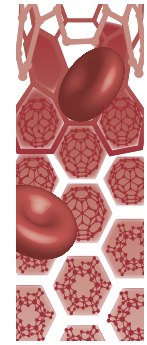
Review

Nanomedicine





# Regulation of stem cell fate by nanomaterial substrates

Stem cells are increasingly studied because of their potential to underpin a range of novel therapies, including regenerative strategies, cell type-specific therapy and tissue repair, among others. Bionanomaterials can mimic the stem cell environment and modulate stem cell differentiation and proliferation. New advances in these fields are presented in this review. This work highlights the importance of topography and elasticity of the nano-/micro-environment, or niche, for the initiation and induction of stem cell differentiation and proliferation.

**Keywords:** biomimicking • differentiation • nanotechnology • niche • stem cells • substrates

Omid Mashinchian[1], Lesley-Anne Turner[2], Matthew J Dalby[2], Sophie Laurent[3,4], Mohammad Ali Shokrgozar[5], Shahin Bonakdar[5], Mohammad Imani[6] & Morteza Mahmoudi*[,7,8,9]

[1]Department of Medical Nanotechnology, School of Advanced Technologies in Medicine (SATiM), Tehran University of Medical Sciences, PO Box 14177–55469, Tehran, Iran

[2]Centre for Cell Engineering, Joseph Black Building, Institute of Biomedical & Life Sciences, University of Glasgow, Glasgow G12 8QQ, Scotland, UK

[3]Department of General, Organic & Biomedical Chemistry, NMR & Molecular Imaging Laboratory, University of Mons, Avenue Maistriau 19, B-7000 Mons, Belgium

[4]CMMI – Center for Microscopy & Molecular Imaging, Rue Adrienne Bolland, 8, B-6041 Gosselies, Belgium

[5]National Cell Bank, Pasteur Institute of Iran, PO Box 13169–43551, Tehran, Iran

[6]Novel Drug Delivery Systems Department, Iran Polymer & Petrochemical Institute (IPPI), PO Box 14965/115, Tehran, Iran

[7]Department of Nanotechnology & Nanotechnology Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, PO Box 14155–6451, Tehran, Iran

[8]Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA 94305–5101, USA

[9]Cardiovascular Institute, Stanford University School of Medicine, Stanford, CA 94305–5101, USA

*Author for correspondence: mahmoudi@stanford.edu

Over the last few decades, biomaterial-based therapeutic approaches have been successfully employed in regenerative medicine for the repair of tissues [1–5]. This novel approach creates new opportunities for stem cell-based regenerative therapies and the advancement of drug delivery and discovery [2,6–8]. Inevitably, numerous people lose a part or function of their organs and tissues due to a diverse range of diseases, birth defects or accidental trauma; thus, a tremendous clinical demand exists to promote the regeneration of injured/diseased tissues. Continuous advances in the field of cell and tissue engineering give scientists hope for future developments of implantable tissues, for example, for skin and cartilage, which have already been commercialized or possess high commercialization potential [9]. Stem cells, including embryonic (ESCs), adult and induced pluripotent stem cells (iPSCs), are promising cell sources to underpin these novel therapies [10,11]. One of the most challenging aspects of regenerative medicine, both *in vitro* and *in vivo*, is how to guide stem cell differentiation toward a specific desired lineage [12–15]. *In vivo*, appropriate differentiation, proliferation and maintenance of potency are regulated by stem cells and their specific microenvironments (niches) [16–18]. Biomaterials can mimic the niches of stem cells and specifically effect the *in vitro* differentiation that is necessary for clinical application. Consequently, research efforts have been principally devoted to understanding how a wide range of well-recognized differentiation factors (e.g., growth factors, low molecular weight chemicals, extracellular matrix [ECM] components, cell shape, matrix stiffness and mechanical forces) contribute to the stem cell microenvironment [16,19–23]. In this review, the topography that stem cells encounter will be a particular focus. The roles of niche components and architecture in regulating cell behaviors can be elucidated by simplifying the niche structure using well-defined synthetic microenvironments as artificial bioinspired models. It is possible to biomimic the 3D structures that support tissue growth and direct cell behavior through cell-ECM interactions by using natural or artificial polymeric matrices, as will be discussed [24,25]. In this review, we highlight new advances in the bionanomaterials field, focusing on stem cell fate together with a perspective on future applications in the next generation of regenerative medicine.

## Stem cells & their niches

A biological niche is an instructive multidimensional microenvironment that supplies both chemical and physical guidance to stem

Future Medicine part of fsg

cells growth and development (Figure 1). This environment must exhibit several anatomic and functional features. The niche should facilitate the differentiation or self-renewal of stem cells as required [26–29]. Specific cues instruct stem cells to preserve or change their fate and to modulate their functions under diverse physiological conditions. Adult stem cell niches are composed of mixtures of extracellular cues that are largely produced by supporting cells and ECM, both of which the stem cells may adhere to [30]. Several mammalian niches have been well characterized, such as those of the skin and intestine and to some extent the bone marrow [30–32]. Close physical contact between supporting cells and stem cells can provide diverse biochemical and mechanical signals via soluble or membrane-bound factors. Similarly biochemical and mechanical signals can be conferred from bound or inherent factors of the protein and sugar rich ECM. In addition, stem cells are able to react with nervous system impulses inside the niche, suggesting circadian regulation [32,33]. Stem cell metabolism also appears to be closely controlled by the niche; reactive oxygen species and ions are known to effect stem cell function [34] and metabolomically quiescent mesenchymal stem cells (MSCs) tend to pool unsaturated metabolites to allow redox plasticity upon differentiation [35,36]. Our focus is not really on soluble factor regulation, although we acknowledge the important role of this regulation, for example, Wnt glycoproteins, hedgehog proteins and fibroblast growth factors [37–39]. In addition, while we acknowledge the role of adhesive interactions via adhesion proteins located on the cell surface, which act as an anchor between support and stem cells (i.e., retaining stem cells in close proximity to self-renewal signals), it is not a main focus of our review (see Figure 1) [40,41].

## Cell–substrate signaling at the nanoscale

Cells communicate with the ECM through cell-adhesion structures, such as integrin proteins that cluster and combine with other proteins to form focal adhesions. Focal adhesions are large macromolecular structures responsible for transmitting mechanical forces to cells. These cell adhesion structures mediate cell signaling and affect cell shape, cell motility and cell-ECM attachment. They also act as trigger points for

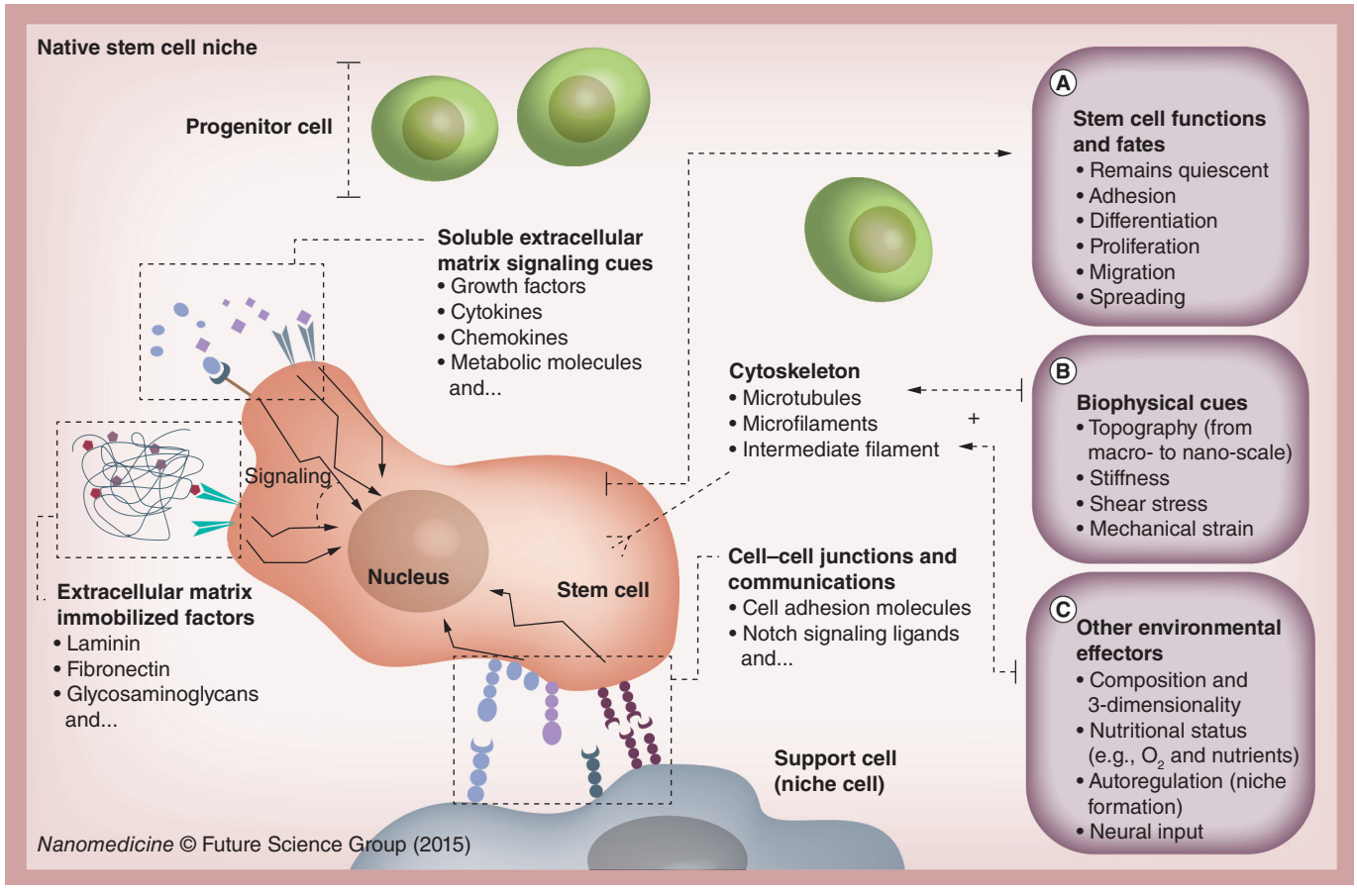



**Figure 1. Schematic illustration of the stem cell niche.** Adult stem cells are contained within a 3D microenvironment comprised of complex mixtures of extracellular cues (biochemical, physical and mechanical) **(A–C).** The niche includes, amongst other components, cell–cell adhesion proteins, soluble factors and extracellular matrix.
For color figures, please see online at www.futuremedicine.com/doi/full/10.2217/NNM.14.225

cascades of intracellular signaling pathways and affect cellular performance at many levels including cellular/molecular processes, stem cell–matrix interactions and differentiation [42]. For example, the pathway of RhoA/Rho-associated kinase regulates focal adhesion formation and the cytoskeletal organization of cells [43]. A number of molecular structures are known to be particularly important in stem cell responses to topography, including focal adhesion kinase, paxillin and talin, factors in the ECM (such as surface chemistry, lateral spacing and geometry of nanofeatures) and in the medium (such as the existence of nanoparticles [NPs]) [44]. Therefore, bioinspired nanomaterials must be designed to mimic ECM structural complexity including specialized, textured topography (which as described previously are known to direct stem cell behaviors including maintenance of multipotency and differentiation).

## Limitations for chemical factors

Although potent regulators of cell behavior (discussed further in the section 'Effects of chemical properties'), chemical factors are limited in that they are thought to be only one component of the 'triangle' of cell control, whereby in addition to chemical factors inducing stem cell differentiation inside the niche, topography and matrix elasticity are other chief factors influencing cell fate [45–50]. Other important aspects that significantly influence the attachment of cells to biomaterials *in vivo* are surface moisture retention [51], material structure [52] and preparation methodologies [53]. Feeder cells, which are obtained primarily from animal sources, are routinely used for the differentiation and maintenance of ESCs as they provide suitable chemical factors for stem cell maintenance. In addition to the risk of immune recognition, there is another prominent hazard due to the different sources of stem cells and feeder cells that can spread viruses and other unsafe infectious agents [54]. In this context, accurate/safe controlling of the stem cells fate in the laboratory/clinical setting is a crucial issue and also alternative strategies besides a conventional method (e.g., growth factors) should be considered in order to completely induce the mature cells.

