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Nanoparticles Toxicity Examined In Vivo in Animals

► In Vivo Toxicity of Titanium Dioxide and Gold Nanoparticles

Nanopatterned Substrata

► Nanopatterned Surfaces for Exploring and Regulating Cell Behavior

Nanopatterned Surfaces for Exploring and Regulating Cell Behavior

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Abbreviations

1D	One-dimensional
2D	Two-dimensional
3D	Three-dimensional
AFM	Atomic force microscopy
ECM	Extracellular matrix
ES	Embryonic stem cell
MSC	Mesenchymal stem cell
RGD	Argine-glycine-aspartic acid
SAM	Self-assembled monolayer

Synonyms

Nanopatterned substrata

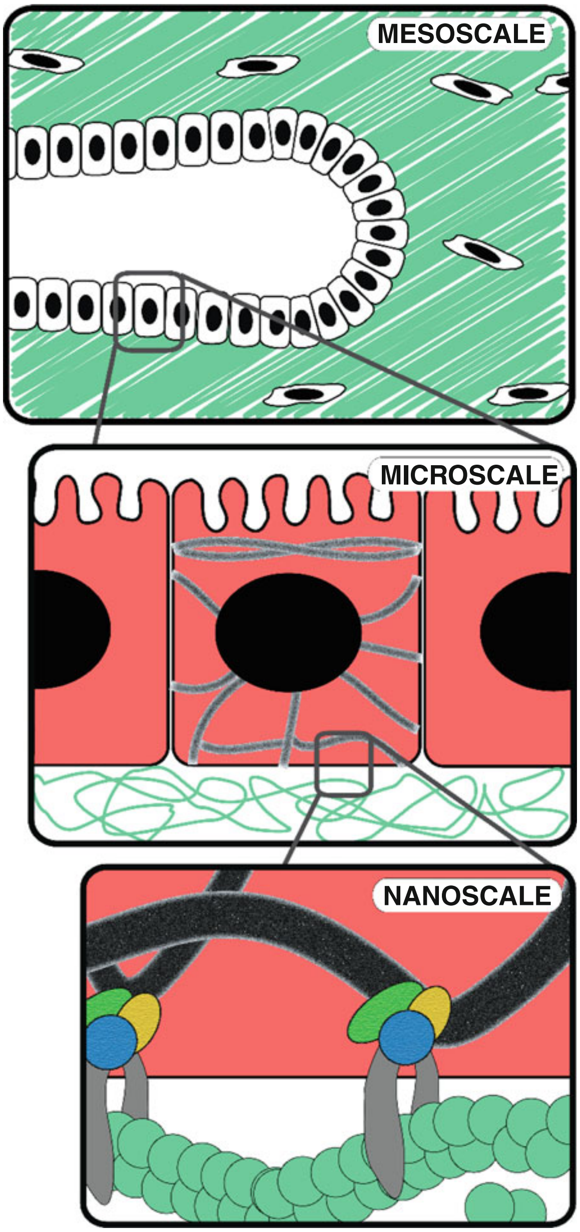
Definition

Surfaces (substrata) that are fabricated to have chemically or topographically nanometer-sized features, which are used to investigate cell morphology, phenotype, and/or behavior.

Overview

Cells Sense and Respond to Their Environment

Cellular environments consist of complex mechanical, chemical, electrical, and topological gradients ranging



