Embryonic and Induced Pluripotent Stem Cells: Understanding, Creating, and Exploiting the Nano-Niche for Regenerative Medicine

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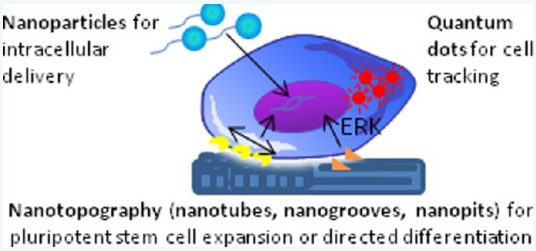
ABSTRACT Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) have the capacity to differentiate into any specialized cell type of the human body, and therefore, ESC/iPSC-derived cell types offer great potential for regenerative medicine. However, key to realizing this potential requires a strong understanding of stem cell biology, techniques to maintain stem cells, and strategies to manipulate cells to efficiently direct cell differentiation toward a desired cell type. As nanoscale science and engineering continues to produce novel nanotechnology platforms, which inform, infiltrate, and impinge on many aspects of everyday life, it is no surprise that stem cell research is turning toward developments in nanotechnology to answer research questions and to overcome obstacles in regenerative medicine. Here we discuss recent advances in ESC and iPSC manipulation using nanomaterials and highlight future challenges within this area of research.



KEYWORDS: embryonic stem cells · nanomaterials · nanoparticles · nanotopography · regenerative medicine · niche

The term nanomaterial encompasses a variety of materials with nanoscale structural features which include nanoparticles, nanofibers, nanosurfaces, and nanocomposites. As nanomaterials become increasingly more sophisticated in their range of physical properties (2D surfaces, 3D structures, variable porosity, stiffness, and biodegradability), their diversity of use for medical applications continues to expand. Both physical and chemical properties of biomaterials are now more readily altered, providing opportunities to improve efficacy.[1]

Stem cells can be isolated from a variety of sources and consequently differ in their ease of *in vitro* culture, proliferation rates, and capacity to form specialized cell types. Regardless of stem cell type, current focus remains on stem cell expansion, maintenance of the stem cell state, differentiation, and, ultimately, transplantation and clinical application. Enhanced understanding and manipulation of stem cells to produce cell types of interest or transplantable tissues is the predominant goal of regenerative medicine. Here we restrict predominantly to investigations of nanoscale physical properties and their use in embryonic stem cell (ESC) and ESC-like-induced pluripotent stem cell (iPSC) research. Furthermore, we assess how nanomaterials may hold the key for future advances in regenerative medicine.

Embryonic Stem Cells and Induced Pluripotent Stem Cells. *Derivation and Properties.* ESCs are isolated from the inner cell mass (ICM) of blastocyst stage embryos (Figure 1). *In vivo*, the ICM develops to form ectodermal, endodermal, and mesodermal tissues of the embryo proper. ESCs exhibit the unique property of pluripotency, the ability to form any specialized, differentiated cell types of the organism from which they are derived. Hence, ESCs can differentiate to form any cell type of the adult organism (Figure 1). In order to maintain pluripotency, ESCs must proliferate while suppressing differentiation, a process known as self-renewal.

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In 1981, the first ESCs, derived from mice (mESCs), were isolated and grown in culture.[2] Almost two decades later, the isolation of human ESCs (hESCs) was reported by Thomson and colleagues.[3] For mouse or human ESCs, pluripotency is demonstrated by injection into immune-deficient mice to produce teratomas containing cells expressing markers of each of the three primary germ layers.[3,4] Pluripotency of mESCs can also be demonstrated by injection into a mouse blastocyst to form a chimeric mouse and subsequent assessment of offspring to confirm incorporation of these cells into the germline.[5] However, the conditions required for maintaining pluripotency and self-renewal of mESC and hESCs *in vitro* are quite different, and thus studies in one animal ESC line are not always transferable to another.

While adult stem cells are ethically preferable, sources of human adult stem cells are somewhat limited, and isolation can prove complex and can be painful for the patient. The limited capacity of adult stem cells to self-renew makes their expansion *in vitro* a significant challenge, and unlike hESCs, adult stem cells are lineage restricted. Evidence exists to suggest that hESC-derived cell populations display low immunogenicity and could, potentially, be transplanted with minimal immunosuppression.[6−8] Similarly, mesenchymal stem cells and indeed hESC-derived mesenchymal stem cells are also reported to provide immunosuppressive properties.[9,10] Consequently, ESCs offer significant potential to treat a wider range of diverse pathological disorders.