## Synthetic substrates & artificial niches: the three generations of biomaterials

Hench and Polak define the evolution for biomaterials as first generation (structural, e.g., titanium orthopedic implants), second generation (bioactive, e.g., hydroxyapatite and bioglass) and third generation (reproducible molecular control of cells, e.g., nanotopography) [55]. A third generation biomaterial designed for use in tissue engineering requires incorporation of multiple types of signals, which increases the complexity of the system and further complicates the interpretation of results. In this regard, cells are capable of complex tissue engineering, able, ultimately to form organs given the right cues. However, once the body is formed, the capacity of cells for self-renewal and regeneration is limited; many factors are responsible for healing, including supporting nutrition and environmentally optimized conditions. Thus, tissue engineering can be used to assist the body by various biomimetic strategies. A combination of cells, material(s) and physico-chemical factors have emerged as important in the tissue engineering field to assist the body in this healing process [56,57]. Early studies focused on the nontoxicity of materials and the preservation of cell viability, whereas later studies have focused on cell functions, including proliferation, migration and expression. The physical and chemical properties of materials play an important role in regulating cell function and thus chemical characteristics and physical structures must be mimicked [58].

Both specialized (fully differentiated) and nonspecialized stem cells can be isolated from tissues *in vitro* using mechanical or enzymatic techniques. For tissue engineering to be a success, cells should be incorporated into an appropriate scaffold as soon as possible in order to retain their native features. The scaffold and culture medium must be precisely selected; in this way, chemical and physico-mechanical properties are mimetically correlated with the natural environment [59]. For example, cartilage tissue (largely an avascular hydrogel) contains specialized cells, chondrocytes that are nourished by a synovial fluid flow of nutrients through it. Hydrogels from different materials, such as alginate, chitosan, polyvinyl alcohol and PEG, have been used to mimic this structure [60,61]. Although preliminary observations showed that chondrocytes cultured in 3D hydrogels can keep some of their functional characteristics, chemical induction by TGF-β or physical stimulation by hydrostatic pressure enhance cellular responses [62]. Thus, MSCs exposed to similar stimulation (chemical and/or physical) might be differentiated to exhibit the chondrocyte phenotype [63]. In general, cells can lose their original natural phenotype after transportation and culture *in vitro*. The most prevalent containers for *in vitro* cell cultures are fabricated from hydrophobic transparent polystyrene, which has been used for many years for a range of cell types and is not designed specifically for stem cells. Because adherent cells attach loosely to the uncharged polystyrene plate, the modification of its surface by introducing carboxyl and amine functional groups is normally performed using, for example, plasma. The rigidity of this substrate is not ideal for stem cell growth, often resulting in random and spontaneous differentiation

(e.g., MSCs differentiating into fibroblasts). In some cases, especially in ESC or keratinocyte cultures, supporting feeder layer cells must be used to provide biochemical signal cues. For example, a skin substitute developed by Genzyme (MA, USA; Epicel®) employs mouse fibroblasts as supporting cells for keratinocyte cultures. However, the separation of these two different cell types requires additional equipment and, from an immunological stand point, this strategy does not appear to be safe in clinical applications. Matrigel, a basal lamina ECM mix from murine origin, is now routinely used in stem cell culture; however, it is poorly defined and from an animal source. Although several protocols have been assessed for feeder-free culturing of diverse types of stem cells [64], the coculture procedure is sometimes utilized because it significantly improves the identification of what actually organizes a stem cell native niche [65]. In this context, chemically cross-linked feeder cells (i.e., chemical fixation technique) have been fabricated as culture substrates for stem cells which could reduce significant time and effort in feeder cell preparation [66]. Based on research carried out in 1995 [67], chemically cross-linked donor cells were employed in order to investigate the biological effects of membrane-anchored EGF on the acceptor cells. Further studies have revealed keratinocyte growth-promoting activity on glutaraldehyde-fixed fibroblast feeder cells [68]. Accordingly, chemically cross-linked feeder cells can be utilized to maintain the undifferentiated state of stem cells. In this respect, chemically cross-linked human stromal cells have been applied as *ex vivo* supporter for expansion of the human cord blood hematopoietic progenitor cells [69]. Moreover, the process of decellularizing tissues is another important consideration for tissue engineering, because it retains the physical complexity of the ECM thus presenting essential information to diverse cells. With regard to efficiency of decellularized ECMs on cell commitment, a specific decellularized ECM derived from mouse embryonic fibroblasts have been shown to maintain human ES cells in serum-free medium [70]. Although many studies have been conducted recently, we are still far from suggesting an appropriate cell culture substrate for *in vitro* use. These results suggest that, biomimicked surfaces such as cell-formed ECM-derived substrates or decellularized ECMs, can be recognized as potential alternatives to maintain the undifferentiated state and/or enhance the differentiation induction of stem cells with a normal karyotype.

## The 'triangle' of control

### Effects of chemical properties

Chemically inductive molecules have been shown to control cell behavior, reprograming cells and directing their differentiation both *in vitro* and *in vivo* [71–73]. These molecules can be incorporated into the scaffold structure or delivered to the substrate for retaining or reprograming cell fate [74]. Other signals that are important in controlling cell behavior include metal ions, inorganic substances, organic molecules and other physicochemical factors such as temperature and pH. For example, hydroxyapatite is a well-known calcium phosphate derivative that naturally contains variable amounts of impurities that affect its physicochemical properties [75,76]. Elements such as magnesium, fluoride or strontium can be introduced into hydroxyapatite, and their presence shown to influence cell behavior [77–79], for example, fluoride increases osteoblast proliferation and alkaline phosphatase secretion (which is an osteoblastic bone formation indicator) [80]. It is thought that these ions may affect the protein adsorption profile and cell attachment properties of the hydroxyapatite [81].

Cell-matrix adhesions and interfacial interactions between cells and substrates are important for cell functions [82,83]. Some of the most important molecules in natural ECMs are polypeptides (such as collagen, elastin, laminin and fibronectin) and polysaccharides, (including chondroitin sulfate, heparan sulfate and hyaluronic acid). Using these components in the fabrication of artificial ECMs is a very promising method to mimic the biochemical properties of the natural ECM. Although these strategies may not preserve the biochemical activity of pure ECM components, promising results in different cases have been reported [10,84–86]. Other natural or artificial polymers, such as silk, spider silk [87], chitosan or polyurethanes, may be used to regulate stem cell behavior. For instance, a blend composed of silk and cellulose (25:75) can induce chondrogenesis in MSCs [88]. Polymer networks composed of silk fibroin for wound dressing applications showed noncytotoxic effects when evaluated by cell proliferation methods [89] and specifically, they promoted the maintenance of soft tissue *in vivo* by providing longer-term structural integrity [90]. Several peptide sequences, such as RGD, IKVAV and YIGSR, found in ECM proteins are recognized as cell mediators; surfaces can, for example, be chemically modified with RGD to enhance cell attachment or alter cell morphology in addition to promoting osteogenesis or chondrogenesis [14,91]. These peptides can directly regulate stem cell functions through interactions between adhesions molecules such as integrins on the cell surface and ligands on ECM molecules [92].

Surface modification of cell culture substrates can alter cell attachment, morphology, proliferation and gene expression. For example, differences in cell morphology and spatial distribution of fibronectin or vitro-

nectin were reported on glass substrates modified with carboxyl and amine functional groups [93]. Curran *et al.* [94] developed silane-modified surfaces with different functional groups, including methyl ($-CH_3$), amino ($-NH_2$), silane (–SH), hydroxyl (–OH) and carboxyl (–COOH), to evaluate MSC differentiation. Osteogenicity on amine and silane-modified surfaces and chondrogenicity on hydroxyl and carboxyl-modified surfaces were reported, whereas the MSC phenotype was maintained on methyl-modified surfaces. Carboxylic acid functional groups abundant in cartilage glycosaminoglycan, phosphate groups prevalent in mineralized bone and hydrophobic groups such as t-butyl that are found in lipids were, respectively, chosen to induce chondrogenesis, osteogenesis and adipogenesis in functionalized PEG encapsulated MSCs, respectively [95]. The differentiation of bone marrow derived MSCs was investigated on a variety of silane modified surfaces *in vitro* [96].

Additionally, the surface hydrophobic/hydrophilic properties of substrates play an important role on the kinetics of protein adsorption and their folded conformation, which in turn could influence cell activities [97,98]. Interestingly, the hydrophilic properties of the substrate can also have an impact on apoptosis, while a reduced expression of proinflammatory cytokines have been demonstrated on hydrophilic surfaces [99]. A recent study has demonstrated that small changes in matrix hydrophobicity, a result of adding or removing of $CH_2$ groups, can modify cell–matrix interactions and subsequently have a profound influence on various cellular behaviors (e.g., adhesion, motility, shape, cytoskeletal organization and differentiation fate) [100]. As a consequence, it can be concluded that the chemical aspect of cell/stem cell–material interactions is a crucial direction for biological studies and applications. Additionally, surface chemistry analysis and chemical modification processes should be considered in order to design 3D functional biointerfaces that appropriately control different biological phenomena (e.g., cell adhesion, migration, recognition and uptake, among others).

## Effects of physical & mechanical properties

Considered to be a supporting structure, in addition to chemical cues the ECM provides physical and mechanical signals for cell functions, including adhesion, migration and differentiation [21,101,102]. ECM physical cues can be mimicked by using, for example, porous and/or fibrillar structures with dimensions similar to the natural ECM. For example, the potential of bioinspired nanofibers based on polyvinyl alcohol-chondroitin sulfate for the differentiation of MSCs has been recently investigated [103]. Proteins are adsorbed to these fibrous structures in a different profile compared with nonfibrous substrates [104]. As a result, the attachment, spreading and migration of cells were altered, demonstrating how the physical architecture of fibrous meshes, such as their diameter or porosity (or bead formation), affect protein adsorption and cell fate [105]. Studies show that different distributions of fiber diameters in tissues containing fibrils are related to the ages of those tissues or the types of mechanical loading [106,107]. For example, characterization of the fibrous structure of aortic heart valve leaflets showed regional differences in the hinge (rectilinear patterns) and belly (radially oriented striped pattern) parts [108] and Abrams *et al.* [109] showed that fibrous features on the human corneal basement membrane varied from 22 to 191 nm in diameter. A technique that can be used to fabricate fibers is electrospinning; however, its application is limited due to several drawbacks, including: denaturation of natural proteins (e.g., collagen) by organic solvents (e.g., hexafluoro-2-propanol) during electrospinning [110] and the formation of fibers with larger diameters than native tissues, which also limit porosity [111]. Scaffold porosity is considered to be another important physical cue that can be adjusted to resemble the natural ECM. For example, accordion honeycomb scaffolds were fabricated with varying anisotropy for myocardial repair applications [112].

It is desirable to produce substrates with mechanical properties that mimic those of the ECM. However, due to the heterogeneity of mechanical properties between species, tissues and even tissue ages in the same person, it is extremely difficult to predict the viscoelasticity of the cellular environment. Preliminary studies (in the 1920s) confirmed that cells cultured in clots with different stiffnesses showed dissimilar shapes [113]. It is now commonly accepted that cells sense and respond to the stiffness of their environment [114,115]. Substrate mechanical properties are important for the regulation of cell shape, growth and even cell survival [116,117]. Reduced cell spreading and higher motility rates have been observed in cells cultured on flexible substrates compared with rigid ones [118]. Stroka *et al.* [119] showed that the motility of neutrophils decreased with increasing surface rigidity due to the stronger attachments formed. It can be concluded that matrix elasticity can initially trigger the induction of differentiation in stem cells but is likely to be insufficient to complete terminal differentiation [120]. Trapmann *et al.* [12] cultured stem cells on polydimethylsiloxane and polyacrylamide hydrogel surfaces with different stiffnesses (0.1 kPa–2.3 MPa). They found that cell spreading and differentiation was modulated by the elastic modulus of polyacrylamide.

