide expression has provided an enormous body of data, but has yet to elucidate any clear mechanisms. Studies that investigate individual signaling pathways that are likely to be implicated in cell–nanotopography interactions may provide greater utility in understanding the origin of these responses. These studies can be conducted by examining cell–topography interactions in the presence of other signaling modalities—a trend that is evident in more recent studies that investigate the coupled effect of soluble factors and substrate nanotopography on cell function.[21,31,97] Future studies should ideally span multiple cell types and substrates to confirm the generality of subsequent findings. Genetic manipulation[102] and gene knockdown using siRNA[103] are other techniques that would serve to identify and investigate the role of specific organelles and signaling pathways implicated in cell–nanotopography interactions.

5. Summary and Outlook

5.1. Cell–Nanotopography Signaling

Cell–nanotopography interactions could serve as an alternative signaling mechanism to precisely control cell function. Cells respond to numerous chemical, physical, mechanical, and electrical stimuli, which can be engineered to control cell function. Substrate engineering encompasses several of these factors in an attempt to utilize cell–biomaterial interactions to control and tune such functions as cell fate,[104] differentiation,[105] and genetic manipulation.[106] Nanotopographic cues are a subset of substrate engineering, which can be used to control many aspects of cell behavior. Nanotopographic cues can be incorporated into large areas with relative ease, thus allowing for large-scale cell culture. Synthetic nanotopography could act synergistically with soluble factors to enhance the efficiency of differentiation protocols. For example, synergistic cues from soluble factors and cell–nanotopography interactions could have the

potential to dramatically increase the specificity and efficiency of lineage-specific stem-cell differentiation.[22] Nanotopography could also be superimposed upon other modes of substrate engineering, because it can be incorporated into many engineering and biomedical materials without significantly impacting the physicochemical properties of the bulk material. Therefore nanotopography could be utilized in addition to other types of cell–biomaterial surface conditions, including microcontact printed chemistries[107] and bulk mechanical properties of the substrate, two additional examples of cues to guide cell function.[13,104,105]

5.2. Nanotopography in Basic Science and Engineering

Engineering substrates to induce desired cell phenotype and genotype has the potential to become an important component of scaffold design for tissue-engineering applications. Substrate nanotopography may also be utilized as a tool to study complex cell functions, such as adhesion, migration, cytoskeleton reorganization, and cell polarization. For example, nanograting substrates can be used to study contact guidance and migration in vitro. Nanopost or nanopit substrates can be used to study the role of filopodia dynamics, focal adhesion formation, and other cytoskeleton functions in a controlled manner. As nanotopography aids in unveiling new discoveries in basic cell function, engineers could used these new discoveries as a basis for the design and fabrication of next-generation synthetic nanotopographic substrates. These advances could then be used to iteratively improve the impact of cell–nanotopography interactions for use in tissue-engineering applications.[108]

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[1] S. L. Goodman, P. A. Sims, R. M. Albrecht, *Biomaterials* **1996**, *17*, 2087.
[2] G. A. Abrams, S. L. Goodman, P. F. Nealey, M. Franco, C. J. Murphy, *Cell Tissue Res.* **2000**, *299*, 39.
[3] E. Pamula, V. De Cupere, Y. F. Dufrene, P. G. Rouxhet, *J. Colloid Interface Sci.* **2004**, *271*, 80.
[4] L. Bozec, G. van der Heijden, M. Horton, *Biophys. J.* **2007**, *92*, 70.
[5] K. Wolf, R. Muller, S. Borgmann, E. B. Brocker, P. Friedl, *Blood* **2003**, *102*, 3262.
[6] J. Sutherland, M. Denyer, S. Britland, *J. Anat.* **2005**, *206*, 581.
[7] H. Haga, C. Irahara, R. Kobayashi, T. Nakagaki, K. Kawabata, *Biophys. J.* **2005**, *88*, 2250.
[8] P. Friedl, E. B. Bröcker, *Dev. Immunol.* **2000**, *7*, 249.
[9] P. Friedl, *Curr. Opin. Cell Biol.* **2004**, *16*, 14.