Nanopatterned Surfaces for Exploring and Regulating Cell Behavior, Fig. 1 Cell interaction and behavior are governed over multiple length scales. Cell-cell and cell-matrix interactions collectively form tissue architecture and function at the mesoscale. Microscale interactions guide cell shape and connectivity, whereas nanoscale interactions influence cell processes such as adhesion, motility, and gene expression

in size from the nanoscale (1–100 nm) to the macroscale (>1 mm) (Fig. 1). Cell behavior is governed by these multiscale environmental properties, which, when disrupted, lead to aberrant tissue function and disease. At the mesoscale (100–1,000 μm), aggregate cell behavior resulting from cell-cell interactions, coupled with properties of the extracellular matrix (ECM), create the tissue architecture and sculpt the mechanical and chemical microenvironment. Heterogeneity of the tissue architecture at the mesoscale produces unique microenvironments. Cell-cell and integrin-dependent cell-ECM interactions at the microscale (1–100 μm) regulate cell shape and adhesion, and consequently affect cell survival, growth, differentiation, and migration. At the nanoscale, molecular and subcellular processes such as integrin activation, focal adhesion formation, actin polymerization, and cytoskeletal organization enable the cell to be physically coupled to its ECM. Additionally, membrane-bound receptors signal through intracellular pathways to modulate gene expression and protein synthesis in response to chemical signals such as morphogens.

Cells interact with and regulate their surroundings in a relationship referred to as dynamic reciprocity [1]. Through dynamic and reciprocal crosstalk, cell behavior is modulated by cell-cell and cell-ECM interactions, and that behavior sculpts and defines the microenvironment. One of the many ways in which the cell senses and reacts to its environment is through integrin-dependent signaling. Transmembrane integrins attach and mechanically couple to several ECM ligands, including the prototypic sequence arginine-glycine-aspartic acid (RGD). Integrin engagement leads to recruitment and clustering to form focal adhesions. Intracellularly, integrins within the focal adhesion associate with several proteins including vinculin, talin, tensin, α-actinin, paxillin, Src, and focal adhesion kinase. These proteins couple the focal adhesion to the actin cytoskeleton. This physical linkage enables the cell to adhere to, move along, and interact mechanically with the ECM. In addition, the focal adhesion activates several kinase cascades that biochemically transduce information about the microenvironment into the nucleus, which leads to changes in gene expression. These gene expression changes can result in the production and secretion of soluble signaling molecules, enzymes, and ECM components that shape and maintain the extracellular environment. The formation, maturation, and disassembly of focal adhesions are not only key for cell spreading and migration, but also appear to be central modulators of many cellular functions including proliferation and differentiation.

Engineering Model Substrata

The tissue microenvironment is complex and the responses of cells to microenvironmental interactions can occur through individual and synergistic mechanisms. As a result, model substrata are often employed to decouple and isolate the effects of various microenvironmental factors. These model surfaces typically fit into one of two categories: chemically or topologically patterned. Chemically patterned surfaces contain regions of active and inactive (inert) biomolecules and thus present spatial patterns of chemical ligands to the cells of interest. ECM proteins such as fibronectin, vitronectin, laminin, and collagen or their active binding motifs are often used. In contrast, topologically patterned surfaces alter the surface topology that is presented to the cells. In these systems, etched pits, posts, grooves, and gratings are used to characterize cell behavior. Regardless of the type of model substratum used, advances in micro- and nanotechnology have enabled the creation of such tailored surfaces to investigate the interactions between cells and their microenvironment at multiple length scales.

Because of the existence of several simple, well-established techniques, micropatterned surfaces have become a popular tool to interrogate cell behavior. Micrometer-scale topology can be created using standard photolithography coupled with traditional etching processes; however, at the micrometer scale most studies have focused on chemically patterned substrata. For chemically patterned surfaces, photolithographic techniques can be used to spatially deposit or remove different materials and thus define regions for chemical functionalization. Microcontact printing is the most common method of microscale chemical functionalization. This technique employs the use of micropatterned elastomeric stamps that are "painted" with the ECM ligand of interest. The coated stamps are then pressed onto a treated surface, which deposits the cell-adhesive ECM ligands onto the surface in the same spatial pattern as that of the stamp.