Although the outcome of recent studies clarifies the ability of elasticity to affect cell differentiation, more studies on chemically similar structures with different elasticities must be performed. Other research has shown that myogenic markers of MSCs were upregulated when cells were grown on softer gels coated with type I collagen that simulated muscle elasticity; conversely, stem cells showed osteogenic differentiation when grown on more rigid gels [19]. It should be mentioned that polydimethylsiloxane is broadly known as a suitable substrate for cell growth and proliferation, due to its biocompatibility, mechanical stability and also nontoxicity [121–123]. This influence of the hardness or softness of matrices has also been shown to affect the differentiation of neuronal stem cells [124]. Blau *et al.* fabricated hydrogels with different rigidities (2, 12 and 42 kPa) by altering the amount of PEG in the hydrogel structure. The self-renewal potential of muscle stem cells *in vitro* depended on the elasticity of the substrate, and these cells retained their regenerative potential after transplantation [125]. In addition to MSCs, ESC differentiation can be influenced depending on how tight or loose adhesions are, which in turn dependent on surface stiffness. ESCs were found to respond to stiffness on polyelectrolyte films; poly(L-lysine) and hyaluronan multilayered native films reduced mouse ESC (mESC) proliferation in comparison to highly crosslinked nanofilms; however, the surface stiffness of these nanomaterials did not affect the expression of mESC markers, such as *Nanog*, *Sox2* and *Oct-4*. A low proliferation rate of mESCs has been demonstrated on less stiff polydimethylsiloxane substrates. In addition to the varying expression levels of self-renewal markers, the expression levels of mesodermal markers, such as brachyury and goosecoid, were found to be enhanced on nanofilm substrates [126].

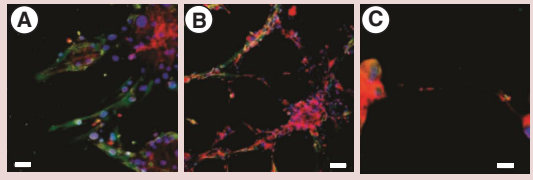



**Figure 2. Spontaneously differentiated neural stem cells analyzed by immunostaining on heat-treated single-walled carbon nanotube/laminin thin films.** Blue color highlights nuclei. **(A)** The presence of neurons (GFAP staining, red; MAP-2 staining, green) in this image provides clues as to which successful neuronal connections could be formed between cells grown on the carbon nanotube substrates and the surrounding tissue. **(B)** GFAP staining, red; nestin staining, green. **(C)** Synapsin staining, red. Synapsin is present in differentiated cells; therefore, its presence confirms that neuronal cell growth and differentiation on the SWNT/laminin thin films results in functional neuronal networks. Scale bars: 2 μm.
Reprinted with permission from [130] © American Chemical Society (2009).

The mechanical profile of bone is well known as an important factor in bone/cartilage tissue engineering. Because of the exceptional mechanical strength of carbon nanotubes and carbon nanofibers, they can be utilized as reinforcing agents in composite materials/scaffolds [127,128]. In one study, biodegradable nanocomposites reinforced by single-wall carbon nanotubes were reported to be lighter, less dense and much stronger than metallic-based bone substitutes, for example, the titanium and stainless steel that are typically used in orthopedics [129]. In another study, laminin-single-wall carbon nanotubes films were shown to have a potential effect on the growth/proliferation of neural stem cells. These thin composite films can serve as biological substrates for promoting cellular adhesion and differentiation (Figure 2) [130]. A recent article by Pulskamp *et al.* [131] demonstrated that human cells treated with commercial carbon nanotube (CNT) products revealed no acute toxicity on viability, which was performed with different assays (WST-1, PI-staining) and also, none of them induced inflammatory mediators. On the other side, some reports showed inconsistency between research findings on the effects of both refined and raw CNTs on mice lungs [132,133]. These findings illustrate the difficulty of assessing toxicity of CNTs due to their agglomerative features in aqueous solutions and consequently, mechanical blockage of airways is the main result of mortality [134].

From another point of view, mechanical properties are thought to be important in cancer research; studies have been reported that tumors are stiffer than normal tissue and may be detected by physical palpation [135]. This rigidity is reported to have an inductive effect on the invasiveness of tumor cells [136]. Although the physical mechanisms employed by metastatic cancer cells in their functions (migration) and gene expression are poorly understood, studies in this field can be helpful for the development of new therapies that target tumor ECM and for understanding how normal tissue differentiation is achieved [135,137,138].

The lack of appropriate 3D models is a hindrance in tissue engineering, largely a result of 2D *in vitro* culture methods providing different conditions for cells in comparison to *in vivo* conditions. An increased number of actin-myosin fibers as a result of cell culture on tissue culture polystyrene has been reported by Discher *et al.* [139]. Alternatives to 2D culture systems, which more closely resemble the nat-

ural 3D architecture of tissues are being sought. For example, a simple protocol was proposed by Fischer *et al.* [140] to produce 3D structures with stiffness similar to the ECM, in which the cells are sandwiched between a 3D fibrillar ECM and a polyacrylamide gel [141]. In summary, physico-mechanical properties such as stiffness, elastic and viscous moduli should be precisely tuned to entirely mimic biological tissues. In particular, in native structures (i.e., surrounding matrix) some important biological variables could be affected by the complexities of weak or strong cues such as: confinement/nonconfinement of the environment, softness and/or rigidity of the ECM and also continuity/discontinuity of the targeted ECM.

## Effects of topography

More recently the effects of surface topography on cell behaviors have been investigated [142–148]. With reference to a comprehensive review by Langer *et al.,* [149] topographies can be used as important signaling modalities to control cell fate and can be designed to mimic the structure of natural ECM (see Figure 3).

Substrates with microscale patterning of greater than 100 µm can be utilized for colony shape-induced differentiation [150]. Khademhosseini *et al.*[151] manufactured concave microwell arrays with widths between 200 and 1000 µm, with greater neuronal and cardiomyocyte differentiation observed in ESC colonies grown on larger microwells. Other reports also suggest that the differentiation of embryonic stem cells can be regulated by the size of the suspended aggregates (i.e., embryoid bodies) [152]. For example, the gene and protein expression levels of ectodermal markers were increased in aggregates with an initial microwell size of 100 µm in comparison to 500 µm, in which higher levels of mesodermal and endodermal markers were observed [152]. When the aggregate size was constrained to 200 µm, cardiac differentiation was enhanced [153]. Tissue culture plates could be produced with these well-like topographies to control the proliferation or differentiation of ESCs. Features with dimensions greater than 100 µm are well suited for aggregations, whereas single cells can sense smaller micro- or nanoscale topographies and respond to the shape (ridge, groove and pillar) of surface features.

Surface roughness is known to impact cell behaviors. Studies on silicon substrates with different roughness and similar surface energies demonstrated that moderately rough substrates could boost cell proliferation [154]. Vandrovcova *et al.* [155] reported a larger spreading area and a reduced rate of proliferation for osteosarcoma cells cultured on $TiO_2$ films with increased surface roughness. In addition, collagen accumulation and/or related gene expression were positively modulated in MSCs cultured on a titanium rough surface [156]. In another study, enhancement in the activation of Wnt/β-catenin signaling in cells seeded on titanium surfaces (i.e., rough surface topography) was observed [157]. Human ESCs (hESCs) have been observed to exhibit spontaneous differentiation on vitronectin-coated nanorough glass surfaces. In this experiment, hESCs that adhered selectively to smooth surface fragments showed high expression levels of OCT-4 (a self-renewal marker) with enhanced proliferation rates, whereas the nanorough surfaces tended to induce hESCs to spontaneously differentiate. A mouse embryonic fibroblast-conditioned media supplemented with a specific synthetic polymer coating of poly(2-(methacryloyloxy)-ethyl dimethyl-(3-sulfopropyl) ammonium hydroxide) (PMEDSAH) could maintain the proliferation/pluripotency of hESCs when cultured on roughened surfaces (Figure 4) [20].

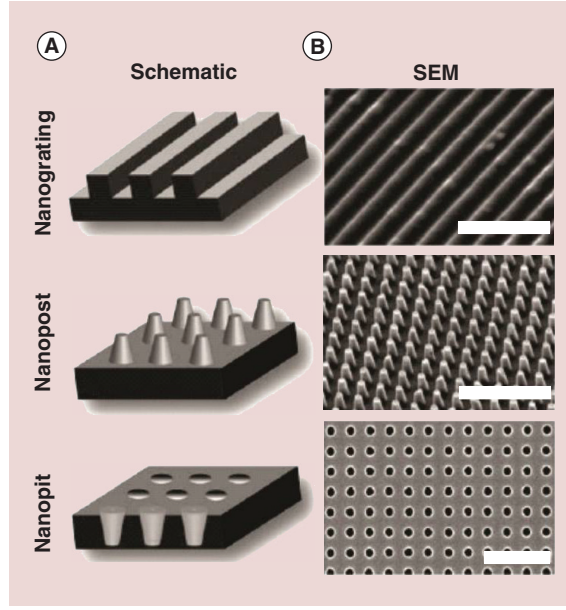



**Figure 3. Three main nanotopographic geometries.**
Schematic illustrations **(A)** and SEM images **(B)** of topography geometries, specifically gratings (scale bar 5 mm), postarrays (scale bar 5 mm) and nanopit arrays (scale bar 1 mm). Schematics are not drawn to scale.
SEM: Scanning electron microscope.
Reprinted with permission from [149] © John Wiley and Sons (2009).

Cells can be embedded in structures formed by micron scaled columns, pits, grooves or pillars, and their function changes according to these topographic configurations. Migration is also effected by micron scale topographies, for example the migration rate of fibroblasts was increased when cultured on a microscale pillar patterned structure compared with a flat surface [158]. It was proposed that the strength of local adhesion and contraction was regulated by increasing the sur-

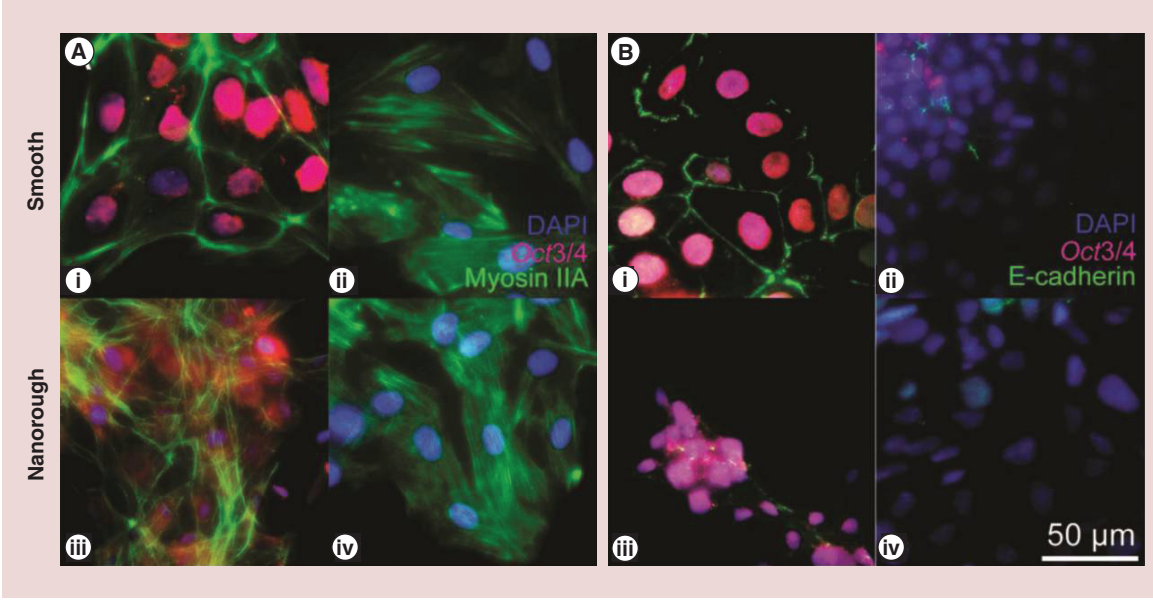



**Figure 4. Assessment of nonmuscle myosin IIA and E-cadherin expressions in human embryonic stem cells cultivated on different glass substrates.** Illustration of immunofluorescence images of Oct3/4+ **(i & iii)** and Oct3/4- **(ii & iv)** human embryonic stem cells expanded on smooth ($R_q$ =1 nm) or nanorough ($R_q$ = 100 nm) glass surfaces after 48 h culture. Cells were stained for nuclei (DAPI; blue), Oct3/4 (red) and myosin IIA **([A]**: green) or E-cadherin **([B]**: green).
Reprinted with permission from [20] © American Chemical Society (2012).

face contact area [158]. Nanoscaled topographies have also been shown to regulate cell behaviors, for example, a wide range of cell types, including nerve cells and MSCs, respond to both nano- and micro-scaled grooved substrates by elongating in the direction of the groove [159]. Alterations in focal adhesion molecules and cytoskeletal organization of cells cultured on different nanotopographies has been observed by Yim *et al.* [160]. Bucaro *et al.* [161] reported altered mouse ESC morphology when cultured on different patterned silicon nanopillars (diameter of 400 nm, length of 5 µm). The cells showed spindle-like morphology on surfaces with lower interpillar spacing (0.8–1.25 µm) but appeared spherical on surfaces with higher spacing (1.5–2.5 µm). Improved alignment and elongation of hESCs when cultured on poly(dimethyl siloxane) substrates with nanoscale line-gratings further confirm the effects (i.e., geometry and dimensions) of surface topography on ESC behaviors [162]. Additionally, vertically aligned $TiO_2$ nanotubes with diameters of 15 nm are able to increase MSC differentiation into the osteogenic lineage (see Figure 5) [163]. In human MSCs, 100 nm diameter $TiO_2$ nanotubes were seen to be more osteoinductive [164]. In the presence of osteogenic supplements in the medium, mouse ESCs cultured on randomly oriented fibrous scaffolds showed higher degrees of osteogenic differentiation compared with flat films [165]. Electrospun nanofibers (both random and aligned) displayed an inductive potential for stem cells to differentiate into specific neural lineages and initiate neurite outgrowth [166,167]. For instance, multiwalled carbon nanofibers can induce osteoblast proliferation compared with flat glass surfaces [168]. Additionally, these nanofibers can increase alkaline phosphatase activity, indicating osteoblastic bone formation [169].