Adult somatic cell-derived iPSCs are increasingly being investigated as a patient-specific alternative to hESCs with less controversy. Seminal papers from the Yamanaka group demonstrated that mouse fibroblasts could be reprogrammed to mESC-like cells by the expression of four mESC-specific transcription factor genes (Klf4, c-Myc, Oct-3/4, and Sox2).[11,12] More recently, adult human fibroblasts have been genetically manipulated to form human iPSCs.[13,14] Since these initial publications, further reports describe iPSCs formed from nonpluripotent, somatic adult cells, and additional strategies have been developed to limit genetic manipulation or to incorporate reprogramming factor-free methods.[15] Critically, a high degree of similarity exists between iPSCs and ESCs, offering new hope for the use of pluripotent stem cells for regenerative therapies with fewer ethical concerns and, potentially, enhanced patient specificity.[16−18]

VOCABULARY: **Nanomaterials** - share the defining characteristic of feature size within the nanoscale range of 1−100 nm but can be made of metals, ceramics, polymers, or composite materials and include nanoparticles, nanofibers, nanosurfaces, and nanocomposites.; **Nanotopography** - the roughness or spatial features displayed by a surface with nanoscale dimensions.; **Niche** - the *in vivo* or *in vitro* environment which provides both chemical and physical cues to maintain self-renewal or to direct differentiation.; **Pluripotency** - the ability of a stem cell to form any specialized, differentiated cell types of the organism from which it is derived.; **Regenerative Medicine** - the replacement or regeneration of cells or tissues to repair or replace aged, diseased, or injured tissue.; **Self-renewal** - encompasses the proliferation of stem cells while maintaining the stem cell state.;

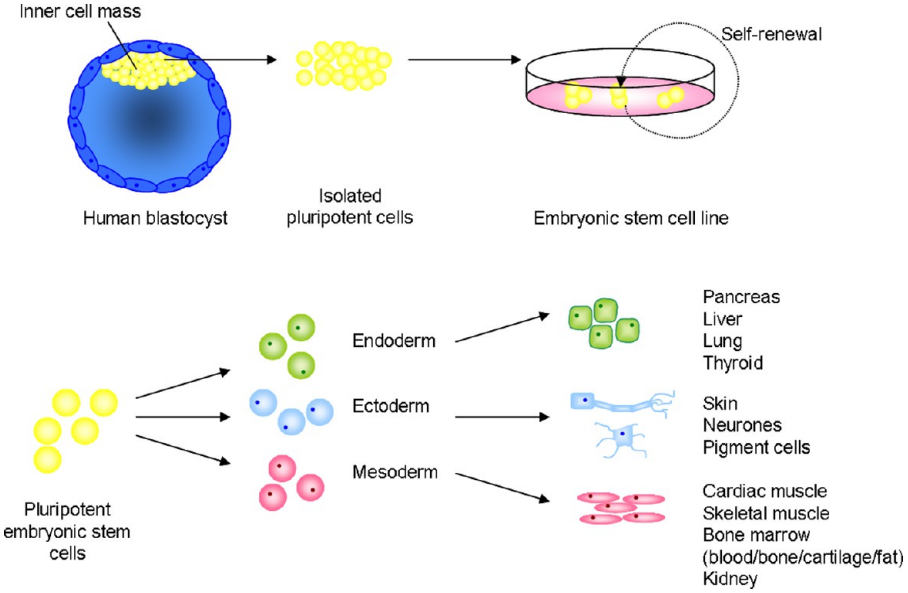

Figure 1. Derivation of human ESC lines and their pluripotency ESC lines are derived from the inner cell mass of blastocyst stage embryos and, when maintained in culture, undergo self-renewal, proliferation with retention of the stem cell state. *In vitro*, these cells are pluripotent in their ability to differentiate to any cell type of the three germ layers (endoderm, ectoderm, and mesoderm) that comprise the embryo proper. Theoretically, embryonic stem cells could be manipulated to form any cell type of the human body, but in reality, this requires a detailed understanding of the chemical and physical cues necessary to direct differentiation to a specific cell type.

Therapeutic Potential. It is the property of pluripotency, the possibility of producing any of the cell types that comprise the human body, to which hESCs and human iPSCs owe their therapeutic and research potential. Within the field of regenerative medicine, significant focus is placed on the expansion of ESC/iPSCs and directed differentiation into homogeneous cultures *ex vivo*, followed by transplantation. In this sense, ESC/iPSC-derived cell types would be transplanted with the view to repairing or replacing cells lost or damaged through disease and injury for regenerative medical strategies. However, the concept of regenerative medicine has been expanded beyond purely the use of ESC-derived cell types and tissue structures. Genetic manipulation prior to transplantation may not only reduce the risk of rejection but transplanted cells may also provide a novel vehicle for gene therapy to treat genetic diseases and cancer.[19] Furthermore, ESC/iPSC-derived cell types may prove to be a valuable source of cells on which to test new candidate drugs, an enterprise that has previously relied on animal models, limited human cell lines, and human volunteers. Thus drug toxicity testing and candidate drug screening on ESC/iPSC-derived cell types could significantly benefit from this area of research.[20]

The therapeutic potential of hESCs and iPSCs is vast; however, to realize this essentially requires mass expansion of a homogeneous, self-renewing, pluripotent population of cells to obtain sufficient cell numbers, followed by efficient specific differentiation to a desired precursor or terminally differentiated cell type prior to clinical application. For iPSCs, in addition to these challenges, the efficient induction of pluripotency in adult cells without compromising safety by genetic manipulation that might result in greater risk to the patient subsequent to reintroduction of cells must also be overcome.

