

An update of nanotopographical surfaces in modulating stem cell fate: a narrative review

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biomaterials; mechanotransduction; nanotopographical surfaces; stem cell; tissue regeneration

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ABSTRACT

Stem cells have been one of the ideal sources for tissue regeneration owing to their capability of self-renewal and differentiation. In vivo, the extracellular microenvironment plays a vital role in modulating stem cell fate. When developing biomaterials for regenerative medicine, incorporating biochemical and biophysical cues to mimic extracellular matrix can enhance stem cell lineage differentiation. More specifically, modulating the stem cell fate can be achieved by controlling the nanotopographic features on synthetic surfaces. Optimization of nanotopographical features leads to desirable stem cell functions, which can maximize the effectiveness of regenerative treatment. In this review, nanotopographical surfaces, including static patterned surface, dynamic patterned surface, and roughness are summarized, and their fabrication, as well as the impact on stem cell behaviour, are discussed. Later, the recent progress of applying nanotopographical featured biomaterials for altering different types of stem cells is presented, which directs the design and fabrication of functional biomaterial. Last, the perspective in fundamental research and for clinical application in this field is discussed.

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Introduction

Stem cells, including mesenchymal stem cells (MSCs), neural stem cells (NSCs), and induced pluripotent stem cells (iPSCs), have exhibited great potential as promising solutions for tissue regeneration owing to their ability to self-renewal and differentiation into specific cells lineages.¹ Therefore, directing stem cells into a specific lineage with defined mature functions is vital for the treatment of tissue-specific degenerative diseases.^{2, 3} In vivo, stem cells are sensitive to their surrounding environment, which includes soluble cues, insoluble cues, and physical stimuli.^{4, 5} Enormous research and reviews have focused on the significance of the biochemical cues such as soluble factors and adhesion ligands in controlling stem cells' behaviour, such as cell adhesion, migration, proliferation, apoptosis, and differentiation.^{6, 7} In recent years, biophysical cues⁸ have attracted great attention due to their significant capability of manipulating stem cell fate.⁹ Engineering the biomaterial

interface with biophysical factors is favoured by several advantages: low cost, the feasibility of operation, high efficiency of cell differentiation, and controllable material fabrication. Thus, biophysical factors functionalized biomaterial exhibited great potential in the application of tissue engineering and regenerative medicine.¹⁰⁻¹³

Stem cells respond to extracellular matrix (ECM)-like biomaterials at the cell-materials interface via mechanotransduction-mediated gene regulation. Specifically, stem cells are responsible for the microscale features due to their comparable size to the cells.^{14, 15} Thus, modulating the stem cell fate can be achieved by controlling the nanotopographic features on synthetic surfaces.¹⁶ Nanotopography refers to nanoscopic scale (1–100 nm) morphological features and is within the same order of magnitude as cell receptors, such as integrins.¹⁷ When the implant interacts with the biological environment, the first biological event is the absorption of proteins on the surface of implants,^{18, 19} the implant interface also has a

great impact on other biological events such as the interactions with blood such as platelet adhesion, and hemostasis, inflammation,²⁰ and osteogenic cell responses.²¹

The goal of this review is to update the recent advances in the understanding of how the nanotopographical features modulate stem cell behaviour. We first provide a review of three different types of functional nanotopographical surfaces. We then highlight recent progress in designing and fabrication of nanotopographical surfaces in modulating stem cell fate. Last, we elaborate on the future developing perspectives in this field, more specifically, the opportunities and challenges in developing smart materials for engineering stem cell fate and function. However, in this review, we mainly focused on the biophysical aspects of nanotopographical surfaces that regulate stem cell fate, though the modulation of stem cell fate is synergy effects from biochemical and biophysical cues of biomaterials.

Search Strategy

The articles about nanotopographical surfaces and materials for modulating stem cell behaviours were obtained using the following conditions: ((nanotopographical surfaces) OR (topographical surfaces) OR (topography) OR (patterned surfaces) OR (topography features)) AND ((stem cells) OR (cell)). All these searches were retrieved on PubMed and Web of Science databases before November 2021. The results were further screened by title and abstract. Finally, 94 articles were included in this review.