As a concluding point, the biological response of stem cells to nanotopographical stimuli is the result of complex mechanotransduction pathways that accommodate new loading settings from the environment. Mechanotransduction is a specific process whereby physical cues, such as topography and rigidity, exert forces on cells that can alter biochemical signaling and also induce adaptive cellular functional changes. In this respect, some cues that originated from cell–substrate interactions can directly transduce to the nucleus which in turn may effect the cell phenotype. For instance, it has been demonstrated that β-catenin signaling is involved in topography induced mechanotransduction [170]. These findings further define 'complicated topography' as a new benchmark for optimizing the growth of stem cell-based engineered tissues [171].

## Nanoscale engineering: toward niche manipulation

ECM elements of the niche have diverse but specific properties, for example, surface chemistry and

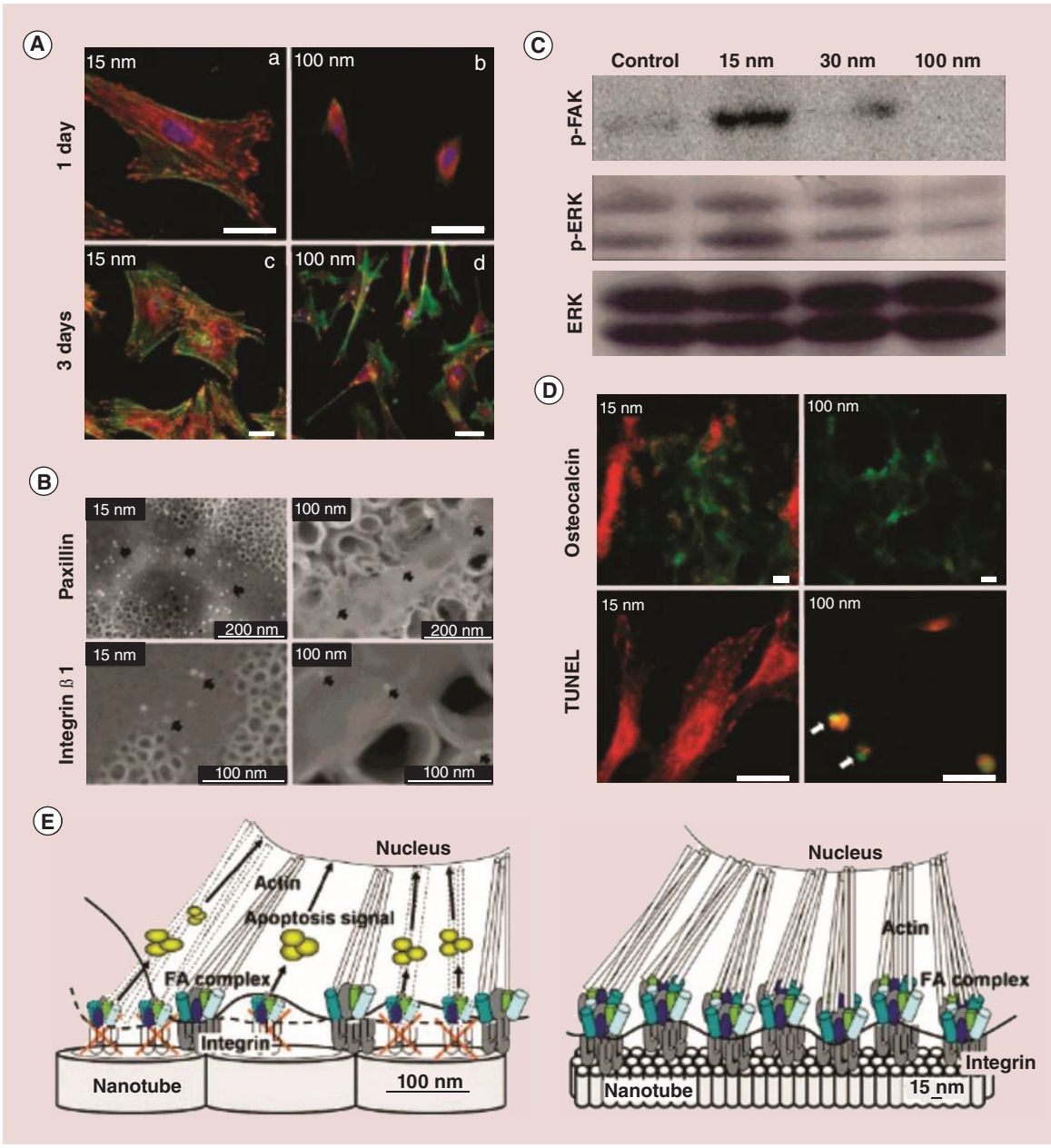



**Figure 5. Focal contact formation, apoptosis, and differentiation of mesenchymal stem cells on 15- and 100-nm nanotubes.** **(A)** Focal contact formation and stress fiber assembly was apparent 1 and 3 days after plating on 15-nm nanotubes **(A & C)** but was reduced on 100-nm nanotubes **(B & D)**. Antipaxillin staining in red: **(A–D)**; antiactin staining in green, and DAPI nuclear staining in blue. **(B)** Scanning electron microscope micrographs (immunogold staining with paxillin and β1-integrin antibodies) were used for analyzing the focal contacts, which revealed dense packing of paxillin in focal contacts on 15-nm tubes. **(C)** Analysis of FAK and ERK phosphorylation confirmed the high extent of focal contact signaling on nanotubes smaller than 30-nm compared with 100-nm nanotubes. **(D)** Osteogenic differentiation occurred on 15-nm nanotubes as observed by osteocalcin staining (red, upper panels) but was infrequently detectable on 100-nm nanotubes. **(E)** Hypothetical model demonstrating the lateral spacing of focal contacts on nanotubes with different diameters. A 15-nm spacing was optimal for integrin assembly into focal contacts, inducing the assembly of actin filaments and signaling to the nucleus, but diameters greater than 70 nm could not support focal contact formation, resulting in apoptosis.
Reprinted with permission from [163] © American Chemical Society (2007).

topography with nanoscale features that present cues to guide cell behavior/fate [8,28]. Nanotopographical features inside the niche (e.g., pores, whorls, pits, ridges and grooves) and their symmetry are important factors affecting the differentiation of stem cells toward specific lineages. For instance, highly ordered

nanopits can maintain MSC multipotency [36,172]. However, add in just a small amount of disorder to the pattern and MSCs are stimulated to form osteoblasts [173]. ESCs have been seen to respond to similar disordered nanopits by differentiating along the mesodermal lineage [174]; they have also been shown to have enhanced self-renewal on highly ordered surfaces [175] and other reports illustrate similar findings [20,176]. Artificial nanoscale banding patterns have been shown as stimulatory; MSCs cultured on nanohelices with a band pattern similar to collagen (~67 nm) form osteoblasts; however, if banding periodicity is increased to a less physiologically relevant approximately 100 nm, this is not observed [177]. These examples illustrate how both embryonic and adult stem cells show unique differentiation behavior in response to nanoscale features. We note that proteins adsorb to materials, both *in vivo* and *in vitro*, and that this is important as cells do not interact directly with materials [178]. Materials can be used to organize the nanonetworks of proteins, for example, to enhance cell binding through opening of fibronectin binding sites [179], and this can cause changes in cell response [180].

In a different kind of study of this phenomenon, the aggregation of embryoid bodies was observed with no adverse effects after the penetration and accumulation of poly(lactic-co-glycolic acid) NPs in human ESC colonies [181]. In another report, the proteins fibronectin and E-cadherin-Fc were embedded in carbonate apatite NPs to accelerate transgene expression in ESCs upon nonviral transgene delivery [182,183]. Biodegradable NPs were used to increase transfection efficiency of plasmid DNA incorporated in poly(β-amino esters) and were shown to maintain hESC viability and pluripotency following transfection [184]. More recently, polyamidoamine dendrimer-modified magnetic NPs have been demonstrated to increase the delivery of lentivirus expression plasmids for reprograming of human dermal fibroblasts to iPSCs [185].

A commercial 3D nanofibrillar scaffold (applied on coverslips) called Ultra-Web has been shown to produce larger mESC colonies with enhanced proliferation characteristics. This is important because developing artificial proliferation-promoting scaffolds is one of the key strategies to providing nanomaterial-niche composites. The Ultra-web nanofibers were prepared to reconstruct the fibrous network of the ECM, which improves stem cell attachment and transplantation efficiency [186]. Numerous features make nanofibrillar constructs distinctive, such as large surface area, tailored chemistry and the high density of epitopes achievable [187]. Consequently, the chemical, mechanical and 3D properties of these fibrous scaffolds may be able to influence the activation of different signaling pathways and control cell fate and differentiation.

### Effects of topography on protein attachment

During *in vitro* culture, cells are suspended in a liquid medium containing serum proteins. Most cells require a few hours for attachment to the substrate and this attachment is affected by serum protein adsorption to the substrate. It seems that surface topography indirectly influences the composition, orientation or conformation of adsorbed serum proteins and thus can also influence cell adhesion. The exact mechanisms and general trends of protein activity and adsorption on such surface features are not well understood [188]. Probably because of the larger number of possible adsorption-promoting interactions, surfaces with a hydrophobic nature are more frequently used in protein adsorption studies compared with hydrophilic surfaces [189,190]. For instance, albumin protein strongly adsorbs on hydrophobic self-assembled monolayers [191]. Most *in vitro* studies determined a favorable cellular response to charged and hydrophilic surfaces [178]. It should be noted that some studies report no alteration in protein adsorption on different surface topographies [192]. Table 1 provides a brief summary of reported alterations in protein adsorption according to topographical features [188].