Stem Cell Niche. The stem cell niche is the cellular and non-cellular environment surrounding stem cells and can comprise both chemical (soluble) and physical (nonsoluble or topographical) features of micro- and nanoscale proportions. Through interaction with this niche, stem cell fate is regulated. It is widely accepted that establishment of a stem cell niche is important both to maintain the population of stem cells and to provide an environment for the differentiation of stem cells into specialized cell types for contribution to functional tissues.[21,22] Just as a cell detects the chemical components of its environment including proteins, hormones, and growth factors, the physical properties of the surroundings can also influence cell fate. *In vivo*, the physical environment is provided by the extracellular matrix (ECM) with varying degrees of stiffness and topographical features depending on the stem-cell-specific niche. Similarly, *in vitro*, factors such as mechanical properties, surface stiffness, chemistry, and nanotopography have all been reported to influence stem cell maintenance and lineage commitment.[23−32]

Directed differentiation protocols involve the addition or removal of factors to culture media in a sequential manner.[33] This approach serves to activate or inhibit intracellular signaling pathways in a temporal fashion to induce changes in gene expression, driving cell differentiation down a specific lineage. *In vivo* differentiation is a complex process requiring both chemical and physical cues (both temporally and spatially), whereas standard chemical-induced methods neglect the importance of the physical environment experienced by a cell. Thus, *in vitro* techniques for directed differentiation combining chemical cues and topographical cues may be more efficient and allow for the production of a wider range of cell types. In the first instance, an understanding of cell type and characteristics is informative in any niche development.

Nanomaterials. *Nanomaterials for ESC Research and Regenerative Medicine.* Early approaches using nanomaterials predominantly focused on adult terminally differentiated or adult stem cell types and implemented surfaces displaying nanoscale topography that mimicked the ECM. Collagen is a major component of the ECM, and nanoscale collagen fiber structures were found to enhance the cell−matrix interaction.[34] Furthermore, there are several publications describing the successful replacement of feeder cells with extracellular matrix components in order to support self-renewal or to promote differentiation of ESCs.[35−39] Subsequently, synthetic nanoscale surfaces and scaffolds have been used to investigate the contribution of niche topography to maintain the stem cell state or to direct differentiation in the absence of chemical factors. Protocols for directed differentiation have applications for the derived cell types in drug toxicity screening and regenerative medicine.

Due to continual advances in fabrication methods, nanomaterials are developing with a strong regenerative medicine focus (reviewed in ref 40 and references therein). Specifically, nanomaterials have been implemented to address surface molecule tracking and *in vivo* tracking of transplanted cells, while nanoparticles have been investigated for their potential to deliver molecules (DNA, RNAi, protein) intracellularly.

Intracellular Delivery to ESCs or Somatic Cells Using Nanomaterials. The genetic reprogramming of somatic cells toward a stem-cell-like state has garnered considerable attention and has the remarkable potential to overcome both the limitations of adult stem cells and the ethical issues surrounding ESC use. Of the currently used methods, viral transfection of genes is less desirable due to the safety concerns related to genome-integrating viral DNA, while nonviral lipid or polymer transfection agents often yield lower percentages of successfully transfected cells.[41] Nanoparticle transfer of genetic material resolves these issues, and

the range of nanoparticles available may allow this method to be optimized to a higher efficiency than other gene delivery methods.