[10] R. D. Campbell, B. A. Marcum, *J. Cell Sci.* **1980**, *41*, 33.
[11] E. W. Dent, F. B. Gertler, *Neuron* **2003**, *40*, 209.
[12] R. G. Flemming, C. J. Murphy, G. A. Abrams, S. L. Goodman, P. F. Nealey, *Biomaterials* **1999**, *20*, 573.
[13] D. E. Discher, P. Janmey, Y.-l. Wang, *Science* **2005**, *310*, 1139.
[14] Q. P. Pham, U. Sharma, A. G. Mikos, *Tissue Eng.* **2006**, *12*, 1197.
[15] F. Variola, J.-H. Yi, L. Richert, J. D. Wuest, F. Rosei, A. Nanci, *Biomaterials* **2008**, *29*, 1285.
[16] J. Y. Lim, J. C. Hansen, C. A. Siedlecki, R. W. Hengstebeck, J. Cheng, N. Winograd, H. J. Donahue, *Biomacromolecules* **2005**, *6*, 3319.
[17] M. J. Dalby, M. O. Riehle, H. Johnstone, S. Affrossman, A. S. G. Curtis, *Biomaterials* **2002**, *23*, 2945.
[18] C. J. Bettinger, Z. Zhang, S. Gerecht, J. T. Borenstein, R. Langer, *Adv. Mater.* **2008**, *20*, 99.
[19] C. J. Bettinger, B. Orrick, A. Misra, R. Langer, J. T. Borenstein, *Biomaterials* **2006**, *27*, 2558.
[20] J. Lu, M. P. Rao, N. C. MacDonald, D. Khang, T. J. Webster, *Acta Biomater.* **2008**, *4*, 192.
[21] S. Gerecht, C. J. Bettinger, Z. Zhang, J. Borenstein, G. Vunjak-Novakovic, R. Langera, *Biomaterials* **2007**, *28*, 4068.
[22] E. K. F. Yim, S. W. Pang, K. W. Leong, *Exp. Cell Res.* **2007**, *313*, 1820.
[23] X. Wang, C. A. Ohlin, Q. Lu, J. Hu, *Biomaterials* **2008**, *29*, 2049.
[24] E. Rebollar, I. Frischauf, M. Olbrich, T. Peterbauer, S. Hering, J. Preiner, P. Hinterdorfer, C. Romanin, J. Heitz, *Biomaterials* **2008**, *29*, 1796.
[25] E. K. F. Yim, R. M. Reano, S. W. Pang, A. F. Yee, C. S. Chen, K. W. Leong, *Biomaterials* **2005**, *26*, 5405.
[26] C.-H. Choi, S. H. Hagvall, B. M. Wu, J. C. Y. Dunn, R. E. Beygui, C.-J. Kim, *Biomaterials* **2007**, *28*, 1672.
[27] L. Chou, J. D. Firth, V. J. Uitto, D. M. Brunette, *J. Cell Sci.* **1995**, *108*, 1563.
[28] A. I. Teixeira, G. A. Abrams, P. J. Bertics, C. J. Murphy, P. F. Nealey, *J. Cell Sci.* **2003**, *116*, 1881.
[29] N. W. Karuri, S. Liliensiek, A. I. Teixeira, G. Abrams, S. Campbell, P. F. Nealey, C. J. Murphy, *J. Cell Sci.* **2004**, *117*, 3153.
[30] B. A. Dalton, F. Walboomers, M. Dziegielewski, M. D. M. Evans, S. Taylor, J. A. Jansen, J. G. Steele, *J. Biomed. Mater. Res.* **2001**, *56*, 195.
[31] A. M. Rajnicek, L. E. Foubister, C. D. McCaig, *Dev. Biol.* **2007**, *312*, 448.
[32] J. D. Foley, E. W. Grunwald, P. F. Nealey, C. J. Murphy, *Biomaterials* **2005**, *26*, 3639.
[33] M. Biggs, R. Richards, N. Gadegaard, C. Wilkinson, M. Dalby, *J. Mater. Sci. Mater. Med.* **2007**, *18*, 399.