## Synthetic nanoenvironments & artificial niches

Advanced nanomaterials with nanospecific features, such as nanofilms, nanotubes, nanofibers and bioscaffolds, can be produced by a range of techniques, for example, chemical/physical/electrochemical vapor deposition, electrospinning, electron-beam lithography, dip-pen nanolithography and electrohydrodynamic lithography [42,201–203]. As previously described, nanotechnological approaches can provide surfaces that mimic natural ECM features, which are also on the nanometer scale. This approach has specific potential for the manufacture of an environment for stem cells and adult cells. For example, graphene-coated glass surfaces can restrict the loss of *Nanog* and *Oct4* expression in mouse iPSCs without the need for any chemical reagents such as leukemia inhibitory factor (LIF) [204]. Recently, Mahmoudi *et al.* [22] fabricated smart nanoenvironments using soft lithography, whereby *in vitro* cell cultures were used as templates for the transfer of cell-imprinted patterns into artificial polymer substrates. The imprinted substrates were used to induce stem cell differentiation toward

Table 1. A brief list of reports describing the effects of different topographical features on protein adsorption.

| Nanotopographic feature | Fabrication technique and/or material | Feature dimensions (nm) | Protein effect | Ref. |
|---|---|---|---|---|
| Stochastic roughness | Oblique angle deposition, tantalum | 12–44 | Fibronectin adsorption increased with increasing surface roughness, however, conformational change was increased | [193] |
| | Colloidal silica particle | 7–11 | Less fibrinogen adsorption on 11-nm rough surfaces compared with other surfaces Fibronectin conformation changed on nanorough surfaces compared with flat | [194] |
| | Polymer de-mixing, alumina | 32 | Increased unfolding of vitronectin compared with conventional alumina (mean roughness 16 nm) | [195] |
| Particles | Silica | Diameter less than 20 | Lysozyme adsorbed in a native conformation | [196] |
| Grooves | Electron beam lithography, silicon | Spacing 90 | Fibronectin conformation altered on grooved substrates as determined by reduced osteoblast adhesion compared with planar controls | [197] |
| Pits | Colloidal lithography, titanium | Diameter 40, depth 10 | Fibrinogen conformation altered on nanopits as determined by platelet adhesion | [198] |
| Nanoporous surface | β-type Ti–25Nb–25Zr alloy | Pore size <15 | Surface nanotopography did not alter the surface roughness or hydrophilicity of the Ti25Nb25Zr alloy but was capable of inducing biological responses, such as, protein adsorption, cell adhesion, cell migration, cell proliferation and cell mineralization | [199] |
| Average surface roughness | Solution casting, β-phase PVDF | 50–300 | Increased fibronectin adsorption toward the cell–material interface was demonstrated on β-phase PVDF films | [200] |

PVDF: Poly(vinylidene fluoride).
Reprinted with permission from [188] © Elsevier (2010).

desired matured cell types, which had themselves been used as templates for the cell-imprinting. These results suggest that the dynamic plasma membrane of cultivated stem cells is capable of adopting the shape of matured cells imprinted in artificial polymer substrates [205].

## Conclusion & future perspective

Despite extensive ongoing studies to design biomimetic materials for regenerative medicine, few biofunctionalized biomaterials have been successfully translated into the clinical setting. The complete mechanisms governing the regulation of stem cell differentiation into specific desired lineages remain unclear due to the difficulty of reproducing a complicated biological microenvironment and the regulatory factors of tissue repair. In this regard, stem cell culture materials need to be equipped with physicochemical inductive cues to completely control the diverse phases of healing. Many innovative efforts have been made to enhance the efficacy of products for tissue regeneration. However, the lack of success thus far emphasizes the importance of engineered biomaterials to provide numerous signals in concert. These 'bioinspired' platforms can be effectively tuned to direct stem cell fate according to a defined application. Continued advances in the design of sophisticated devices will lead to improvements in patient care and quality of life. Therefore, in the future, highly integrated nano/microdevices will find increasing use in biomedical and pharmaceutical activities.

### Financial & competing interests disclosure

L-A Turner and MJ Dalby are funded by the BBSRC and BBSRC, MRC and EPSRC, respectively. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

## Executive summary

**Stem cells & their niches**

- The native biological stem cell niche is a multidimensional nano- to micro-scale environment, which supplies both chemical and physical guidance to control stem cell quiescence, proliferation and development.
- Different cues instruct stem cells under diverse physiological environments.
- Numerous mammalian niches have been studied (e.g., skin, intestine and bone marrow).

**Cell–substrate signaling at the nanoscale**

- Cell adhesions transmit external mechanical forces intracellularly and potentially mediate cell signaling effecting behaviors such as cell shape, motility and differentiation.
- One type of cell–substrate adhesion is the focal adhesion; FAK, paxillin and talin are important components and play a role in stem cell responses to substrate topography.

**Limitations for chemical factors**

- *In vivo* translation of chemically controlled cell behaviors may present problems, for example, feeder cells can spread viruses and other unsafe infectious agents to the culture.
- Topography and elasticity are other principal factors influencing stem cell fate, making a 'triangle' of control.

**Synthetic substrates & artificial niches: the three generations of biomaterials**

- The evolution of biomaterials has been described as falling into three different generations: first generation (structural), second generation (bioactive) and third generation (reproducible molecular control of cells).
- A combination of cells, material(s) and physico-chemical factors have emerged in the tissue engineering field as crucial in the bodys healing process. Thus the chemical and/or physico-mechanical properties of synthetic substrates should be mimetically correlated with the native environment of the cell.

**The 'triangle' of control: effects of chemical properties**

- Chemically inductive molecules have been shown to regulate cell behavior, inducing their differentiation and could affect the reprogramming process.
- Other important signals in controlling the cell behavior include: metal ions, inorganic substances, organic molecules, temperature and pH.
- Modified surfaces on cell culture substrates can affect cell attachment, morphology and proliferation (e.g., substrates modified with carboxyl and amine functional groups have altered the spatial distribution of fibronectin or vitronectin).
- Cell-matrix adhesions and interfacial interactions between cells and natural/synthetic substrates are important factors in cell function.

**The 'triangle' of control: effects of physical & mechanical properties**

- Extracellular matrix (ECM) physical and mechanical cues can be imitated using porous and/or fibrillar structures with dimensions similar to the natural ECM.
- Physical architecture of fibrous meshes (e.g., diameter or porosity) can effect protein adsorption and cellular fate.
- Scaffold porosity is considered to be an important physical cue which can be adjusted to mimic the natural ECM.
- One well-known example where the mechanical profile of the tissue is known to be important is in bone/cartilage tissue engineering.

**The 'triangle' of control: effects of topography**

- Topography can be used as a signaling modality in order to control cell fate.
- It has been demonstrated that moderately rough silicon substrates can boost cell proliferation.
- Migration can be stimulated by micron scale topographies.
- A wide range of cell types, such as nerve cells and mesenchymal stem cells, respond to nano-to-micro-scaled grooved substrates by extending in the direction of the groove.

**Nanoscale engineering: toward niche manipulation**

- Biomaterials can be manipulated in order to organize the nanonetworks of diverse proteins, for example, to improve cell binding through opening of fibronectin binding sites thus altering cell responses to the substrate.

**Effects of topography on protein attachment**

- Surface topography can influence cell adhesion by indirectly affecting the composition, orientation and conformation of adsorbed serum or plasma proteins.

**Synthetic nanoenvironments & artificial niches**

- Nanomaterials with nanospecific features (e.g., nanofilms, nanotubes, nanofibers and bioscaffolds) can be fabricated by a range of methodologies such as lithography-based approaches, chemical/physical/electrochemical vapor deposition and electrospinning.

## References


Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Shi J, Votruba AR, Farokhzad OC, Langer R. Nanotechnology in drug delivery and tissue engineering: from discovery to applications. *Nano Lett.* 10(9), 3223–3230 (2010).

2 Petros RA, Desimone JM. Strategies in the design of nanoparticles for therapeutic applications. *Nat. Rev. Drug Discov.* 9(8), 615–627 (2010).

3 Serpooshan V, Zhao M, Metzler SA *et al.* The effect of bioengineered acellular collagen patch on cardiac remodeling and ventricular function post myocardial infarction. *Biomaterials* 34(36), 9048–9055 (2013).

4 Badylak SF. Decellularized allogeneic and xenogeneic tissue as a bioscaffold for regenerative medicine: factors that influence the host response. *Ann. Biomed. Eng.* 42 (7), 1517–1527 (2014).

5 Jungebluth P, Alici E, Baiguera S *et al.* Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study. *Lancet* 378(9808), 1997–2004 (2011).

6 Caiazzo M, Dell'anno MT, Dvoretskova E *et al.* Direct generation of functional dopaminergic neurons from mouse and human fibroblasts. *Nature* 476(7359), 224–227 (2011).

7 Hubbell JA, Chilkoti A. Nanomaterials for drug delivery. *Science* 337(6092), 303–305 (2012).

8 Ranga A, Gjorevski N, Lutolf MP. Drug discovery through stem cell-based organoid models. *Adv. Drug Deliv. Rev.* 69–70, 19–28 (2014).

9 Khademhosseini A, Vacanti J, Langer R. Progress in tissue engineering. *Sci. Am.* 300(5), 64–71 (2009).

10 Higuchi A, Ling Q-D, Hsu S-T, Umezawa A. Biomimetic cell culture proteins as extracellular matrices for stem cell differentiation. *Chem. Rev.* 112(8), 4507–4540 (2012).

11 Goodridge HS. Induced pluripotent stem cell derived myeloid phagocytes: disease modeling and therapeutic applications. *Drug Discov. Today* 19(6), 774–780 (2014).

12 Trappmann B, Gautrot JE, Connelly JT *et al.* Extracellular-matrix tethering regulates stem-cell fate. *Nat. Mater.* 11(7), 642–649 (2012).

13 Nakagawa M, Koyanagi M, Tanabe K *et al.* Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat. Biotechnol.* 26(1), 101–106 (2007).

14 Marklein RA, Burdick JA. Controlling stem cell fate with material design. *Adv. Mater.* 22(2), 175–189 (2010).

15 Mihaylova MM, Sabatini DM, Yilmaz ÖH. Dietary and metabolic control of stem cell function in physiology and cancer. *Cell Stem Cell* 14(3), 292–305 (2014).

16 Alberti K, Davey RE, Onishi K *et al.* Functional immobilization of signaling proteins enables control of stem cell fate. *Nat. Methods* 5(7), 645–650 (2008).

17 Higuchi A, Ling Q-D, Chang Y, Hsu S-T, Umezawa A. Physical cues of biomaterials guide stem cell differentiation fate. *Chem. Rev.* 113(5), 3297–3328 (2013).

18 Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. *Nature* 505(7483), 327–334 (2014).

19 Engler AJ, Sen S, Sweeney HL, Discher DE. Matrix elasticity directs stem cell lineage specification. *Cell* 126(4), 677–689 (2006).

20 Chen W, Villa-Diaz LG, Sun Y *et al.* Nanotopography influences adhesion, spreading, and self-renewal of human embryonic stem cells. *ACS Nano* 6(5), 4094–4103 (2012).

21 Guilak F, Cohen DM, Estes BT, Gimble JM, Liedtke W, Chen CS. Control of stem cell fate by physical interactions with the extracellular matrix. *Cell Stem Cell* 5(1), 17–26 (2009).

22 Mahmoudi M, Bonakdar S, Shokrgozar MA *et al.* Cell-imprinted substrates direct the fate of stem cells. *ACS Nano* 7(10), 8379–8384 (2013).

23 Gattazzo F, Urciuolo A, Bonaldo P. Extracellular matrix: a dynamic microenvironment for stem cell niche. *Biochim. Biophys. Acta* 1840(8), 2506–2519 (2014).

24 Kretlow JD, Mikos AG. From material to tissue: biomaterial development, scaffold fabrication, and tissue engineering. *AIChE J.* 54(12), 3048–3067 (2008).

25 Fong EL, Watson BM, Kasper FK, Mikos AG. Building bridges: leveraging interdisciplinary collaborations in the development of biomaterials to meet clinical needs. *Adv. Mater.* 24(36), 4995–5013 (2012).

26 Spradling A, Drummond-Barbosa D, Kai T. Stem cells find their niche. *Nature* 414(6859), 98–104 (2001).

27 Yamashita YM, Fuller MT, Jones DL. Signaling in stem cell niches: lessons from the *Drosophila* germline. *J. Cell Sci.* 118(4), 665–672 (2005).

28 Scadden DT. The stem-cell niche as an entity of action. *Nature* 441(7097), 1075–1079 (2006).

29 Yamashita YM, Tumbar T. Stem cells and their niche in homeostasis/regeneration and disease. *Mol. Biol. Cell* 25(6), 736–736 (2014).

30 Moore KA, Lemischka IR. Stem cells and their niches. *Science* 311(5769), 1880–1885 (2006).

31 Li L, Xie T. Stem cell niche: structure and function. *Annu. Rev. Cell Dev. Biol.* 21, 605–631 (2005).

32 Ehninger A, Trumpp A. The bone marrow stem cell niche grows up: mesenchymal stem cells and macrophages move in. *J. Exp. Med.* 208(3), 421–428 (2011).

33 Bianco P. Bone and the hematopoietic niche: a tale of two stem cells. *Blood* 117(20), 5281–5288 (2011).

34 Adams GB, Chabner KT, Alley IR *et al.* Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. *Nature* 439(7076), 599–603 (2005).

35 Yanes O, Clark J, Wong DM *et al.* Metabolic oxidation regulates embryonic stem cell differentiation. *Nat. Chem. Biol.* 6(6), 411–417 (2010).

36 Mcmurray RJ, Gadegaard N, Tsimbouri PM *et al.* Nanoscale surfaces for the long-term maintenance of mesenchymal stem cell phenotype and multipotency. *Nat. Mater.* 10(8), 637–644 (2011).