Ferreira *et al.* demonstrated the internalization and perinuclear accumulation of PLGA nanoparticles within hESC colonies and aggregated hESCs forming embryoid bodies (EBs). Importantly, no adverse effects on cell viability or proliferation were reported in cells containing nanoparticles.[42] Focusing on gene delivery, Kutsuzawa *et al.* demonstrated that nanoparticles formed of carbonate apatite crystals mixed with cell adhesive proteins fibronectin and E-cadherin-Fc enhanced transgene delivery and accelerated expression in recipient mESCs compared to carbonate apatite alone.[43,44] These mixed organic and inorganic nanoparticle composites bind to the cell surface and become internalized by mESCs, providing an efficient nonviral method of gene delivery.[44,45] Green *et al.* reported the development and use of self-assembling, biodegradable nanoparticles of poly(β-amino esters) that incorporate plasmid DNA within the nanoparticle for successful, higher efficiency transfection of hESCs. Transfected hESCs reportedly maintained their viability, undifferentiated state, and pluripotency following transfection with nanoparticles.[46] The authors noted that modification of the end group of the polymer used to form the nanoparticles altered the size and positive ζ-potential of the nanoparticles with a resultant impact on transfection efficiency[46] and, critically, the potential for cell-specific polymer modification to modulate transfection efficiency in undifferentiated hESCs compared to human umbilical vein cord cells.[46,47] More recently, nanomaterials have been reported to aid reprogramming in the preparation of iPSCs. Thus polyamidoamine dendrimer-modified magnetic nanoparticles were reported to enhance the delivery of lentivirus expression plasmids and to induce the reprogramming of human dermal fibroblasts to pluripotent cells.[48] Furthermore, Lee *et al.* reported efficient induction of pluripotency in mouse fibroblasts using biodegradable cationic polymer PEI-coated superparamagnetic nanoparticles to produce transient expression of four ESC transcription factors.[49] Consequently, this magnetic nanofection technique led to over 60% of the resulting mouse iPSC population being free from integrated exogenous DNA.

Tracking Transplanted Cells with Nanoparticles. Nanoparticle labeling of ESCs prior to transplantation into animal models offers a unique insight into cell migration. Furthermore, nanoparticle tracking may also allow an assessment of the functional contribution to replacing lost cells or to the repair of the diseased state. Thus, by transfecting mESCs with superparamagnetic dextran-coated iron oxide nanoparticles followed by transplantation into rat brains, Hoehn and co-workers were able to track mESC migration to a site of transient cerebral ischemic lesion, performed 2 weeks previously (Figure 2A).[50] Interestingly, migration of labeled mESCs, using magnetic resonance imaging (MRI), was observed over an 11 day period from the site of implantation to the opposite brain hemisphere where the lesion had been performed. During this time, the morphology of migrated mESCs had altered to neural-like cells in contrast to the rounded morphology of mESCs at the implantation site (Figure 2A).[50] Arai *et al.* used a superparamagnetic iron oxide agent to label mESCs prior to injection into a murine model of acute myocardial infarction and showed partial restoration of left ventricular volume end fraction and a reduction in thinning of the anterior and anterolateral region of the myocardium in comparison to non-mESC-treated mice.[51] Importantly, neither study reported adverse effects following mESC labeling with these nanoparticles, with Hoehn *et al.* describing no distinguishable toxic or proliferation effects to the cells treated with dextran-coated iron oxide nanoparticles and Arai *et al.* reporting a nanoparticle-independent ability of mESCs to partially restore myocardium function in this model.[50,51] Additionally, the uptake of iron oxide nanoparticles did not affect the cardiogenic or calcium handling capacity of mESC-derived cardiomyocytes.[52]

The application of nanoparticles for medical applications holds significant challenges since the chemical composition of the nanoparticle can impact toxicity. For example, silver nanoparticles were reported to induce apoptosis and enhance expression of cell cycle checkpoint protein p53 and DNA damage repair protein *Rad51* in mESCs.[53] Silica nanoparticles were reported to inhibit differentiation to contractile myocardial cells in mouse EBs at concentrations at which cytotoxic effects were not observed in a test typically used to assess the embryotoxic potential of chemicals.[54] Polystyrene nanoparticles were reported to reduce mESC viability and enhance fibroblastic columnar cell-type morphologies.[55] These studies highlight not only the importance of adequate testing of nanoparticles in relation to cell proliferation and differentiation potential but also the possible deleterious long-term effects that nanoparticle accumulation could have on patients receiving nanoparticle-based therapies.[56,57] Furthermore, maternal–fetal risks associated with nanoparticles and the potential for nanoparticle accumulation to transfer cumulatively to offspring are areas that clearly should also be addressed (an excellent review on nanotoxicology is provided in ref 58). However, these challenges may be overcome by the implementation of biodegradable nanoparticles such as those championed by Green and colleagues.[46,47]

Quantum dots are nanoscale particles with sustained fluorescence and good cellular uptake. The ability to detect quantum dot fluorescence by a number of methods including *in vivo* detection allows biodistribution following transplantation to be scrutinized.[59]