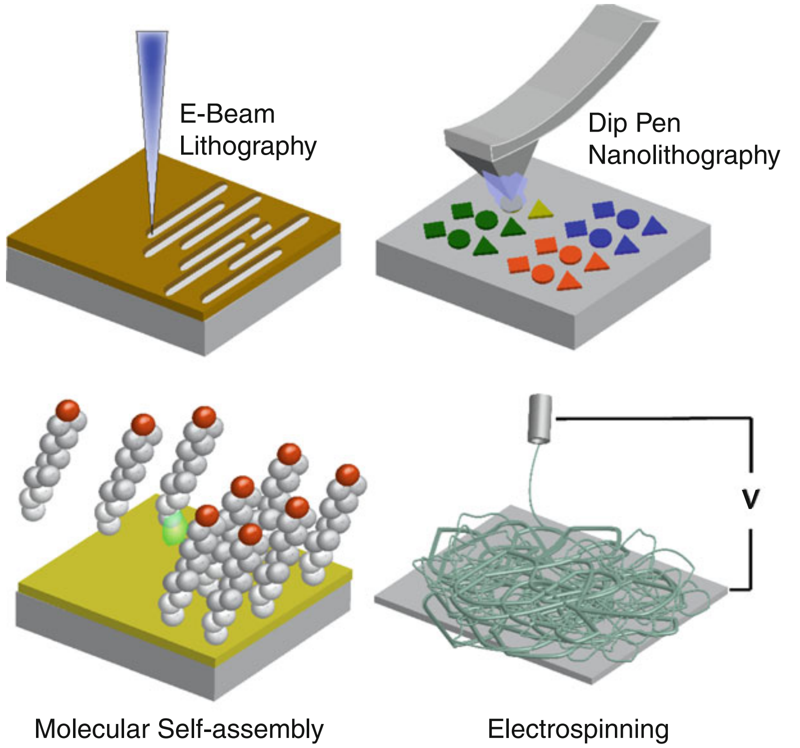
Micrometer-sized features can be used to control cell position and connectivity; and thus micropatterning is often employed to investigate both mesoscale and microscale cell behavior. Micropatterned surfaces have been used to coculture fibroblasts and hepatocytes to maintain normal hepatocyte phenotype [2]. Additional mesoscale behavior has been investigated with epithelial sheets grown on micropatterned islands of varying size and shape. Patterns of cell proliferation emerge that result from cell-cell and cell-ECM interactions within the epithelial sheet [3]. Chemical micropatterning has also been used with single cells to identify the role of cell shape in determining cell fate [4]. Geometric control of cell growth and viability was demonstrated by varying the size of chemically patterned islands of ECM.

Although micropatterning is a powerful tool to investigate cell behavior, particularly at the mesoscale, this approach has its limitations. Micropatterning techniques do not afford the ability to control the surface density of the patterned ligand. Additionally, the minimum feature sizes achieved with these techniques do not permit the exposure of multiple different ligands to a single cell. These drawbacks make it difficult to probe cell-ECM interactions at the scale of integrin engagement or focal adhesion formation. Additionally, cells in vivo are not exposed to flat two-dimensional (2D) surfaces but rather to a complex topological microenvironment. The ECM consists primarily of interwoven protein fibers ranging from 10 to 300 nm in diameter. Basement membranes consist of complex mixtures of nanoscale pits, pores, and fibers ranging in size from 5 to 200 nm with peaks and valleys of approximately 100 nm in height [5]. The investigation of cell behavior in response to these native nanotopologies cannot be explored using micropatterned surfaces.

The advent of new tools and fabrication techniques has enabled the creation of spatial patterns in chemistry and topology that can vary greatly over the length scale of a cell. Nanoscale differences in surface roughness, fiber diameter, and ligand spacing can be produced using micropatterned substrata to more closely recapitulate the native tissue microenvironment. Thus, the goal of nanoscale patterning is to investigate molecular mechanisms and subcellular processes that determine fundamental cell behaviors including adhesion, proliferation, gene expression, and differentiation.