Different Types of Nanotopographical Surfaces

In vivo, cells interact with ECM in a very complex three-dimensional environment,⁵ the architectures vary from porous fibrous networks to membranes, which indicate diverse topographical local microenvironments. Vast types of biomaterials with micro/nanoscale surface topographic features have been applied to understand how cells sense cell-ECM interfaces and develop their morphology and function. Different cell phenotypes that are sensitive to topographical features have been revealed by synthetic substrates with varied topographic features, this, in return, inspires a new aspect of mechano-responsive cellular properties and illustrates fundamental understanding for novel biomaterials with unique topographic features to program cellular behaviours.

Therefore, as one of the most popular biophysical features used for the manipulation of stem cell fate,²² researchers have studied different types of nanotopographical surfaces to modulate stem cell fate.¹⁵ *In vitro* studies have revealed that interaction of the interface of nanotopographical surfaces and stem cells can manipulate stem cell activity, specifically their adhesion, migration, proliferation, and differentiation.^{21, 23, 24} By controlling the topology of biomaterials, attempts have been made to manipulate the differentiation of stem cells towards the desired lineage. These nanotopographical features can be mainly divided into three types, i.e., static patterned surface, dynamic patterned surface, and roughness surfaces. In this section, we will mainly discuss these three types of nanotopographical biomaterials, and the techniques applied

for their fabrication.

Static patterned surface

Static patterned surfaces are surfaces with regular patterns. The fast development of microfabrication technologies has facilitated the fabrication of synthetic surfaces with regular patterned micro/nanoscale topography. In the 1990s, the Whitesides group from Harvard University proposed using soft lithography to create patterned surfaces.²⁵ Later, researchers started to apply photolithography, microcontact printing, self-assembly monolayer, ion milling, focused electron-beam-induced deposition, dop-pen nanolithography, electron beam lithography, chemical etching, micromachining, and reactive ion etching to prepare nano/microscale topographic surface.²⁶⁻³¹ For the static patterned surfaces, we divided them into isotropic patterned surfaces and anisotropic patterned surfaces.

Isotropic patterned surfaces are randomly oriented nanostructures that are proved to affect cell differentiation. These nanostructures are pillars, tubes, poles, wires, and random fibres. Among all these structures, we will mainly focus on high aspect ratio nanostructured surfaces, as they possess a similar length scale to cellular components and exhibit their great potential in altering the interaction between cells and materials.³²

Before the discussion, to be clear, we define "high aspect ratio" as structures with an aspect ratio equal to or greater than 10:1,^{33,34} including nanoneedles, nanopillars, nanowires, nanostraws, nanotubes, nanoelectrodes, nanobars, nanoblades, nanopikes, nanoposts, nanowhiskers, vertically aligned nanostructures. Nanopillars have been verified to promote cells elongation and differentiation rather than cell spreading. Rasmussen et al.³⁵ studied the influence of pillar arrays on the behaviour of human embryonic stem cells (hESCs), their research revealed that soft polycarbonate pillars which bear with high aspect ratio could promote the differentiation of hESCs into endoderm cells, whereas hESCs were prone to differentiate into pancreatic lineage on the stiff pillar and planar control. Further study has also revealed the mechanism may lay on the stimulation of transcriptional coactivator with PDZ-binding motif (TAZ) expression in hESCs.³⁶

Unlike nanopillars, nanopits possess a different shape, the pit size is also critical for stem cell adhesion and responses. Karazisis et al.³⁷ have claimed that nanopits with 4 μm depth and pit diameter of 200 nm can promote osteogenesis of MSCs by providing larger surface traction forces to promote the formation of adhesion complexes and the expression of osteogenesis-related genes. Further studies have also demonstrated that nanopit cues could regulate the differentiation of hESCs and iPSCs into functional pancreatic endocrine cells to produce insulin.³⁸ Moreover, researchers have declaimed that nanopore has been proved to prohibit cell attachment and limit cell migration thus retaining the stemness of multiple stem cells.^{36,39}