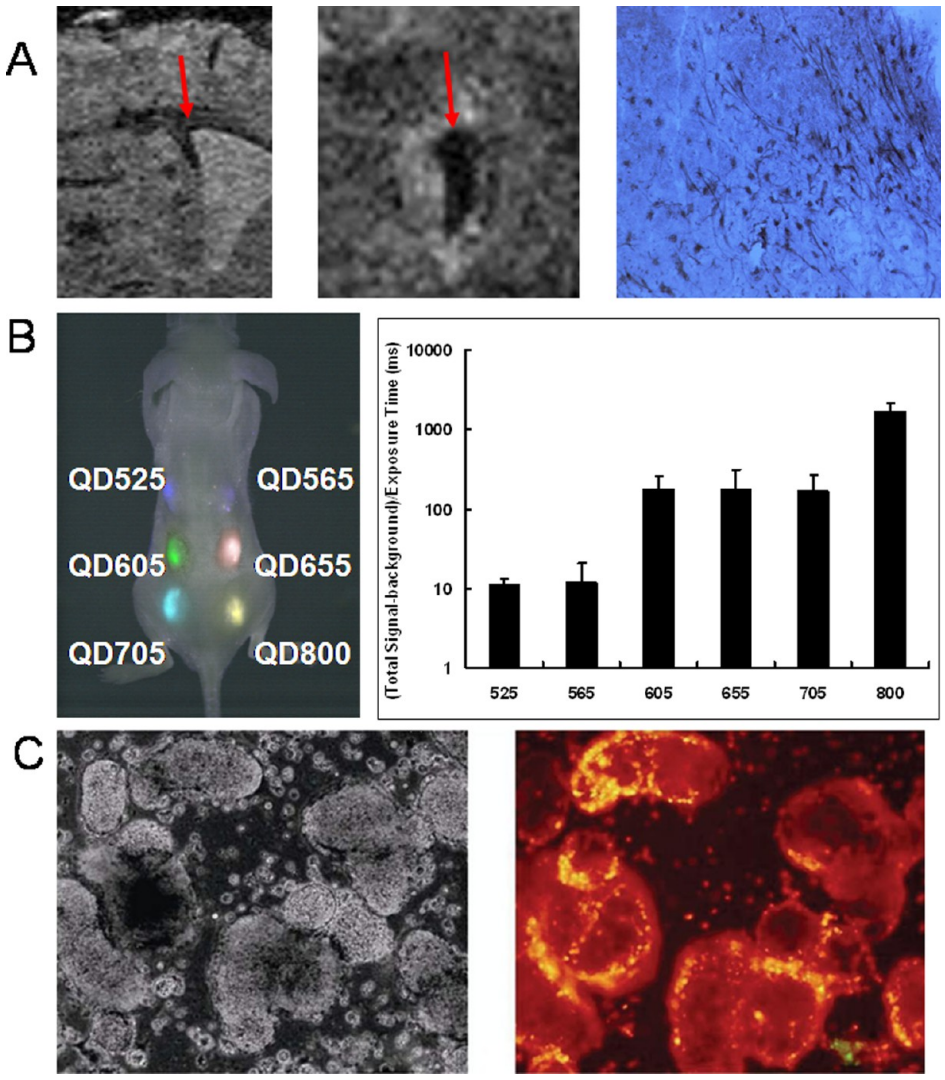

Figure 2. Quantum dot labeling for embryonic and induced pluripotent stem cell tracking. (A) Dextran-coated iron oxide nanoparticles were used to label mESCs for detection by magnetic resonance imaging (MRI). Two weeks after transient focal cerebral ischemia in Wistar rats, labeled cells were implanted into the normal contralateral hemisphere. Cells were observed to migrate from the normal hemisphere to the lesioned hemisphere and line the ventricular wall (left) and accumulate in the choroid plexus of the lateral ventricle (middle). Additionally, mESCs within the periphery of the ischemic lesion adopted a neural-like morphology (right). Reprinted with permission from ref 50. Copyright 2002 National Academy of Sciences, U.S.A. (B) Quantum dots emitting light of various wavelengths were used for intracellular labeling of mESCs prior to subcutaneous injection of athymic nude mice. The fluorescent signals emitted following a single excitation wavelength were detected by imaging, shown as a stacked image (left). Fluorescent signal intensity for each quantum dot was quantified and is represented graphically as total signal background/exposure time (ms) (right). Reprinted with permission from ref 60. Copyright 2007 BioMed Central. (C) Human dermal fibroblasts were reprogrammed to iPSCs and fully characterized (bright-field image of iPSCs shown left). Using fluorescent magnetic nanoparticles (FMNPs), iPSCs were labeled, and after 4 h, fluorescence was detected intracellularly (right). Reprinted with permission from ref 48. Copyright of 2011 Dove Medical Press Ltd.