37 Reya T, Duncan AW, Ailles L *et al.* A role for Wnt signalling in self-renewal of haematopoietic stem cells. *Nature* 423(6938), 409–414 (2003).

38 Murdoch B, Chadwick K, Martin M *et al.* Wnt-5A augments repopulating capacity and primitive hematopoietic development of human blood stem cells *in vivo*. *Proc. Natl Acad. Sci. USA* 100(6), 3422–3427 (2003).

39 De Haan G, Weersing E, Dontje B *et al. In vitro* generation of long-term repopulating hematopoietic stem cells by fibroblast growth factor-1. *Dev. Cell* 4(2), 241–251 (2003).

40 Lutolf MP, Blau HM. Artificial stem cell niches. *Adv. Mater.* 21(32–33), 3255–3268 (2009).

41 Rice JJ, Martino MM, De Laporte L, Tortelli F, Briquez PS, Hubbell JA. Engineering the regenerative microenvironment with biomaterials. *Adv. Healthc. Mater.* 2(1), 57–71 (2013).

42 Sniadecki NJ, Desai RA, Ruiz SA, Chen CS. Nanotechnology for cell–substrate interactions. *Ann. Biomed. Eng.* 34(1), 59–74 (2006).

43 Seo CH, Furukawa K, Montagne K, Jeong H, Ushida T. The effect of substrate microtopography on focal adhesion maturation and actin organization via the RhoA/ROCK pathway. *Biomaterials* 32(36), 9568–9575 (2011).

44 Ferreira L, Karp JM, Nobre L, Langer R. New opportunities: the use of nanotechnologies to manipulate and track stem cells. *Cell Stem Cell* 3(2), 136–146 (2008).

45 Watt FM, Huck WT. Role of the extracellular matrix in regulating stem cell fate. *Nat. Rev. Mol. Cell Biol.* 14(8), 467–473 (2013).

• **Illustration of environmental (niche) signals that regulate stem cell behavior in order to maximize the efficiency of cell-based therapies.**

46 Teo BKK, Wong ST, Lim CK *et al.* Nanotopography modulates mechanotransduction of stem cell and induces differentiation through focal adhesion kinase. *ACS Nano* 7(6), 4785–4798 (2013).

47 Trappmann B, Chen CS. How cells sense extracellular matrix stiffness: a material's perspective. *Curr. Opin. Biotechnol.* 24(5), 948–953 (2013).

48 Worley K, Certo A, Wan LQ. Geometry–force control of stem cell fate. *BioNanoSci* 3(1), 43–51 (2013).

49 Conti MA, Even-Ram S, Liu C, Yamada KM, Adelstein RS. Defects in cell adhesion and the visceral endoderm following ablation of nonmuscle myosin heavy chain II-A in mice. *J. Biol. Chem.* 279(40), 41263–41266 (2004).

50 Saha K, Keung AJ, Irwin EF *et al.* Substrate modulus directs neural stem cell behavior. *Biophys. J.* 95(9), 4426–4438 (2008).

51 Ponsonnet L, Reybier K, Jaffrezic N *et al.* Relationship between surface properties (roughness, wettability) of titanium and titanium alloys and cell behaviour. *Mat. Sci. Eng.* 23(4), 551–560 (2003).

52 Anselme K, Bigerelle M, Noel B *et al.* Qualitative and quantitative study of human osteoblast adhesion on materials with various surface roughnesses. *J. Biomed. Mat. Res.* 49(2), 155–166 (2000).

53 Ahmad M, Gawronski D, Blum J, Goldberg J, Gronowicz G. Differential response of human osteoblast-like cells to commercially pure (cp) titanium grades 1 and 4. *J. Biomed. Mat. Res.* 46(1), 121–131 (1999).

54 Xu C, Inokuma MS, Denham J *et al.* Feeder-free growth of undifferentiated human embryonic stem cells. *Nat. Biotechnol.* 19(10), 971–974 (2001).

55 Hench LL, Polak JM. Third-generation biomedical materials. *Science* 295(5557), 1014–1017 (2002).

56 Langer R, Vacanti JP. Tissue engineering. *Science* 260(5110), 920–926 (1993).

57 Harrison R, St-Pierre J-P, Stevens M. Tissue engineering and regenerative medicine: a year in review. *Tissue Eng. Part B Rev.* 20(1), 1–16 (2014).

58 Lutolf MP, Gilbert PM, Blau HM. Designing materials to direct stem-cell fate. *Nature* 462(7272), 433–441 (2009).

59 Fisher OZ, Khademhosseini A, Langer R, Peppas NA. Bioinspired materials for controlling stem cell fate. *Acc. Chem. Res.* 43(3), 419–428 (2010).

60 Drury JL, Mooney DJ. Hydrogels for tissue engineering: scaffold design variables and applications. *Biomaterials* 24(24), 4337–4351 (2003).

61 Buwalda SJ, Boere KW, Dijkstra PJ, Feijen J, Vermonden T, Hennink WE. Hydrogels in a historical perspective: from simple networks to smart materials. *J. Control. Release* 190, 254–273 (2014).

62 Miyanishi K, Trindade MC, Lindsey DP *et al.* Effects of hydrostatic pressure and transforming growth factor-β 3 on adult human mesenchymal stem cell chondrogenesis *in vitro*. *Tissue Eng.* 12(6), 1419–1428 (2006).

63 Bosnakovski D, Mizuno M, Kim G, Takagi S, Okumura M, Fujinaga T. Chondrogenic differentiation of bovine bone marrow mesenchymal stem cells (MSCs) in different hydrogels: influence of collagen type II extracellular matrix on MSC chondrogenesis. *Biotechnol. Bioeng.* 93(6), 1152–1163 (2006).

64 Hasegawa Y, Tang D, Takahashi N, Hayashizaki Y, Forrest AR, Suzuki H. CCL2 enhances pluripotency of human induced pluripotent stem cells by activating hypoxia related genes. *Sci. Rep.* 4, 5228 (2014).

65 Sun Y, Chen CS, Fu J. Forcing stem cells to behave: a biophysical perspective of the cellular microenvironment. *Ann. Rev. Biophys.* 41, 519–542 (2012).

66 Perestrelo AR, Grenha A, Rosa Da Costa AM, Belo JA. Locust bean gum as an alternative polymeric coating for embryonic stem cell culture. *Mat. Sci. Eng.* 40, 336–344 (2014).

67 Higashiyama S, Iwamoto R, Goishi K *et al.* The membrane protein CD9/DRAP 27 potentiates the juxtacrine growth factor activity of the membrane-anchored heparin-binding EGF-like growth factor. *J. Cell Biol.* 128(5), 929–938 (1995).

68 Verfaillie C, Catanzaro P. Direct contact with stroma inhibits proliferation of human long-term culture initiating cells. *Leukemia* 10(3), 498–504 (1996).

69 Joddar B, Ito Y. Artificial niche substrates for embryonic and induced pluripotent stem cell cultures. *J. Biotechnol.* 168(2), 218–228 (2013).

70 Klimanskaya I, Chung Y, Meisner L, Johnson J, West MD, Lanza R. Human embryonic stem cells derived without feeder cells. *Lancet* 365(9471), 1636–1641 (2005).

71 Lyssiotis CA, Lairson LL, Boitano AE, Wurdak H, Zhu S, Schultz PG. Chemical control of stem cell fate and developmental potential. *Angew. Chem. Int. Ed. Engl.* 50(1), 200–242 (2011).

72 Madl CM, Mehta M, Duda GN, Heilshorn SC, Mooney DJ. Presentation of BMP-2 mimicking peptides in 3D hydrogels directs cell fate commitment in osteoblasts and mesenchymal stem cells. *Biomacromolecules* 15(2), 445–455 (2014).

73 Kiessling L. Probing and perturbing stem cells with chemical biology. *ACS Chem. Biol.* 9(1), 1–2 (2014).

74 Cha C, Liechty WB, Khademhosseini A, Peppas NA. Designing biomaterials to direct stem cell fate. *ACS Nano* 6(11), 9353–9358 (2012).

75 Perloff A, Posner AS. Preparation of pure hydroxyapatite crystals. *Science* 124(3222), 583 (1956).

76 Moreno EC, Kresak M, Zahradnik RT. Fluoridated hydroxyapatite solubility and caries formation. *Nature* 247(5435), 64–65 (1974).

77 Montazeri L, Javadpour J, Shokrgozar MA, Bonakdar S, Javadian S. Hydrothermal synthesis and characterization of hydroxyapatite and fluorhydroxyapatite nano-size powders. *Biomed. Mater.* 5(4), 045004 (2010).

78 Yan J, Sun J-F, Chu PK, Han Y, Zhang Y-M. Bone integration capability of a series of strontium-containing hydroxyapatite coatings formed by micro-arc oxidation. *J. Biomed. Mat. Res. Part A*, 101(9), 2465–2480 (2013).

79 Bertoni E, Bigi A, Cojazzi G, Gandolfi M, Panzavolta S, Roveri N. Nanocrystals of magnesium and fluoride substituted hydroxyapatite. *J. Inorgan. Biochem.* 72(1–2), 29–35 (1998).

80 Qu H, Wei M. The effect of fluoride contents in fluoridated hydroxyapatite on osteoblast behavior. *Acta Biomater.* 2(1), 113–119 (2006).

81 Montazeri L, Javadpour J, Shokrgozar MA, Bonakdar S, Khayyat Moghaddam M, Asgary V. The interaction of plasma proteins with nano-size fluoride-substituted apatite powders. *Ceramics International* 39(6), 6145–6152 (2013).

82 Khetan S, Guvendiren M, Legant WR, Cohen DM, Chen CS, Burdick JA. Degradation-mediated cellular traction directs stem cell fate in covalently crosslinked three-dimensional hydrogels. *Nat. Mater.* 12(5), 458–465 (2013).

83 Liu X, Wang S. Three-dimensional nano-biointerface as a new platform for guiding cell fate. *Chem. Soc. Rev.* 43(8), 2385–2340 (2014).

84 Shokrgozar MA, Fattahi M, Bonakdar S *et al.* Healing potential of mesenchymal stem cells cultured on a collagen-based scaffold for skin regeneration. *Iran Biomed. J.* 16(2), 68–76 (2012).

85 Reilly GC, Engler AJ. Intrinsic extracellular matrix properties regulate stem cell differentiation. *J. Biomech.* 43(1), 55–62 (2010).

86 Abraham S, Eroshenko N, Rao RR. Role of bioinspired polymers in determination of pluripotent stem cell fate. *Regen. Med.* 4(4), 561–578 (2009).

87 Lewicka M, Hermanson O, Rising AU. Recombinant spider silk matrices for neural stem cell cultures. *Biomaterials* 33(31), 7712–717 (2012).

88 Singh N, Rahatekar SS, Koziol KKK *et al.* Directing chondrogenesis of stem cells with specific blends of cellulose and silk. *Biomacromolecules* 14(5), 1287–1298 (2013).

89 Kweon H, Yeo J-H, Lee K-G *et al.* Semi-interpenetrating polymer networks composed of silk fibroin and poly (ethylene glycol) for wound dressing. *Biomed. Mater.* 3(3), 034115 (2008).

90 Mauney JR, Nguyen T, Gillen K, Kirker-Head C, Gimble JM, Kaplan DL. Engineering adipose-like tissue *in vitro* and *in vivo* utilizing human bone marrow and adipose-derived mesenchymal stem cells with silk fibroin 3D scaffolds. *Biomaterials* 28(35), 5280–5290 (2007).

91 Ruoslahti E, Pierschbacher MD. New perspectives in cell adhesion: RGD and integrins. *Science* 238(4826), 491–497 (1987).

92 Jiang J, Papoutsakis ET. Stem-cell niche based comparative analysis of chemical and nano-mechanical material properties impacting *ex vivo* expansion and differentiation of hematopoietic and mesenchymal stem cells. *Adv. Healthc. Mater.* 2(1), 25–42 (2012).

93 Curran JM, Chen R, Hunt JA. Controlling the phenotype and function of mesenchymal stem cells *in vitro* by adhesion to silane-modified clean glass surfaces. *Biomaterials* 26(34), 7057–7067 (2005).

94 Curran JM, Chen R, Hunt JA. The guidance of human mesenchymal stem cell differentiation *in vitro* by controlled modifications to the cell substrate. *Biomaterials* 27(27), 4783–4793 (2006).