Compared to nanopillars, nanopikes are regular sharp needles that are aligned vertically.⁴⁰ These structures have been widely used in controlled drug delivery systems, as they can deliver

Nanotopographical surfaces modulate stem cell behaviours

therapeutic compounds into cells with less invasion, as well as transmit biophysical cues to the intracellular microenvironment via specific signal transduction molecules without damaging the cell membrane. Recent study has successfully synthesized nanospine arrays of the topographical hydrogel by using the photolithography technique.⁴⁰ The hydrogel with uniform geometries exhibited high flexibility and adaptability which ultimately enhanced the osteogenic process of dental pulp stem cells. These high aspect ratio nanostructures interact with multiple cell organelles by precisely stimulating biophysical cues. Therefore, provide an ideal platform for the designing of implantable scaffolds for regenerative medicine.

In contrast to isotropic patterned surfaces, anisotropic patterned surfaces withstand intrinsic structures that mimic native ECM, for example, fibrils and collagen are prone to assemble into ordered structures along with certain directions according to their collective effects. Specifically, these anisotropic nanostructures commonly occur in the functional tissues of the myocardium, muscle, blood vessels, and bone tissue.⁴¹ Cells respond and adapt to anisotropic structures in a similar way as that of hierarchically structured ECM, thereby anisotropic structures are superior to modulate stem cell fate.⁴² So far, both inorganic biomaterials like titanium alloy and organic hydrogels, have been engineered with aligned, grooved, or wrinkled surfaces, to regulate stem cell behaviour for tissue regeneration.^{43,44}

Overall, wrinkled, or grooved surfaces are widely used to promote stem cell elongation, orientation, and differentiation. Take MSCs as an example, MSCs are prone to proliferate and migrate under the contact guidance of parallel structures.⁴⁵ Moreover, anisotropic patterning demonstrated nonnegligible properties to induce the functional regeneration of the nervous system and myocardium by promoting cellular communication, signal transmission, and substrate exchange.^{46, 47} However, introducing anisotropic features on the surface of *in vivo* implants is still challenging.

Dynamic patterned surface

In vivo, the microenvironment of ECM is in a continuously dynamic state. Thus, static patterned surfaces are limited to spatiotemporally mimicking the ECM topography either in the aspect of structure or function. In this case, dynamic topographic patterns are superior to static patterning in arranging the ECM-mimetic biointerface.^{48, 49} Thanks to the fast development of synthetic chemistry, enormous stimuli-responsive materials have been investigated. These stimuli-sensitive biomaterials change their physicochemical properties according to external conditions. Among the external stimulations, electro-, photo-, and thermo-fields are widely used. For the electrical triggered dynamic patterned surfaces, Wei et al.⁵⁰ have fabricated electrically conductive polymer arrays on a titanium substrate, which nanotopographic features can be altered from hydrophobic nanotubes to hydrophilic nanotips by applying redox-electro-chemical potentials, thereby preventing MSC focal adhesion and later activated intracellular mechanotransduction. As such, azobenzene possesses cyclic trans-cis-trans photoisomerization when

exposed to ultraviolet light, which is superior to facilitating polymers as light-responsive materials.⁵¹ The conformation change of azobenzene functionalized polymers led to the topological change from round-top nanopillars to elongated nanobars, which ultimately modulate the cell activities by interfering with the curvature-relevant biological process. Furthermore, shape memory polymer is also an ideal candidate as a dynamic patterned surface substrate as they can change their shape dynamically in a time-dependent manner once triggered by external stimuli such as light or heat. Researchers have applied thermo-responsive shape memory polymers to reveal how a dynamic microenvironment influences cellular behaviour by a topographic transition from flat to wrinkle.⁵²

Though the dynamic patterning surfaces have attracted considerable attention, it is still challenging to develop these dynamic patterning into *in vivo* implants. In addition, the change of surface pattern could also alter the intrinsic biophysical properties,⁵³ which might be difficult to carry out an in-depth study of individual factors that affect cellular behaviour.