Fabricating Nanopatterned Substrata

Unlike micropatterned surfaces, fabrication of nanopatterned surfaces large enough for multicellular studies is technically challenging as well as time and labor intensive. As a result, studies to date have focused primarily on the behavior of single cells. The types of fabrication methodologies discussed herein are by no means comprehensive, but rather



Nanopatterned Surfaces for Exploring and Regulating Cell Behavior, Fig. 2 Cartoon representations of various fabrication modalities used to create nanopatterned surfaces

a sampling of commonly used techniques to create chemically and topologically defined nanopatterned surfaces. Fabrication technologies adapted from the semiconductor industry as well as novel nanobiotechnology methods are rapidly changing, enabling faster and simpler creation of nanopatterned features. For a more comprehensive review of fabrication techniques, the reader is directed to specialized reviews [6].

Topologically nanopatterned substrata are created using several techniques. These surfaces can be categorized as unordered or ordered topologies. Unordered topologies typically arise spontaneously as a result of processing methods including chemical etching and polymer demixing. Surfaces with unordered topologies have randomly patterned features and lack orientation and geometrical control, but the fabrication processes are relatively simple, fast, and inexpensive. Ordered topologies, on the other hand, are created with precise control of feature pattern, orientation, and geometry. Electron-beam (e-beam) lithography (Fig. 2) is the most commonly used approach to create nanoscale ordered topologies. E-beam lithography uses an electron beam to develop a layer of resist coated on the substratum. Following e-beam lithography, further processing of the substratum using standard etching techniques results in nanoscale topologies including nanoscale grooves, gratings, or pits.

Dip-pen nanolithography (Fig. 2) is a method to create chemically nanopatterned surfaces using an atomic force microscopy (AFM) tip [7]. The AFM tip is "dipped" into a reservoir, coating the tip with the ligand of interest, and is then used to repeatedly deposit small amounts of the ligand onto the substratum to form chemically patterned regions with an approximate spatial resolution of 5 nm. This technology enables the placement of different adhesive ligands in close proximity in defined shapes with nanoscale precision and dimensions. Additionally, this technology can be multiplexed to create a higher throughput chemical patterning system by combining multiple AFM tips in parallel.

In addition to the "top-down" fabrication strategies used to deposit or remove material from a surface as described above, newer "bottom-up" technologies of molecular self- or templated-assembly (Fig. 2) have been used to create larger chemically nanopatterned surfaces. Molecular self-assembly is a broad category of techniques wherein (supra)molecular building blocks or colloids are spontaneously self-organized as a result of their interactions to form nanoscale topology and/or spatial patterns of cell-adhesive ligands [8]. Similarly, templated-assembly creates the same types of surfaces with the use of a reusable template to define an organization for the molecular building blocks. These newer fabrication techniques (e.g., self-assembled monolayers (SAMs) and phase-separated block copolymers) have enabled the reliable creation of large patterned areas.