95 Benoit DS, Schwartz MP, Durney AR, Anseth KS. Small functional groups for controlled differentiation of hydrogel-encapsulated human mesenchymal stem cells. *Nat. Mater.* 7(10), 816–823 (2008).

96 Rashidi H, Yang J, Shakesheff KM. Surface engineering of synthetic polymer materials for tissue engineering and regenerative medicine applications. *Biomater. Sci.* 2(10), 1318–1331 (2014).

97 Keselowsky BG, Collard DM, García AJ. Integrin binding specificity regulates biomaterial surface chemistry effects on cell differentiation. *Proc. Natl Acad. Sci. USA* 102(17), 5953–5957 (2005).

98 GarcíA AJ, Vega MaD, Boettiger D. Modulation of cell proliferation and differentiation through substrate-dependent changes in fibronectin conformation. *Mol. Biol. Cell* 10(3), 785–798 (1999).

99 Brodbeck WG, Patel J, Voskerician G *et al.* Biomaterial adherent macrophage apoptosis is increased by hydrophilic and anionic substrates *in vivo*. *Proc. Natl Acad. Sci. USA* 99(16), 10287–10292 (2002).

100 Ayala R, Zhang C, Yang D *et al.* Engineering the cell–material interface for controlling stem cell adhesion, migration, and differentiation. *Biomaterials* 32(15), 3700–3711 (2011).

101 Place ES, Evans ND, Stevens MM. Complexity in biomaterials for tissue engineering. *Nat. Mater.* 8(6), 457–470 (2009).

102 Han YL, Wang S, Zhang X *et al.* Engineering physical microenvironment for stem cell based regenerative medicine. *Drug Discov. Today* 19(6), 763–773 (2014).

103 Coburn JM, Gibson M, Monagle S, Patterson Z, Elisseeff JH. Bioinspired nanofibers support chondrogenesis for articular cartilage repair. *Proc. Natl Acad. Sci. USA* 109(25), 10012–10017 (2012).

104 Woo KM, Chen VJ, Ma PX. Nano-fibrous scaffolding architecture selectively enhances protein adsorption contributing to cell attachment. *J. Biomed. Mater. Res. A* 67(2), 531–537 (2003).

105 Chen VJ, Ma PX. Nano-fibrous poly(L-lactic acid) scaffolds with interconnected spherical macropores. *Biomaterials* 25(11), 2065–2073 (2004).

106 Svensson L, Aszodi A, Reinholt FP, Fassler R, Heinegard D, Oldberg A. Fibromodulin-null mice have abnormal collagen fibrils, tissue organization, and altered lumican deposition in tendon. *J. Biol. Chem.* 274(14), 9636–9647 (1999).

107 Parry DAD, Squire JM. *Fibrous Proteins: Coiled-Coils, Collagen and Elastomers.* Elsevier, CA, USA (2005).

108 Tseng H, Grande-Allen KJ. Elastic fibers in the aortic valve spongiosa: a fresh perspective on its structure and role in overall tissue function. *Acta Biomater.* 7(5), 2101–2108 (2011).

109 Abrams GA, Schaus SS, Goodman SL, Nealey PF, Murphy CJ. Nanoscale topography of the corneal epithelial basement membrane and Descemet's membrane of the human. *Cornea* 19(1), 57–64 (2000).

110 Zeugolis DI, Khew ST, Yew ES *et al.* Electro-spinning of pure collagen nano-fibres: just an expensive way to make gelatin? *Biomaterials* 29(15), 2293–2305 (2008).

111 Schnell E, Klinkhammer K, Balzer S *et al.* Guidance of glial cell migration and axonal growth on electrospun nanofibers of poly-ε-caprolactone and a collagen/poly-ε-caprolactone blend. *Biomaterials* 28(19), 3012–3025 (2007).

112 Engelmayr GC, Cheng M, Bettinger CJ, Borenstein JT, Langer R, Freed LE. Accordion-like honeycombs for tissue engineering of cardiac anisotropy. *Nat. Mater.* 7(12), 1003–1010 (2008).

113 Fletcher DA, Mullins RD. Cell mechanics and the cytoskeleton. *Nature* 463(7280), 485–492 (2010).

114 Discher DE, Janmey P, Wang YL. Tissue cells feel and respond to the stiffness of their substrate. *Science* 310(5751), 1139–1143 (2005).

115 Shah N, Morsi Y, Manasseh R. From mechanical stimulation to biological pathways in the regulation of stem cell fate. *Cell Biochem. Funct.* 32(4), 309–325 (2014).

116 Wang HB, Dembo M, Wang YL. Substrate flexibility regulates growth and apoptosis of normal but not transformed cells. *Am. J. Physiol. Cell Physiol.* 279(5), C1345–C1350 (2000).

117 Vichare S, Sen S, Inamdar MM. Cellular mechanoadaptation to substrate mechanical properties: contributions of substrate stiffness and thickness to cell stiffness measurements using AFM. *Soft Matter* 10(8), 1174–1181 (2014).

118 Pelham RJ, Wang Y-L. Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc. Natl Acad. Sci. USA* 94(25), 13661–13665 (1997).

119 Stroka KM, Aranda-Espinoza H. Neutrophils display biphasic relationship between migration and substrate stiffness. *Cell Motil. Cytoskeleton* 66(6), 328–341 (2009).

120 Even-Ram S, Artym V, Yamada KM. Matrix control of stem cell fate. *Cell* 126(4), 645–647 (2006).

121 Leclerc E, Corlu A, Griscom L, Baudoin R, Legallais C. Guidance of liver and kidney organotypic cultures inside rectangular silicone microchannels. *Biomaterials* 27(22), 4109–4119 (2006).

122 Bani-Yaghoub M, Tremblay R, Voicu R *et al.* Neurogenesis and neuronal communication on micropatterned neurochips. *Biotechnol. Bioeng.* 92(3), 336–345 (2005).

123 Jeon K, Oh H-J, Lim H *et al.* Self-renewal of embryonic stem cells through culture on nanopattern polydimethylsiloxane substrate. *Biomaterials* 33(21), 5206–5220 (2012).

124 Leipzig ND, Shoichet MS. The effect of substrate stiffness on adult neural stem cell behavior. *Biomaterials* 30(36), 6867–6878 (2009).

125 Gilbert PM, Havenstrite KL, Magnusson KE *et al.* Substrate elasticity regulates skeletal muscle stem cell self-renewal in culture. *Science* 329(5995), 1078–1081 (2010).

126 Blin G, Lablack N, Louis-Tisserand M, Nicolas C, Picart C, Pucéat M. Nano-scale control of cellular environment to drive embryonic stem cells selfrenewal and fate. *Biomaterials* 31(7), 1742–1750 (2010).

127 Spadaccio C, Rainer A, Trombetta M *et al.* Poly-L-lactic acid/hydroxyapatite electrospun nanocomposites induce chondrogenic differentiation of human MSC. *Ann. Biomed. Eng.* 37(7), 1376–1389 (2009).

128 Park J, Bauer S, Schlegel KA, Neukam FW, Von Der Mark K, Schmuki P. $TiO_2$ nanotube surfaces: 15 nm – an optimal length scale of surface topography for cell adhesion and differentiation. *Small* 5(6), 666–671 (2009).

129 Shi X, Hudson JL, Spicer PP, Tour JM, Krishnamoorti R, Mikos AG. Injectable nanocomposites of single-walled carbon nanotubes and biodegradable polymers for bone tissue engineering. *Biomacromolecules* 7(7), 2237–2242 (2006).

130 Kam NWS, Jan E, Kotov NA. Electrical stimulation of neural stem cells mediated by humanized carbon nanotube composite made with extracellular matrix protein. *Nano Lett.* 9(1), 273–278 (2008).

131 Pulskamp K, Diabaté S, Krug HF. Carbon nanotubes show no sign of acute toxicity but induce intracellular reactive oxygen species in dependence on contaminants. *Toxicol. Lett.* 168(1), 58–74 (2007).

132 Huczko A, Lange H, Całko E, Grubek-Jaworska H, Droszcz P. Physiological testing of carbon nanotubes: are they asbestos-like? *Fullerene Sci. Technol.* 9(2), 251–254 (2001).

133 Shvedova AA, Kisin ER, Mercer R *et al.* Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 289(5), L698–L708 (2005).

134 Warheit DB, Laurence B, Reed KL, Roach D, Reynolds G, Webb T. Comparative pulmonary toxicity assessment of single-wall carbon nanotubes in rats. *Toxicol. Sci.* 77(1), 117–125 (2004).

135 Paszek MJ, Zahir N, Johnson KR *et al.* Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8(3), 241–254 (2005).

136 Alexander NR, Branch KM, Parekh A *et al.* Extracellular matrix rigidity promotes invadopodia activity. *Curr. Biol.* 18(17), 1295–1299 (2008).

137 Paszek MJ, Weaver VM. The tension mounts: mechanics meets morphogenesis and malignancy. *J. Mammary Gland Biol. Neoplasia* 9(4), 325–342 (2004).

138 Lu P, Weaver VM, Werb Z. The extracellular matrix: a dynamic niche in cancer progression. *J. Cell Biol.* 196(4), 395–406 (2012).

139 Discher DE, Mooney DJ, Zandstra PW. Growth factors, matrices, and forces combine and control stem cells. *Science* 324(5935), 1673–1677 (2009).

140 Fischer RS, Myers KA, Gardel ML, Waterman CM. Stiffness-controlled three-dimensional extracellular matrices for high-resolution imaging of cell behavior. *Nat. Protoc.* 7(11), 2056–2066 (2012).

141 Rehfeldt F, Engler AJ, Eckhardt A, Ahmed F, Discher DE. Cell responses to the mechanochemical microenvironment: implications for regenerative medicine and drug delivery. *Adv. Drug Deliv. Rev.* 59(13), 1329–1339 (2007).

142 Martino S, D'angelo F, Armentano I, Kenny JM, Orlacchio A. Stem cell-biomaterial interactions for regenerative medicine. *Biotechnol. Adv.* 30(1), 338–351 (2012).

143 Saldaña L, Crespo L, Bensiamar F, Arruebo M, Vilaboa N. Mechanical forces regulate stem cell response to surface topography. *J. Biomed. Mat. Res. Part A* 102(1), 128–140 (2014).

144 Ahn EH, Kim Y, An SS *et al.* Spatial control of adult stem cell fate using nanotopographic cues. *Biomaterials* 35(8), 2401–2410 (2014).

145 Rosa A, Kato R, Castro Raucci L *et al.* Nanotopography drives stem cell fate toward osteoblast differentiation through $\alpha_1\beta_1$ integrin signaling pathway. *J. Cell. Biochem.* 115(3), 540–548 (2014).

146 Lü D, Luo C, Zhang C, Li Z, Long M. Differential regulation of morphology and stemness of mouse embryonic stem cells by substrate stiffness and topography. *Biomaterials* 35(13), 3945–3955 (2014).

147 Ajami E, Mahno E, Mendes V, Bell S, Moineddin R, Davies J. Bone healing and the effect of implant surface topography on osteoconduction in hyperglycemia. *Acta Biomater.* 10(1), 394–405 (2014).

148 Bae D, Moon S-H, Park BG *et al.* Nanotopographical control for maintaining undifferentiated human embryonic stem cell colonies in feeder free conditions. *Biomaterials* 35(3), 916–928 (2014).

149 Bettinger CJ, Langer R, Borenstein JT. Engineering substrate topography at the micro- and nanoscale to control cell function. *Angew. Chem. Int. Ed. Engl.* 48(30), 5406–5415 (2009).

150 Kolind K, Leong KW, Besenbacher F, Foss M. Guidance of stem cell fate on 2D patterned surfaces. *Biomaterials* 33(28), 6626–6633 (2012).

151 Choi YY, Chung BG, Lee DH, Khademhosseini A, Kim J-H, Lee S-H. Controlled-size embryoid body formation in concave microwell arrays. *Biomaterials* 31(15), 4296–4303 (2010).

152 Park J, Cho CH, Parashurama N *et al.* Microfabrication-based modulation of embryonic stem cell differentiation. *Lab Chip* 7(8), 1018–1028 (2007).

153 Sasaki D, Shimizu T, Masuda S *et al.* Mass preparation of size-controlled mouse embryonic stem cell aggregates and induction of cardiac differentiation by cell patterning method. *Biomaterials* 30(26), 4384–4389 (2009).