Roughness surface

Roughness reflects the degree of depression or protrusion at the biomaterial surface, which is one of the most intuitive properties. Thus, the extensive focus has been given by researchers in the past few years. Surface roughness can enhance the effectiveness of engineered scaffolds by promoting cell adhesion, morphology, differentiation, and metabolism. To fabricate considerable surface roughness, many biocompatible components have been applied, such as titanium, polydimethylsiloxane, hydroxyapatite, polylactic acid, and polyurethane acrylate.⁵⁴⁻⁵⁶ Yang et al.⁵⁶ demonstrated that the optimal range of the roughness of hydroxyapatite disk ranges from 0.77 to 1.09 μm for the sake of osteogenesis induction of human MSCs. More specifically, materials with a roughness of $\sim 1.09 \mu\text{m}$ are more effective compared with that of 0.77 μm in modulating the expression of the osteogenic marker. In addition, Chen et al.⁵⁷ extended the study from microscale to nanoscale, in which, they fabricated nanorough surface by utilizing poly(ethylene oxide terephthalate)/poly(butylene terephthalate) electrospun porous scaffolds. They have also demonstrated notable improvement of osteogenic gene expression when MSCs were seeded on the scaffold with high average microroughness (R_a ; $71.0 \pm 11.0 \text{ nm}$), on the contrary, enhancement of chondrogenic gene expression was observed in low surface roughness ($R_a = 14.3 \pm 2.5 \text{ nm}$).⁵⁷

However, the preference of roughness is varied among different stem cells, take iPSCs and hESCs as an example, iPSCs can be guided and oriented into neuronal lineage when cultured on biomaterials with a comparable size in the roughness of iPSCs.⁵⁸ While nanorough surface could induce spontaneous differentiation of hESCs which retained its self-renewal potency on a smooth surface.

Nevertheless, these results are random individual roughness parameters, and a systematic investigation of the relationship between roughness and cell behaviour is required to provide the full phase diagram for the guiding of biomaterial designing.

To reveal the systematic discipline lying behind, researchers have successfully constructed a zirconia surface with roughness gradients at both the microscale and nanoscale by using hydrofluoric acid etching. There they found out that the migration ability of human MSCs is correlated positively to the surface nanoroughness value of biomaterials, albeit no linear correlation between microroughness parameters and the cellular spreading area was found. Using a one-step-tilted dip-coating approach, Hou et al.⁵⁹ fabricated a polymer-based surface with roughness gradient from nano- to micro-scale, then, they investigated the impact of roughness on cell morphology, mechanotransduction and fate modulation systematically. The results revealed that MSCs interact with interfacial roughness through the direct force-dependent mechanism.

The current study has put forward the roughness on stem cells, high-throughput systems have been fabricated by utilizing advanced lithography patterning and microfluid technology to ensure highly efficient and systematic investigation of materials-cell interaction (Table 1).

Nanotopography Controls Stem Cell Fate

Rational design of tissue engineering biomaterials inspired by the physiological environment of the living body provides new opportunities for clinical translation of biomaterials. In recent years, great efforts have been made to develop stem cell based regenerative medicine, as they are one of the main sources in tissue engineering. Nanotopographic surfaces provide a feasible platform to modulate stem cell properties by tailoring parameters of the surrounding microenvironment. In this section, we will mainly focus on the current state of knowledge, updating research, and promising *in vitro* and *in vivo* studies that demonstrate the potential of nanotopographical surfaces for the modulation of stem cell fate.¹⁷

Mesenchymal stem cells

MSCs are stromal cells that are capable of self-renewal and differentiation into multiple cellular lineages when stimulated by a specific microenvironment. These cellular lineages are osteoblastic (bone), adipogenic (fat), myoblastic (muscle), chondrogenic (cartilage), and fibroblastic (connective tissue)