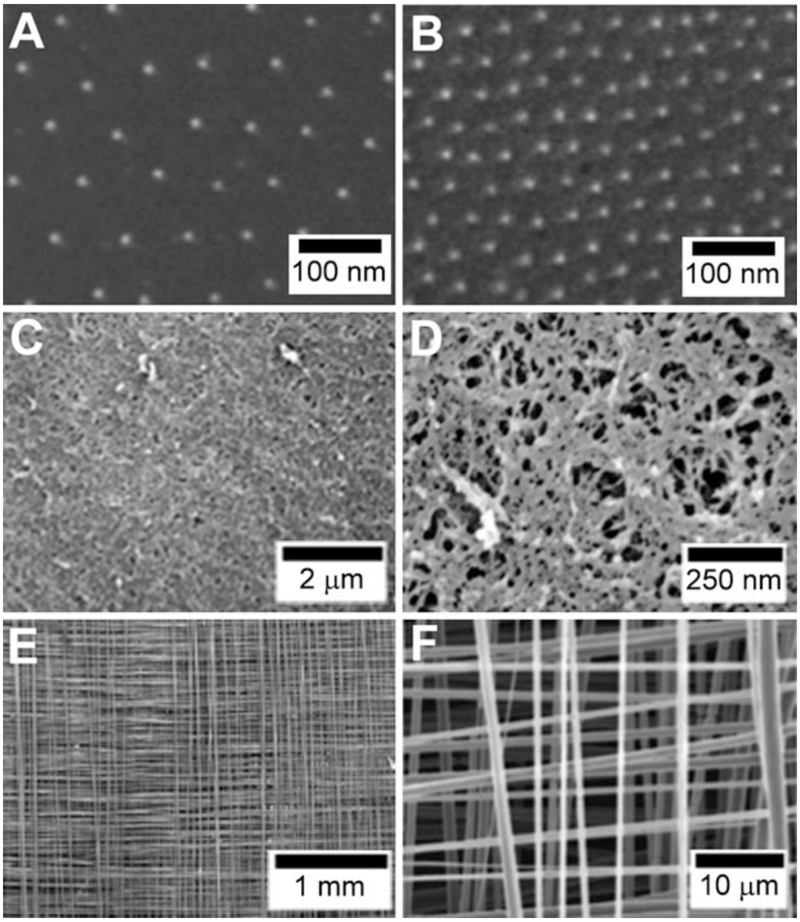
Whereas all of the fabrication techniques described above can be combined to create surfaces with heterogeneously patterned features that vary across length scales, other techniques such as electrospinning [9] do exist to create patterned surfaces that simultaneously expose cells to topologies with widely different dimensions. In electrospinning, an electric field is used to produce polymer fibers from a liquid solution. As the fiber orientation and diameter are easily controlled with simple experimental parameters, fibrous mats containing both nanoscale and microscale fibers can be created with aligned or randomly oriented topologies. Additionally, multiple fibers and complex fiber geometries can be spun simultaneously from different materials or conjugated with different cell-adhesive ligands. This produces substrata that more closely mimic the topology of tissues in vivo: complex fibrous meshes with nano- to micro-sized fibers and randomly sized micropores/pits. Thus, electrospun substrata are widely used to determine how cell behavior is regulated by fiber alignment and multiscale distributions of fiber size and surface ligands.

Key Research Findings

Chemically nanopatterned substrata have been primarily used to investigate integrin engagement and focal adhesion formation in an effort to dissect out cell adhesion properties. The majority of these studies have been carried out on self-assembled surfaces containing RGD-functionalized gold nanoparticles [10] (Fig. 3). Integrin receptors are approximately 10 nm wide and these "nanodots" can be fabricated such that they bind to single integrins. As a result, spacing of bound integrins can be altered by changing the spacing of the nanodots on the substrata. These nanopatterned surfaces have revealed that cell behavior is regulated by the spacing of adhesive ligands. Results from early studies showed that cell motility was affected on surfaces with RGD ligand spacings in the range of 6–300 nm and that spacings from 14 to 25 nm influenced cell-adhesion strength. Subsequent studies explored nanodot spacings between 28 and 85 nm [11] and found that RGD ligand spacings from 58 to 73 nm are optimal for integrin clustering and activation, whereas ligand separation greater than 73 nm causes significantly less cell adhesion, spreading, and integrin-mediated focal contact formation in osteoblasts. Additional studies have measured cell detachment forces from these substrata and demonstrated that spacings greater than 90 nm inhibited focal adhesion formation and decreased detachment forces compared to spacings less than 50 nm [12]. Overall, the data obtained using these chemically nanopatterned surfaces indicate that RGD ligand spacings should be less than approximately 70 nm to stimulate collective cell functions. Additionally, these stimulatory effects tend to increase as the ligand spacing decreases down to approximately 10 nm.