Quantum dots have been used to successfully label mESCs, with minimal effects on viability and proliferation, and to visualize these cells within mice following transplantation (Figure 2B).[60] However, quantum dots are manufactured in the presence of heavy metals and exhibit a blinking phenomenon when visualized. To overcome the limitations of quantum dots, Nagesha *et al.* demonstrated that gold nanoparticles, detected by multiphoton absorption-induced luminescence (MAIL), could be used to visualize mESCs and, by altering the plane of focus, could discriminate them from their mouse embryonic fibroblast feeder layer of cells.[61] Providing further hope for the tracking of transplanted cells, iPSCs were successfully labeled with fluorescent magnetic silica coated quantum dots by Ruan *et al.*[48] (Figure 2C). Furthermore, iPSCs (derived from human dermal fibroblasts) retained their fluorescent signal up to one month after labeling, indicating suitability for longer term detection.[48] These findings demonstrate both the usefulness of nanoparticle tracking of ESCs and iPSCs and for the evaluation of the regenerative capacity of ESCs.

Toward the Generation of a Nanomaterial–ESC Niche. Self-Renewal and Proliferation-Promoting

Nanomaterials. One strategy for the generation of a nanomaterial−ESC niche has centered on the development of proliferation-promoting scaffolds for ESCs. Differentiated cell types typically display slower proliferation rates compared to ESCs, and large numbers of cells would be required for medical applications, emphasizing the need to expand ESCs prior to differentiation. For example, Ultra-Web, a commercially available 3D nanofibrillar scaffold with a nanoporous matrix formed by electrospinning polyamide fibers onto glass or plastic coverslips produces larger mESC colonies which reportedly exhibit enhanced proliferation when grown on an Ultra-Web scaffold.[62] Additionally, the expression of Nanog protein, a marker of self-renewal and an important regulator of the undifferentiated state, was greater in mESCs cultured on Ultra-Web compared with mESCs cultured on non-Ultra-Web-coated coverslips.[62] Interestingly, Nur-E-Kamal *et al.* also reported on the activation of intracellular signaling downstream of phosphoinositide 3-kinase (PI3K) in mESCs cultured on Ultra-Web-coated coverslips. The PI3K signaling pathway is reported to maintain mESC self-renewal and to regulate Nanog expression[63−65] and is known to be important for mESC proliferation,[66,67] explaining the findings obtained by Nur-E-Kamal and colleagues. A topographical surface formed from silica colloidal crystal (SCC) microspheres with diameters of 120, 400, and 600 nm with a type I collagen coating was reported to maintain the expression of mESC self-renewal markers in comparison to flat glass.[68] Colonies of mESCs on microsphere topographies were also reported to form a central pit within the colony and exhibited reduced spreading. Carbon-based materials provide a unique alternative to organic polymeric nanomaterials and have been implemented in pluripotent stem cell research. Thus, for example, mouse iPSCs cultured on graphene-coated glass surfaces exhibited a restricted loss of *Oct4* and *Nanog* expression in the absence of self-renewal maintaining leukaemia inhibitory factor (LIF) compared to graphene-oxide-coated glass or uncoated glass surfaces.[69]

FGF-2, known to be essential for the maintenance of hESC self-renewal,[3,37,70,71] covalently linked to polyamide nanofibrillar surfaces was reported to enhance the proliferation of a number of cell types including hESCs when compared to amine bound to nanofibrillar surfaces,[72] although no assessment was made with regards to hESC self-renewal marker expression (*i.e.*, retention of an undifferentiated state). In the absence of FGF, hESCs were observed to align along poly(dimethyl siloxane) and fibronectin grooves of 600 nm depth, 600 nm width, and 600 nm spacing. Adhesion and contact guidance to this topography resulted in reduced cell surface area, greater elongation, and a decreased rate of proliferation which could be reversed by disruption of the actin cytoskeleton (Figure 3A).[73]

A comparison of biomaterials, extracellular matrix components, and human-derived feeder cells to find a more efficient platform for hESC expansion concluded that the commonly used Matrigel was most supportive for long-term feeder-free culture.[74] Subsequent to this, vitronectin-coated nanorough glass surfaces have been used to demonstrate differential adhesion of hESCs and spontaneously differentiated hESCs demonstrating topographical sensing and selectivity. hESCs expressing the self-renewal marker OCT-4 selectively adhered to regions of smooth surface over nanorough regions (Figure 3B), displayed many filipodia, and exhibited enhanced proliferation rates, whereas cells with reduced expression of OCT-4 correlated with loss of selectivity of surface roughness.[75] More recently, a synthetic polymer coating of poly[2-(methacryloyloxy)-ethyl dimethyl-(3-sulfopropyl)ammonium hydroxide] (PMEDSAH) was reported to maintain the proliferation, self-renewal, and pluripotency of two hESC lines in mouse embryonic fibroblast (MEF)-conditioned media.[76] Moreover, the observation that one of the cell lines could be passaged up to 10 times in commercially available serum-free defined media on this PMEDSAH surface is a significant advancement in achieving scalable expansion of hESCs for biomedical applications.[76] Recent approaches have also implemented the functionalization of synthetic polymer coatings with bioactive components. For example, Klim *et al.* demonstrated that heparin-binding peptide derived from vitronectin supported adhesion, propagation, karyotype, and self-renewal marker expression through interaction with surface glycosaminoglycans for a number of hESC and human iPSC lines.[77] Similarly, synthetic peptide-acrylate surfaces were also reported to support hESC maintenance.[78] Crucially these latter studies utilized a variety of readouts to characterize cells and confirmed pluripotency following an extended number of passages. Additional investigation of the nanotopographical features provided by these coatings both in combination with and dissociated from the chemical interaction bioactive functionalization provide and in comparison to Matrigel will inform future developments in this area.