Table 1. Nanotopographical features and their cellular effect on stem cells

Nanotopographical surfaces	Structure features	Fabrication technique	Cellular effect
Static patterned surfaces	Nanopillar ^{33,35}	Ultraviolet-lithography, injection molding	Promote cells elongation and differentiation
	Nanopits ³⁷	Colloidal lithography	Provide large surface traction forces to promote cell adhesion
	Nanopore ³⁶	Anodization	Prohibit cell attachment and limit cell migration
	Nanospike ⁴⁰	Photolithography	Enhance stem cell differentiation, secretion of growth factors
	Grooved surfaces ^{43,44}	Argon ion plasma, molding	Promote cell adhesion and proliferation
Dynamic patterned surfaces	Electro responsive, nanotubes to nanotips ⁵⁰	Electrochemical polymerization	dynamic attachment and detachment to mesenchymal stem cells
	Ultraviolet responsive, flat to rigid ⁵¹	Spin coating	Induce cyclic cellular and nuclear stretches
	Thermoresponsive, flat to wrinkle ⁵²	Ultraviolet polymerization and spin coating	Dynamic response of focal adhesion
Roughness	Gradient: 0.77–1.09 μm ⁵⁶	Molding	Cellular attachment, F-actin arrangement
	High: 14.3 nm, low: 71 nm ⁵⁷	Electrospinning	Cell morphology, metabolic activity
	Gradient: 200 nm–1.2 μm ⁵⁹	Soft lithography	Enhance cell mechanosensing and osteogenic differentiation of mesenchymal stem cells

lineages. In recent studies, plenty of evidence of the characters of nanotopographical features that modulate MSC fate has been obtained. Nanofiber scaffolds and titanium dioxide nanotubes with a diameter of 15 nm have been observed to enhance the osteogenic differentiation of MSCs.⁶⁰ Notably, Oh et al.⁶¹ proposed that nanotopographic surfaces induced rearrangement of aggregated ECM protein with distinct sizes and spacing, therefore activating integrin-mediated focal adhesion and stem cell function.

Nevertheless, despite two-dimensional nanotopographical features, the nanoscale height of nanotopographical structures has also been investigated to modulate the osteogenesis of MSCs,^{62,63} it is observed that a greater depth of nanotopographical structures could promote osteogenic differentiation of MSCs.

However, the response to nanotopography is cell-type specific, no consistent results were obtained among different types of stem cells. Research has been put forward to study the co-effects of other biochemical or biophysical cues such as stiffness, growth factor, biodegradability, etc., and nanotopographical features to regulate MSCs fate. Wu et al.⁶⁴ have fabricated nano-grating or pillar surface with three different kinds of polyesters to obtain nanotopographic features with different degree of stiffness, thereafter, their effect on MSCs behaviour were thoroughly investigated. The results indicated a negative correlation between surface stiffness and cartilage generation. Moreover, Haag's group⁵⁹ have successfully combined surface roughness and stiffness to systematically study the mechanoreponse and osteogenesis of MSCs.

Nanotopographical surfaces modulate stem cell behaviours

In addition to osteogenesis, great attention has been paid to engineering biomaterials to functionally alter the differentiation of MSCs into neural cells. For example, a hierarchically aligned fibrillar fibrin hydrogel was fabricated by Yao et al.⁶⁵ and its impact on stem cell neurogenic differentiation. They claimed that both the low elasticity and aligned topography of aligned fibrillar fibrin hydrogel promoted the neurogenic differentiation of MSCs in comparison to random fibrin hydrogel and tissue culture plate control after 2 weeks of cell culture in growth medium lacking supplementation with soluble neurogenic induction factors.⁶⁵ In addition, three-dimensional nanostructured microarchitectures have been found to regulate the fate of human MSCs efficiently.⁶⁶ More specifically, they demonstrated that the nanostructured architectures promote alignment and neurogenic differentiation effectively.⁶⁶ Which provides novel biomaterials for the designing of hierarchical architectures featuring micro- and nanotopographical features for the modulation of stem cell differentiation.