[34] D.-H. Kim, P. Kim, K. Y. Suh, A. Seung Kyu Choi, A. Sang Ho Lee, A. Byungkyu Kim in *IEEE Engineering in Medicine and Biology, 27th Annual Conference*, Shanghai, China, September 1 – 4 **2005**, p. 4091.
[35] P. Kim, D. H. Kim, B. Kim, S. K. Choi, S. H. Lee, A. Khademhosseini, R. Langer, K. Y. Suh, *Nanotechnology* **2005**, *16*, 2420.
[36] M. J. Dalby, N. Gadegaard, R. Tare, A. Andar, M. O. Riehle, P. Herzyk, C. D. W. Wilkinson, R. O. C. Oreffo, *Nat. Mater.* **2007**, *6*, 997.
[37] A. Hart, N. Gadegaard, C. Wilkinson, R. Oreffo, M. Dalby, *J. Mater. Sci. Mater. Med.* **2007**, *18*, 1211.
[38] M. J. Dalby, N. Gadegaard, C. D. W. Wilkinson, *J. Biomed. Mater. Res. Part A* **2008**, *84*, 973.
[39] M. J. Dalby, N. Gadegaard, M. O. Riehle, C. D. W. Wilkinson, A. S. G. Curtis, *Int. J. Biochem. Cell Biol.* **2004**, *36*, 2005.

[40] A. S. G. Curtis, N. Gadegaard, M. J. Dalby, M. O. Riehle, C. D. W. Wilkinson, G. A. Aitchison, *IEEE Trans. Nanobiosci.* **2004**, *3*, 61.
[41] M. T. Yang, N. J. Sniadecki, C. S. Chen, *Adv. Mater.* **2007**, *19*, 3119.
[42] A. S. G. Curtis, B. Casey, J. O. Gallagher, D. Pasqui, M. A. Wood, C. D. W. Wilkinson, *Biophys. Chem.* **2001**, *94*, 275.
[43] S. Nomura, H. Kojima, Y. Ohyabu, K. Kuwabara, A. Miyauchi, T. Uemura, *J. Artif. Organs* **2006**, *9*, 90.
[44] S.-h. Hsu, C.-Y. Chen, P. S. Lu, C.-S. Lai, C.-J. Chen, *Biotechnol. Bioeng.* **2005**, *92*, 579.
[45] W. A. Loesberg, J. te Riet, F. C. M. J. M. van Delft, P. Schön, C. G. Figdor, S. Speller, J. J. W. A. van Loon, X. F. Walboomers, J. A. Jansen, *Biomaterials* **2007**, *28*, 3944.
[46] A. I. Teixeira, G. A. McKie, J. D. Foley, P. J. Bertics, P. F. Nealey, C. J. Murphy, *Biomaterials* **2006**, *27*, 3945.
[47] J. Meyle, K. Gültig, W. Nisch, *J. Biomed. Mater. Res.* **1995**, *29*, 81.
[48] L. Bozec, M. Horton, *Biophys. J.* **2005**, *88*, 4223.
[49] K. A. Diehl, J. D. Foley, P. F. Nealey, C. J. Murphy, *J. Biomed. Mater. Res. Part A* **2005**, *75*, 603.
[50] S. Lenhert, M.-B. Meier, U. Meyer, L. Chi, H. P. Wiesmann, *Biomaterials* **2005**, *26*, 563.
[51] T. Tzvetkova-Chevolleau, A. Stéphanou, D. Fuard, J. Ohayon, P. Schiavone, P. Tracqui, *Biomaterials* **2008**, *29*, 1541.
[52] M. J. Dalby, M. O. Riehle, S. J. Yarwood, C. D. W. Wilkinson, A. S. G. Curtis, *Exp. Cell Res.* **2003**, *284*, 274.
[53] J. J. Norman, T. A. Desai, *Ann. Biomed. Eng.* **2006**, *34*, 89.
[54] J. Y. Cheng, A. M. Mayes, C. A. Ross, *Nat. Mater.* **2004**, *3*, 823.
[55] R. Ruiz, R. L. Sandstrom, C. T. Black, *Adv. Mater.* **2007**, *19*, 587.
[56] C. L. Haynes, R. P. V. Duyne, *J. Phys. Chem. B* **2001**, *105*, 5599.