For applications in regenerative medicine, it is important that cell pluripotency is retained at the expansion stage. It was demonstrated that mESCs cultured on the 3D nanofibrillar scaffold could be induced to express neural markers in response to retinoic acid treatment; however, other lineages were not addressed.[62] Furthermore, while OCT-4 expression was reported to be retained by hESCs on a smooth glass surfaces, other self-renewal markers were not investigated and pluripotency was not assessed.[75] For future applications, it would be imperative to fully characterize cells following expansion on nanomaterials to confirm not only pluripotency (by the methods described in section Embryonic Stem Cells and

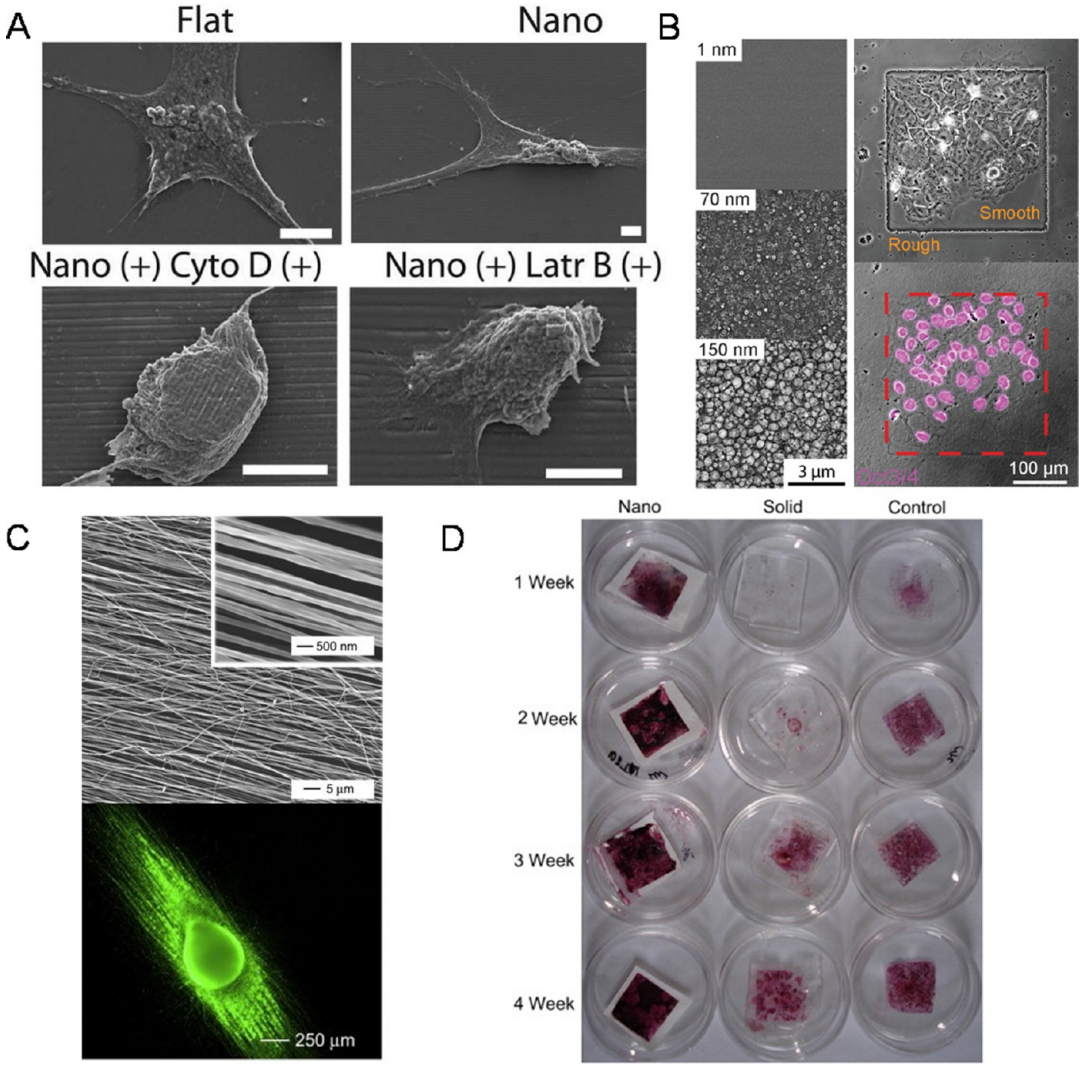

Figure 3. hESCs sense and respond morphologically and phenotypically to nanoscale topographical cues. (A) hESC alignment along poly(dimethyl siloxane) grooves with fibronectin coating (upper panel) is reversed by disruption of the actin cytoskeleton with cytochalasin D (Cyto D) or latrunculin B (Latr B) (lower panel)[73] (scale bars 10 μm). Reprinted with permission from ref 73. Copyright 2007 Elsevier. (B) SEM images of glass surfaces untreated (upper) and treated (middle and lower) with a nanoroughening reactive ion etching technique. hESCs expressing OCT3/4 exhibited preferential attachment and aggregation to smooth regions of glass over nanorough regions of glass.[75] Reprinted with permission from ref 75. Copyright 2012 American Chemical Society. (C) Aligned polymer nanofibers were prepared by electrospinning (upper image). These nanofibers provided a suitable scaffold to guide the outgrowth of neurites from mESC EBs which stain positive for early neural marker Tuj1 (lower image).[84] Reprinted with permission from ref 84. Copyright 2009 Elsevier. (D) Increased calcium mineralization was detected on nanofibrous films seeded with mESC-EBs compared to solid walled or control scaffolds.[100] Reprinted with permission from ref 100. Copyright 2009 Elsevier.

Induced Pluripotent Stem Cells, Derivation and Properties) but also karyotype, proliferation, and viability among other requisite characteristics. These studies demonstrate a unique opportunity to combine physical and chemical characteristics to inform and, critically, recreate the ICM environment of the blastocyst and generate an *in vitro* stem cell niche supportive of self-renewal, expansion, and retention of pluripotency.Nanomaterial-Induced ESC-Directed Differentiation. Following expansion of hESCs, platforms efficient at inducing differentiation are required to reduce the risk of transplanting undifferentiated hESCs into patients, a process that would result in teratoma formation. The notion that physical, topographical features can influence cell fate is not a new area of research[79,80] with seminal observations dating almost 50 years; rather, more novel is the concept that nanoscale platforms can serve as feeder-free, animal-product-free, and supplementary factor-free scaffolds for directed stem cell differentiation.