Neural stem cells

NSCs are self-renewing cells that generate the major cell types of the central nervous system, namely neurons, astrocytes, and oligodendrocytes, during embryonic development and in the adult brain. *In vivo*, unlike other stem cells, NSCs are exposed to a more complex environment including a plethora of signals and morphology varieties, which impeded the development of NSCs based cell therapies for clinical applications. To develop efficient methods for large-scale expansion as well as controlled NSCs differentiation, various approaches that integrate artificial nanotopographical features have been prepared.⁶⁷⁻⁶⁹ In this section we aimed at updating recent progress in developing novel micro- and nano-topographical features to modulate NSCs fate.

Simitzi et al.⁷⁰ have reviewed the recent progress in studying the effect of surface topography in controlling the morphology and outgrowth of NSCs. More specifically, in their review, they divided the topography features into three different types, i.e., continuous, discontinuous, and random topography features. Thoroughly discussed the effect of surface topography on NSCs growth and differentiation into the neuronal and neuroglial lineage. Therefore, engineering naotopographical structures to modulate NSCs fate provide new solutions for neural tissue regeneration. Cho et al.⁶⁹ reported a platform modified with homogeneous nanohole patterns, which was effective in guiding neurogenesis of mouse NSCs. They have fabricated three different nanohole patterns by using laser interference lithography. They claimed that surfaces with 500 nm-sized nanoholes exhibited the best effect on cell adhesion and spreading.⁶⁹ Moreover, Lee et al.⁷¹ demonstrated a combinatorial biophysical cue sensor array combining a surface-modified nanopillar array with a conductive hydrogel micropattern. In which they found that the proliferation of NSCs was enhanced by the silicon dioxide-coated nanopillar array.⁷¹ In addition, exposure time to mechanical cues is important to properly modulate stem cell fate. The time retention effect has attracted researchers' attention to control stem cell differentiation with varied mechanical cues. Yang et

al.⁶⁸ have investigated the effects of time-dependent retention of a nanotopographical cue on differentiating the NSCs by using a stress-responsive and tunable nanowrinkle topography. They concluded that the NSCs could retain the nanotopographical stimuli depending on the dosing time during differentiation, providing a novel methodology in controlling stem cell fate.⁶⁸

Human pluripotent stem cells

Pluripotent stem cells (PSCs) are cells that can self-renew by dividing and developing into the three primary germ cell layers of the early embryo and therefore into all cells of the adult body, but not extra-embryonic tissues such as the placenta.⁷² Nearly a century of mechanobiology research has implicated dynamic nanoscale structural cues from ECM as critical regulators of cellular morphology, differentiation, and functions at each sequential phase of tissue development.^{58, 73-76} Therefore, regulation of PSC behaviours has been studied not only through the integration of biochemical factors but also by designing materials that mimic the stem cell microenvironment including nanotopographic cues. In this part we mainly focus on strategies that applied to modulate the behaviour of hPSCs as an update literature review.

Chen et al.⁷⁷ utilized a large-scale nanofabrication technique based on reactive ion etching to generate random nanoscale structures on glass surfaces with high precision and reproducibility. And they reported that hPSCs are sensitive to nanotopographic cues and can be directed into neuronal differentiation, in addition, they demonstrated early neuroepithelial conversion and motor neuron progenitor differentiation of hPSCs can be promoted using nanoengineered topographic substrates. This study provides an efficient method for the large-scale production of motor neurons from hPSCs, which is useful for regenerative medicine and cell-based therapies.⁷⁷ Despite using random nanoscale structures, Kim's group⁷³ studied the combination of biomimetic topography and electroconductivity for the development of hPSC-derived cardiac tissues. There, they fabricated silk fibroin substrates patterned with anisotropic nanotopography that mimicked myocardial ECM. The patterned substrates promoted both tissue organization and the maturation of the contractile and electrical signal conduction capabilities of cultured cardiomyocytes. Furthermore, this research highlights the benefits of recapitulating the myocardial cell niche *in vitro*.