Studies using topologically nanopatterned substrata can be grouped into two categories, ordered or unordered, based on the layout of the surface features. Ordered surfaces consist of repeated features that are arranged with consistent height, spacing, and orientation. Such surfaces include those with uniform posts or pits and gratings formed by regularly spaced groves. Conversely, surfaces with randomly oriented, varying sized features, such as electrospun meshes, are classified as unordered. The mechanisms by which nanotopographic cues regulate cell behavior are not well understood. The emerging picture is that changes in cytoskeletal organization and structure may drive some of these cell behaviors, potentially in response to the underlying geometry or feature size of the surface. Regardless, it is clear that there is a range of different responses, or differing levels of response that vary by cell type.

Morphology and proliferation are altered when cells are cultured on surfaces with ordered nanotopography [14] (Fig. 4). Human mesenchymal stem cells (MSCs) grown on 350 nm-wide grooves have an aligned cytoskeleton along the direction of the features. Likewise, stem-cell-derived osteoblasts not only aligned and spread in response to nanogrooves of polystyrene, but also showed alignment in actin and

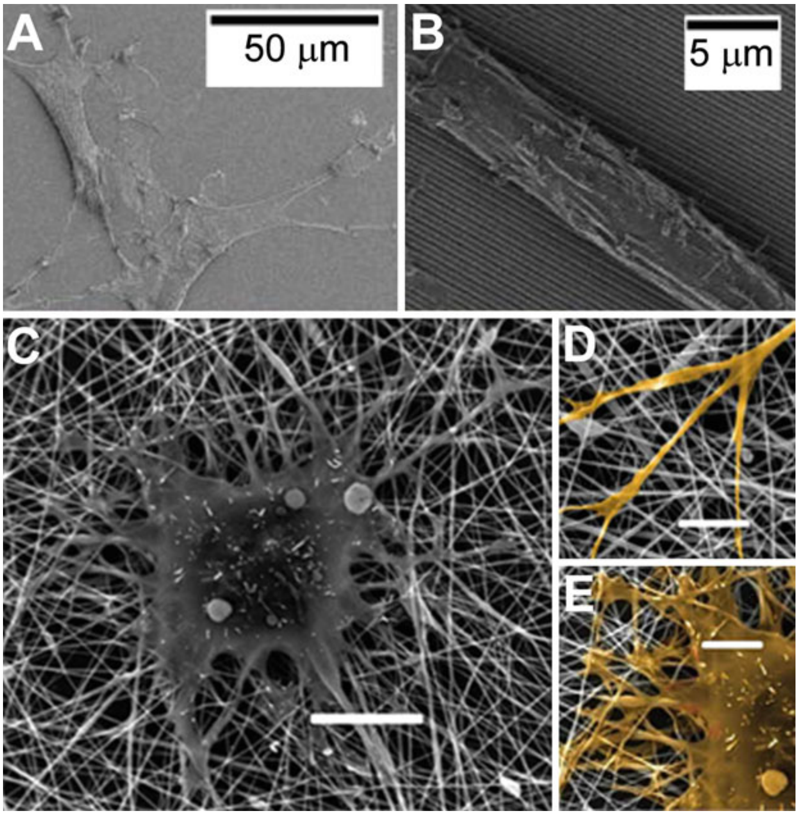


Nanopatterned Surfaces for Exploring and Regulating Cell Behavior, Fig. 3 Example substrata. (**a**, **b**) Ligand-coated gold nanodots can be spaced at varying distances to form chemically nanopatterned substrata (Reproduced with permission from Selhuber-Unkel et al. [12]). (**c**, **d**) The basement membrane of the cornea has topographical variations with both micrometer- and nanometer-sized features (Reproduced with permission from Abrams et al. [5]). Electrospun meshes can be formed to create both unordered and ordered topographies (**e**) and fiber diameters can vary within individual samples (**f**) (Reproduced with permission from Carnell et al. [13])

mineralized matrix. Additional studies with human embryonic stem cells (ES) cultured on 600 nm polydimethylsiloxane (PDMS) ridges have demonstrated an altered organization of cytoskeletal components including F-actin, vimentin, γ-tubulin, and α-tubulin. The alterations to morphology and proliferation were eliminated when the ES cells were exposed to actin-disrupting drugs [15]. Generally, cells seem to be more sensitive to the depth of the groove as opposed to the groove pitch or spacing. Additionally, cell orientation increases with increasing groove depth on grated surfaces but decreases as the groove width or pitch increases.