154 Gentile F, Tirinato L, Battista E *et al.* Cells preferentially grow on rough substrates. *Biomaterials* 31(28), 7205–7212 (2010).

155 Vandrovcova M, Hanus J, Drabik M *et al.* Effect of different surface nanoroughness of titanium dioxide films on the growth of human osteoblast-like MG63 cells. *J. Biomed. Mater. Res. A* 100(4), 1016–1032 (2012).

156 Mendonca DB, Miguez PA, Mendonca G, Yamauchi M, Aragao FJ, Cooper LF. Titanium surface topography affects collagen biosynthesis of adherent cells. *Bone* 49(3), 463–472 (2011).

157 Galli C, Passeri G, Ravanetti F, Elezi E, Pedrazzoni M, Macaluso GM. Rough surface topography enhances the activation of Wnt/beta-catenin signaling in mesenchymal cells. *J. Biomed. Mater. Res. A* 95(3), 682–690 (2010).

158 Frey MT, Tsai IY, Russell TP, Hanks SK, Wang YL. Cellular responses to substrate topography: role of myosin II and focal adhesion kinase. *Biophys. J.* 90(10), 3774–3782 (2006).

159 Recknor JB, Recknor JC, Sakaguchi DS, Mallapragada SK. Oriented astroglial cell growth on micropatterned polystyrene substrates. *Biomaterials* 25(14), 2753–2767 (2004).

160 Yim EK, Darling EM, Kulangara K, Guilak F, Leong KW. Nanotopography-induced changes in focal adhesions, cytoskeletal organization, and mechanical properties of human mesenchymal stem cells. *Biomaterials* 31(6), 1299–1306 (2010).

161 Bucaro MA, Vasquez Y, Hatton BD, Aizenberg J. Fine-tuning the degree of stem cell polarization and alignment on ordered arrays of high-aspect-ratio nanopillars. *ACS Nano* 6(7), 6222–6230 (2012).

162 Gerecht S, Bettinger CJ, Zhang Z, Borenstein JT, Vunjak-Novakovic G, Langer R. The effect of actin disrupting agents on contact guidance of human embryonic stem cells. *Biomaterials* 28(28), 4068–4077 (2007).

163 Park J, Bauer S, Von Der Mark K, Schmuki P. Nanosize and vitality: $TiO_2$ nanotube diameter directs cell fate. *Nano Lett.* 7(6), 1686–1691 (2007).

164 Oh S, Brammer KS, Li YJ *et al.* Stem cell fate dictated solely by altered nanotube dimension. *Proc. Natl Acad. Sci. USA* 106(7), 2130–2135 (2009).

165 Smith LA, Liu X, Hu J, Wang P, Ma PX. Enhancing osteogenic differentiation of mouse embryonic stem cells by nanofibers. *Tissue Eng. Part A* 15(7), 1855–1864 (2009).

166 Xie J, Willerth SM, Li X *et al.* The differentiation of embryonic stem cells seeded on electrospun nanofibers into neural lineages. *Biomaterials* 30(3), 354–362 (2009).

167 Jiang X, Cao HQ, Shi LY, Ng SY, Stanton LW, Chew SY. Nanofiber topography and sustained biochemical signaling

enhance human mesenchymal stem cell neural commitment. *Acta Biomater.* 8(3), 1290–1302 (2012).

168 Elias KL, Price RL, Webster TJ. Enhanced functions of osteoblasts on nanometer diameter carbon fibers. *Biomaterials* 23(15), 3279–3287 (2002).

169 Schindler M, Ahmed I, Kamal J. A synthetic nanofibrillar matrix promotes *in vivo*-like organization and morphogenesis for cells in culture. *Biomaterials* 26(28), 5624–5631 (2005).

170 Schernthaner M, Reisinger B, Wolinski H *et al.* Nanopatterned polymer substrates promote endothelial proliferation by initiation of β-catenin transcriptional signaling. *Acta Biomater.* 8(8), 2953–2962 (2012).

171 Dalby MJ, Gadegaard N, Oreffo RO. Harnessing nanotopography and integrin-matrix interactions to influence stem cell fate. *Nat. Mater.* 13(6), 558–569 (2014).

•• **Comprehensive review article on cell–nanotopography interactions focusing on advancing stem cell culture materials and implantable interfaces, and also discussing the behavior of stem cells in their native niche.**

172 Tsimbouri PM, Mcmurray RJ, Burgess KV *et al.* Using nanotopography and metabolomics to identify biochemical effectors of multipotency. *ACS Nano* 6(11), 10239–10249 (2012).

• **Reports on a specific nanotopographical approach for identification of biochemical modulators and rational selection of biochemical targets in stem cell multipotency.**

173 Dalby MJ, Gadegaard N, Tare R *et al.* The control of human mesenchymal cell differentiation using nanoscale symmetry and disorder. *Nat. Mater.* 6(12), 997–1003 (2007).

•• **Fundamental study that demostrates that nanoscale disorder can stimulate human mesenchymal stem cells to produce bone mineral without any osteogenic supplements *in vitro,* which has implications for cell therapeutic approaches.**

174 Kingham E, White K, Gadegaard N, Dalby MJ, Oreffo RO. Nanotopographical cues augment mesenchymal differentiation of human embryonic stem cells. *Small* 9(12), 2140–2151 (2013).

175 Ji L, Lapointe VL, Evans ND, Stevens MM. Changes in embryonic stem cell colony morphology and early differentiation markers driven by colloidal crystal topographical cues. *Eur. Cell Mater.* 23, 135–146 (2012).

176 Kong YP, Tu CH, Donovan PJ, Yee AF. Expression of Oct4 in human embryonic stem cells is dependent on nanotopographical configuration. *Acta Biomater.* 9(5), 6369–6380 (2013).

177 Das RK, Zouani OF, Labrugère C, Oda R, Durrieu M-C. Influence of nanohelical shape and periodicity on stem cell fate. *ACS Nano* 7(4), 3351–3361 (2013).

178 Wilson CJ, Clegg RE, Leavesley DI, Pearcy MJ. Mediation of biomaterial-cell interactions by adsorbed proteins: a review. *Tissue Eng.* 11(1–2), 1–18 (2005).

179 Llopis-Hernández V, Rico P, Moratal D, Altankov G, Salmerón-Sánchez M. Role of material-driven fibronectin fibrillogenesis in protein remodeling. *BioRes. Open Access* 2(5), 364–373 (2013).

180 González-García C, Moratal D, Oreffo RO, Dalby MJ, Salmerón-Sánchez M. Surface mobility regulates skeletal stem cell differentiation. *Integr. Biol.* 4(5), 531–539 (2012).

181 Ferreira L, Squier T, Park H, Choe H, Kohane DS, Langer R. Human embryoid bodies containing nano-and microparticulate delivery vehicles. *Adv. Mater.* 20(12), 2285–2291 (2008).

182 Kutsuzawa K, Chowdhury E, Nagaoka M, Maruyama K, Akiyama Y, Akaike T. Surface functionalization of inorganic nano-crystals with fibronectin and E-cadherin chimera synergistically accelerates trans-gene delivery into embryonic stem cells. *Biochem. Biophys. Res. Commun.* 350(3), 514–520 (2006).

183 Kutsuzawa K, Akaike T, Chowdhury EH. The influence of the cell-adhesive proteins E-cadherin and fibronectin embedded in carbonate-apatite DNA carrier on transgene delivery and expression in a mouse embryonic stem cell line. *Biomaterials* 29(3), 370–376 (2008).

184 Green JJ, Zhou BY, Mitalipova MM *et al.* Nanoparticles for gene transfer to human embryonic stem cell colonies. *Nano Lett.* 8(10), 3126–3130 (2008).

185 Ruan J, Shen J, Wang Z *et al.* Efficient preparation and labeling of human induced pluripotent stem cells by nanotechnology. *Int. J. Nanomed.* 6, 425 (2011).

186 Ahmed I, Kamal J, Schindler M, Meiners S. Three-dimensional nanofibrillar surfaces promote self-renewal in mouse embryonic stem cells. *Stem Cells* 24(2), 426–433 (2006).

187 Silva GA, Czeisler C, Niece KL *et al.* Selective differentiation of neural progenitor cells by high-epitope density nanofibers. *Science* 303(5662), 1352–1355 (2004).

188 Lord MS, Foss M, Besenbacher F. Influence of nanoscale surface topography on protein adsorption and cellular response. *Nano Today* 5(1), 66–78 (2010).

189 Jönsson U, Ivarsson B, Lundström I, Berghem L. Adsorption behavior of fibronectin on well-characterized silica surfaces. *J. Colloid Interface Sci.* 90(1), 148–163 (1982).

190 Macdonald D, Deo N, Markovic B, Stranick M, Somasundaran P. Adsorption and dissolution behavior of human plasma fibronectin on thermally and chemically modified titanium dioxide particles. *Biomaterials* 23(4), 1269–1279 (2002).

191 Arima Y, Iwata H. Effect of wettability and surface functional groups on protein adsorption and cell adhesion using well-defined mixed self-assembled monolayers. *Biomaterials* 28(20), 3074–3082 (2007).

192 Cai K, Bossert JR, Jandt KD. Does the nanometre scale topography of titanium influence protein adsorption and cell proliferation? *Colloids Surfaces B Biointerfaces* 49(2), 136–144 (2006).

193 Hovgaard MB, Rechendorff K, Chevallier J, Foss M, Besenbacher F. Fibronectin adsorption on tantalum: the influence of nanoroughness. *J. Phys. Chem. B* 112(28), 8241–8249 (2008).

194 Lord M, Cousins B, Doherty P *et al.* The effect of silica nanoparticulate coatings on serum protein adsorption and cellular response. *Biomaterials* 27(28), 4856–4862 (2006).

195 Webster TJ, Schadler LS, Siegel RW, Bizios R. Mechanisms of enhanced osteoblast adhesion on nanophase alumina involve vitronectin. *Tissue Eng.* 7(3), 291–301 (2001).

196 Vertegel AA, Siegel RW, Dordick JS. Silica nanoparticle size influences the structure and enzymatic activity of adsorbed lysozyme. *Langmuir* 20(16), 6800–6807 (2004).

197 Tsai W-B, Ting Y-C, Yang J-Y, Lai J-Y, Liu H-L. Fibronectin modulates the morphology of osteoblast-like cells (MG-63) on nano-grooved substrates. *J. Mater. Sci.* 20(6), 1367–1378 (2009).

198 Sutherland DS, Broberg M, Nygren H, Kasemo B. Influence of nanoscale surface topography and chemistry on the functional behaviour of an adsorbed model macromolecule. *Macromol. Biosci.* 1(6), 270–273 (2001).

199 Huang H-H, Wu C-P, Sun Y-S, Yang W-E, Lin M-C, Lee T-H. Surface nanoporosity of β-type Ti-25Nb-25Zr alloy for the enhancement of protein adsorption and cell response. *Surf. Coat. Technol.* 259(Part B), 206–212( 2014).

200 Low YKA, Zou X, Fang Y *et al.* β-phase poly (vinylidene fluoride) films encouraged more homogeneous cell distribution and more significant deposition of fibronectin towards the cell–material interface compared with α-phase poly (vinylidene fluoride) films. *Mat. Sci. Eng.* 34, 345–353 (2014).

201 Del Campo A, Arzt E. Fabrication approaches for generating complex micro-and nanopatterns on polymeric surfaces. *Chem. Rev.* 108(3), 911–945 (2008).

202 Turner L-A, Downes S, Hill E, Kinloch I. Investigating the suitability of electrohydrodynamic lithography for the fabrication of cell substrates. *J. Mater. Sci.* 49(11), 4045–4057 (2014).

203 Bosworth LA, Turner L-A, Cartmell SH. State of the art composites comprising electrospun fibres coupled with hydrogels: a review. *Nanomedicine* 9(3), 322–335 (2013).

204 Chen G-Y, Pang D-P, Hwang S-M, Tuan H-Y, Hu Y-C. A graphene-based platform for induced pluripotent stem cells culture and differentiation. *Biomaterials* 33(2), 418–427 (2012).

205 Mashinchian O, Bonakdar S, Taghinejad H *et al.* Cell-imprinted substrates act as artificial niche for skin regeneration. *ACS Appl. Mater. Interfaces* 6(15), 13280–13292 (2014).

•• **The results of this pioneering study suggest that the induction of mature cell shapes (through cell-imprinted substrates) onto adipose-derived stem cells can influence nucleus deformation followed by regulation of target genes.**