Nevertheless, many efforts have been made to engineer hPSC-derived cells,⁶⁻¹⁴ further studies have also demonstrated that hPSC-derived cells exhibited similar responses to drug treatment or genetic perturbation.^{8, 9, 15} However, these methods are more likely cell-type specific and limit their *in vivo* application. To overcome this limitation, high-throughput screening platforms that integrate biomimetic cues have been designed and utilized to improve hPSC-derived somatic cell organization and development to multiple cell lineages. For example, a high-throughput nanotopographically-patterned multielectrode array by integrating conductive, ion-permeable, nanotopographic patterns with 48-well multielectrode array plates, has been developed and utilized to investigate the effect of the substrate-mediated cytoskeletal organization on hPSC-

derived cardiomyocyte and neuronal function. This research demonstrated a nanotopographically-patterned multielectrode array as a new tool to facilitate the preclinical analysis of excitable cell function (Table 2).⁷⁸

The Mechanism of Stem Cells Interacts With Nanotopographical Surfaces

Nanotopographical surfaces possess vital physical cues to regulate stem cell behaviour. In this section, we will mainly focus on the mechanism that how stem cells interact with nanotopographical features.

Cellular membrane contact biomaterials directly, it can facilitate cells adapt to nanotopographical surfaces owing to its fluidity. Unlike flat surfaces, nanotopographical surfaces induce seeded cells to form caveolae at the interface, which result in enhanced expression and reorganization of Caveolin and clathrin, thereby modulating cell adhesion and migration.⁷⁹ Moreover, nanotopographical surfaces with a high aspect ratio are prone to induce membrane curvatures to trigger the mechanoreponse of seeded cells. The membrane curvature can modulate the expression of nuclear proteins and yes-associated protein (YAP)

localization along with F-actin polymerization.^{48, 80}

On the other hand, integrin is a transmembrane receptor that mediates the interaction between the cell and the ECM.⁸¹ When cells are exposed to rational designed nanotopographical surfaces, integrin clusters attach to the surfaces and transmit biophysical signals along the cytoskeletal actin. During the process, focal adhesion kinase (FAK), Paxillin, and Vinculin form the adhesion complex.⁸²⁻⁸⁵ Among them, FAK serve as a signalling molecule for mechanosensing, when a cell interacts with external stimuli, FAK undergoes phosphorylation and induce the transportation of intracellular proteins into the nucleus.⁸⁶ In summary, the mechanotransduction pathway starts from integrin induced FAK phosphorylation and focal adhesion formation, then transmit to the intracellular microenvironment via cytoskeleton, thereby activating the MAPK/ERK mechanosensing pathway, which ultimately regulates stem cell differentiation.⁴⁸ In addition, mechanotransduction signals can be also activated by the cytoskeleton and transferred to the nucleus via YAP/TAZ pathway to control cellular differentiation and tissue regeneration (Figure 1).^{87, 88}

Table 2. Examples from the literature of nanotopography controls stem cell fate

Stem cell type	Scaffold	Topographical features	Application
Mesenchymal stem cell	Polyesters ⁶⁴	Nanograting or nanopillars	Cartilage regeneration Neurogenic differentiation
	Fibrin hydrogel ⁶⁵	Hierarchical aligned	
Neural stem cell	Indium tin oxide-coated glass ⁶⁹	Nanopore	Neuronal differentiation
	Silicon oxide surface ⁷¹	Nanopillar arrays	
	Polydimethylsiloxane ⁶⁸	Nanowrinkle	
Induced pluripotent stem cell	Glass surface ⁷⁷	Random nanoscale structures	Neuronal differentiation Cardiac regeneration Preclinical analysis of excitable cell function
	Silk fibroin substrates ⁷³	Anisotropic patterned	
	Multielectrode arrays	Nanoarrays	