Whereas the phenomenon of cell and cytoskeletal alignment is fairly conserved over a wide range of nanogratings of varying dimensions, cell behavior is more varied in response to ordered surfaces with pits and posts. The adhesion of osteoblasts was significantly increased when cultured on 11 nm-high posts compared to either 85 nm-high posts or flat polystyrene [16]. Similarly, fibroblasts also demonstrated increased adhesion and spreading on 13 nm posts compared with 95 nm-high posts. However, endothelial cells displayed less spreading and lower cytokine production when cultured on 100 nm-high posts as compared to flat control surfaces. In general, cells cultured on surfaces that are uniformly or randomly textured with nanometer-sized pits and pillars do not display aligned or preferential orientations.

Although comparing different unordered topologies is difficult due to differences in feature size, shape, uniformity, and chemistry, a trend does seem to emerge. In electrospun fiber meshes ranging from 283 to 1,425 nm in diameter, rat hippocampus-derived adult neural stem cells differentiate and proliferate in response to fiber diameter [17]. For fiber meshes smaller than 283 nm, cells did not demonstrate preferential patterns of spreading; however, as the fiber



Nanopatterned Surfaces for Exploring and Regulating Cell Behavior, Fig. 4 Examples of cell responses to nanotopography. Uniform cell spreading is observed on flat substrata (**a**), whereas cells spread and align along the direction of nanograted substrata (**b**) (Reproduced with permission from Yim et al. [14]). Cells spread, attaching to and around fibers on an unordered electrospun mesh (**c**–**e**). The cell is highlighted in *yellow* and scale bars are 10 μm (**c**) and 5 μm (**d**, **e**) (Reproduced with permission from Christopherson et al. [17])

diameter increased, cells extended along the length of the fiber. In contrast, proliferation increased as fiber diameter decreased. In another study investigating cell adhesion, fibroblasts demonstrated a higher level of adhesion to surfaces with unordered topology as compared to either flat surfaces or those with ordered topology [18]. In summary, the general trend is that cell behaviors such as proliferation, adhesion, and spreading are positively stimulated as the feature size decreases from ~100 to 10 nm.

While almost all of the studies to date have focused exclusively on either chemically or topologically patterned substrata, these parameters are difficult to decouple. Chemically nanopatterned surfaces inevitably have 5–10 nm variations in height across the substratum due to the islands of conjugated ligands. In topographically nanopatterned systems, differences in protein transport and adsorption due to structural features may result in differences in the presentation of adhesive areas and thereby alter cell behavior. Few studies have investigated the competitive or synergistic effects of both chemical and topographic cues [16]. When continuous chemically patterned strips of cell-adhesive ligand were orthogonally overlaid onto nanogrooves, fibroblasts preferentially oriented along the chemical patterns. However, when fibronectin was orthogonally overlaid discontinuously along the top of a nanogroove pattern, osteoblasts preferentially aligned along the topographic pattern. From these limited data it is clear that a variety of systems involving both chemical and topological patterning over varying nanometer-sized scales need to be employed to fully understand interaction effects.

Applications

The basic science studies highlighted herein that seek to understand how nanoscale environmental cues influence cell behavior are fundamental to a range of applications. In particular, these data can be used to engineer biomaterial surface characteristics for improved performance. Materials that interact with the circulatory system, such as those used for stents or cardiac catheters, could be designed with a nanopatterned surface to prevent cell adhesion and clotting. Conversely, orthopedic implants, which need to integrate strongly with the surrounding tissue, could be designed with a nanopatterned bioactive surface to encourage cell adhesion, proliferation, and motility into the surface. Thus, understanding the nano-environmental cues will enable engineers to recreate physiologically desirable microenvironments for various biomaterial-tissue applications.