[57] A. Kosiorek, W. Kandulski, H. Glaczynska, M. Giersig, *Small* **2005**, *1*, 439.
[58] C. N. LaFratta, L. Li, J. T. Fourkas, *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 8589.
[59] A. Pikulin, N. Bityurin, *Phys. Rev. B* **2007**, *75*, 195430.
[60] H. J. In, S. Kumar, Y. Shao-Horn, G. Barbastathis, *Appl. Phys. Lett.* **2006**, *88*, 083104.
[61] K. Seunarine, D. O. Meredith, M. O. Riehle, C. D. W. Wilkinson, N. Gadegaard, *Microelectron. Eng.* **2008**, *85*, 1350.
[62] Y. Xia, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 568; *Angew. Chem. Int. Ed.* **1998**, *37*, 550.
[63] A. Bernard, J. P. Renault, B. Michel, H. R. Bosshard, E. Delamarche, *Adv. Mater.* **2000**, *12*, 1067.
[64] C. C. Lin, C. C. Co, C. C. Ho, *Biomaterials* **2005**, *26*, 3655.
[65] E. Kim, Y. Xia, X. M. Zhao, G. M. Whitesides, *Adv. Mater.* **1997**, *9*, 651.
[66] D. T. Chiu, N. L. Jeon, S. Huang, R. S. Kane, C. J. Wargo, I. S. Choi, D. E. Ingber, G. M. Whitesides, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 2408.
[67] K. Torigoe, H.-F. Tanaka, A. Takahashi, A. Awaya, K. Hashimoto, *Exp. Neurol.* **1996**, *137*, 301.
[68] C. L. E. Nijst, J. P. Bruggeman, J. M. Karp, L. Ferreira, A. Zumbuehl, C. J. Bettinger, R. Langer, *Biomacromolecules* **2007**, *8*, 3067.
[69] C. J. Bettinger, J. P. Bruggeman, J. T. Borenstein, R. S. Langer, *Biomaterials* **2008**, *29*, 2315.
[70] J. P. Bruggeman, C. J. Bettinger, C. L. E. Nijst, D. S. Kohane, R. Langer, *Adv. Mater.* **2008**, *20*, 1922.
[71] L. R. Ellingsworth, F. DeLustro, J. E. Brennan, S. Sawamura, J. McPherson, *J. Immunol.* **1986**, *136*, 877.
[72] M. I. García-Domingo, J. Alijotas-Reig, A. Cisteró-Bahima, F. Tresserra, E. Enrique, *J. Investig Allergol. Clin. Immunol.* **2000**, *10*, 107.
[73] Y. Wang, G. A. Ameer, B. J. Sheppard, R. Langer, *Nat. Biotechnol.* **2002**, *20*, 602.
[74] J. P. Bruggeman, B. J. de Bruin, C. J. Bettinger, R. Langer, *Biomaterials* **2008**, *29*, 4726.
[75] Y. Wang, Y. M. Kim, R. Langer, *J. Biomed. Mater. Res. Part A* **2003**, *66*, 192.
[76] C. J. Bettinger, J. P. Bruggeman, J. T. Borenstein, R. Langer, *J. Biomed. Mater. Res.* **2008**, DOI: 10.1002/jbm.a.32306.
[77] A. Mahdavi, L. Ferreira, C. Sundback, J. W. Nichol, E. P. Chan, D. J. D. Carter, C. J. Bettinger, S. Patanavanich, L. Chignozha, E. Ben-Joseph, A. Galakatos, H. Pryor, I. Pomerantseva, P. T. Masiakos, W. Faquin, A. Zumbuehl, S. Hong, J. Borenstein, J. Vacanti, R. Langer, J. M. Karp, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 2307.
[78] C. J. Bettinger, K. M. Kulig, J. P. Vacanti, R. Langer, J. T. Borenstein, *Tissue Eng.* **2008**, *14*, 1.
[79] D. Hamilton, K. Wong, D. Brunette, *Calcif. Tissue Int.* **2006**, *78*, 314.