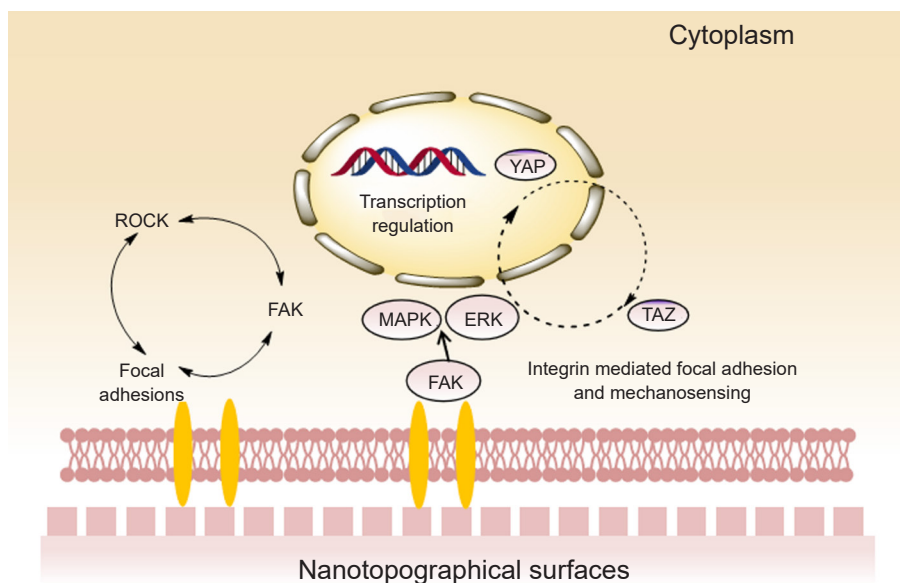


Figure 1. Schematic illustration of cellular response to nanotopographical cues and relevant mechanotransduction. External nanotopographical cues exerting on cell–nanotopography interface mediates the subsequent mechanosensing and focal adhesion, which regulated the downstream molecular expression corresponding to different cell behaviors. FAK: focal adhesion kinase; ERK: extracellular signal regulated kinase; MAPK: mitogen activated protein kinase; MEK: mitogen activated protein kinase; ROCK: Rho-associated protein kinase; YAP: yes-associated protein; TAZ: transcriptional co-activator with PDZ-binding motif.

Conclusion and Perspectives

The development of engineering topographical biomaterials has been a hot research topic reported since the first studies of cellular contact guidance.⁸⁹⁻⁹¹ Nanotopographical regulation of stem cell behaviour has been widely employed while their mechanism remains elusive. Research demonstrated that nanofeatures manipulate cell responses by influencing cytoskeletal structures or mechanotransduction.⁹ Regarding this aspect, Luo et al.⁹² reviewed the mechanism underlying protein physical adsorption on nanotopography, discussed progress in developing advanced nanotopographical biomaterials for guiding the behaviour of cells, in which they mainly focused on the cellular mechanotransductive pathways, therefore it reveals the principle of designing nanotopographical materials for biomedical applications.

However, although nanotopographical features potentially regulate cell fate, challenges remain in optimizing parameters, including size and scale, and introducing them into biomaterials to simulate real ECM structures. Therefore, high-throughput biomaterials that can scale up the parameter range and program the optimal nanotopographical features should be designed.^{78, 93, 94} In addition, most of the research focused on fabricating nanotopographical structures on two-dimensional substrates, while three-dimensional materials with more complex hierarchical structures which can mimic the microenvironment of stem cells are required to put forward the applications of biomaterials with nanotopographical features to a new level. Furthermore, advanced technologies such as computational mathematics, and other biophysical cues can be used for the designing of ideal biomaterials for tissue engineering.

Author contributions

Review design and graphical abstract design: SC, QY; final edits of review: QY. All authors read and approved the final version of the manuscript.

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Conflicts of interest statement

The authors declare that they have no competing interests.

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