Nanopatterning also has applications for diagnostics and cell biology studies such as cell-based biosensors and cell sorting and culture systems. Integration of micro- and nanotechnologies with cell biology has yielded many new and exciting approaches. Use of microfluidics has created cells-on-a-chip or lab-on-a-chip designs where differential cell adhesion based on cell type is critical. Introduction of nanopatterned surfaces can enable a passive physical modification to these systems that would save fabrication time and complexity. Nanopatterned surfaces can be used as passive cell sorting systems that leverage the fact that different cell types respond differently to the same nanotopology. Thus a heterogeneous mixture of cells could be exposed to a nanopatterned surface and only the desired cell population would adhere. Additionally, as understanding of stem cell behavior on nanopatterned substrata grows, one could envision designing substrata that control stem cell adhesion, proliferation, and differentiation – cell behaviors that are difficult to control and that are currently modulated using complex media formulations and cell handling procedures.

Finally, studies with nanopatterned substrata can be integrated into the fields of tissue engineering and regenerative medicine. It is clear that the native in vivo environments and the chemical and physical cues to which cells are exposed are vastly different from those presented by traditional in vitro surfaces such as tissue culture plastic. Examination of signaling cascades within cells on nanopatterned surfaces can reveal how cells detect and respond to chemical and topographic signals in terms of short- and long-term cell functions, such as changes in gene expression and protein synthesis. These details of scaffold architecture and ligand presentation can improve tissue engineering technologies, and provide insight on disease processes where microenvironmental regulation is disrupted, such as in cancer. While some examples of nanopatterned tissue scaffolds exist, particularly with nanoporous materials for bone tissue engineering, the link between cell behavior in 2D and 3D is not straightforward. Cells in 3D environments adhere differently than those in 2D environments and contain different cell-ECM adhesions [19]. A recent study has even demonstrated that cells cultured on 1D patterns (lines) of adhesive ligands behave more similarly to cells in 3D matrices than to those on 2D chemically patterned islands [20]. Thus, while the link between cell behaviors in 2D and 3D is not certain, it is clear that further understanding gained using well-controlled nanopatterned surfaces can contribute to improved 3D tissue models and translational tissue engineering technologies.

Cross-References

▶ Dip-Pen Nanolithography
▶ Electron-Beam-Induced Deposition
▶ Electrospinning
▶ Nanoimprint Lithography
▶ Self-assembly

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Nano-patterning

► BioPatterning

Nanophotonic Structures for Biosensing

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Synonyms

Nano-optical biosensors; Nano-optics biosensing

Definition

Nanophotonic structures for biosensing: one-dimensional, two-dimensional, or three-dimensional structures made of suitable materials, having dimensions ranging from few nanometers to few hundreds of nanometers, said structures showing a well-defined interaction with light providing an optical transduction mechanism for detecting/revealing specific biomolecules in close proximity to the structure itself.

Overview

Optical biosensors [1] constitute powerful detection and analysis tools with wide applications in the biomedical domain. They can provide parallel detection within a single device. Generally speaking, there are two detection methods that are implemented in optical biosensing: fluorescence-based detection and label-free detection. Thanks to the recent advances of technological capabilities for the fabrication of nanometer-sized structures, most of the conventional techniques can be improved, and new original techniques are become feasible.

In fluorescence-based detection, target molecules are labeled with fluorescent tags, typically organic dyes and/or chemically functionalized/decorated quantum dots; the overall intensity of fluorescence indicates the presence of the target molecules. Some quantification can also be inferred, but in general quantitative analysis is challenging due to the fluorescence