[80] R. Kemkemer, S. Jungbauer, D. Kaufmann, H. Gruler, *Biophys. J.* **2006**, *90*, 4701.
[81] M. J. Dalby, M. O. Riehle, D. S. Sutherland, H. Agheli, A. S. G. Curtis, *Eur. J. Cell Biol.* **2004**, *83*, 159.
[82] C. Oakley, D. M. Brunette, *J. Cell Sci.* **1993**, *106*, 343.
[83] J. L. Tan, W. Liu, C. M. Nelson, S. Raghavan, C. S. Chen, *Tissue Eng.* **2004**, *10*, 865.
[84] Y. Ito, *Biomaterials* **1999**, *20*, 2333.
[85] J. Y. Lim, H. J. Donahue, *Tissue Eng.* **2007**, *13*, 1879.
[86] H. K. Song, B. Toste, K. Ahmann, D. Hoffman-Kim, G. T. R. Palmore, *Biomaterials* **2006**, *27*, 473.
[87] A.-S. Andersson, P. Olsson, U. Lidberg, D. Sutherland, *Exp. Cell Res.* **2003**, *288*, 177.
[88] A. Pierres, A.-M. Benoliel, D. Touchard, P. Bongrand, *Biophys. J.* **2008**, *94*, 4114.
[89] B. Wójciak-Stothard, A. S. Curtis, W. Monaghan, M. McGrath, I. Sommer, C. D. Wilkinson, *Cell Motil. Cytoskeleton* **1995**, *31*, 147.
[90] B. Wójciak-Stothard, A. Curtis, W. Monaghan, M. McGrath, I. Sommer, C. Wilkinson, *Cell Biol. Int.* **1995**, *19*, 1861.
[91] X. F. Walboomers, W. Monaghan, A. Curtis, J. A. Jansen, *J. Biomed. Mater. Res.* **1999**, *46*, 212.
[92] M. Nemethova, S. Auinger, J. V. Small, *J. Cell Biol.* **2008**, *180*, 1233.
[93] C. G. Galbraith, K. M. Yamada, J. A. Galbraith, *Science* **2007**, *315*, 992.
[94] A. Hall, *Science* **1998**, *279*, 509.
[95] A. B. Jaffe, A. Hall, *Annu. Rev. Cell Dev. Biol.* **2005**, *21*, 247.
[96] E. Tzima, *Circ. Res.* **2006**, *98*, 176.
[97] M. J. Dalby, A. Hart, S. J. Yarwood, *Biomaterials* **2008**, *29*, 282.
[98] P. Uttayarat, G. K. Toworfe, F. Dietrich, P. I. Lelkes, R. J. Composto, *J. Biomed. Mater. Res. Part A* **2005**, *75*, 668.
[99] R. Wang, R. A. F. Clark, D. F. Mosher, X.-D. Ren, *J. Biol. Chem.* **2005**, *280*, 28803.
[100] S. Jungbauer, H. Gao, J. P. Spatz, R. Kemkemer, *Biophys. J.* **2008**, *95*, 3470.
[101] A. Bershadsky, M. Kozlov, B. Geiger, *Curr. Opin. Cell Biol.* **2006**, *18*, 472.
[102] D. Putnam, *Nat. Mater.* **2006**, *5*, 439.
[103] A. Mullard, *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 513.
[104] C. S. Chen, M. Mrksich, S. Huang, G. M. Whitesides, D. E. Ingber, *Science* **1997**, *276*, 1425.
[105] R. McBeath, D. M. Pirone, C. M. Nelson, K. Bhadriraju, C. S. Chen, *Dev. Cell* **2004**, *6*, 483.
[106] H. J. Kong, J. Liu, K. Riddle, T. Matsumoto, K. Leach, D. J. Mooney, *Nat. Mater.* **2005**, *4*, 460.
[107] J. L. Charest, M. T. Eliason, A. J. Garcia, W. P. King, *Biomaterials* **2006**, *27*, 2487.
[108] A. Khademhosseini, C. Bettinger, J. M. Karp, J. Yeh, Y. Ling, J. Borenstein, J. Fukuda, R. Langer, *J. Biomater. Sci. Polym. Ed.* **2006**, *17*, 1221.