Neural Differentiation. Neural-directed differentiation protocols typically rely on induction of differentiation following the aggregation of ESC to form EBs and the addition of retinoic acid to culture media.[81−83] Xie *et al.* successfully directed the differentiation of mESCs by this method, then transplanted EBs on to biodegradable poly(ε-caprolactone) (PCL) nanofibers. The authors observed migration of neural type cells

(evidenced by the expression of early neural, oligodendrocyte, and astrocyte markers) from EBs along the axis of aligned nanofibers (Figure 3C).[84] Neural differentiation and cell migration on nanofibers were also observed for mESCs seeded directly onto the PCL nanofibers without prior EB formation, although these cells formed aggregates on the nanofibers and retinoic acid was added to the culture media, making it difficult to distinguish the effects of nanofibers alone.[84] Kabiri and colleagues seeded EBs, derived from mESCs in the presence of retinoic acid, onto an electrically conductive composite of carbon nanotubes and electrospun poly(L-lactic acid) nanofibers reporting differential neural marker gene expression dependent on the presence of single- or multiple-walled nanotubes.[85] Consistent with the mESC studies, seeding onto thin film scaffolds based on carbon nanotubes, hESC-derived EBs were reported to have increased neural marker expression and enhanced adhesion,[86] while seeding onto a silk-carbon nanotube composite enhanced neural marker expression and enhanced axonal length compared to silk alone.[87] In the absence of EB formation or retinoic-acid-induced differentiation, hESCs seeded directly onto a composite of type I collagen, and carbon nanotubes exhibited enhanced nestin expression and ectodermal cell morphology compared to cells seeded onto pure collagen matrix. Nestin expression was greatly enhanced compared to spontaneously differentiated cells seeded on gelatin.[88] Furthermore, differentiated cells were aligned in the direction of the collagen/carbon nanotube fibrils,[88] indicating that spatial growth and differentiation of hESCs can be directed using nanomaterials in the absence of chemical factors or specific differentiation medium.

Ridges and grooves of nanoscale proportions (350 nm spacing and 500 nm height) were reported to be sufficient to induce neural differentiation of hESCs. Although the nanotopography surfaces required gelatin coating and treatment with oxygenated plasma to improve hESC adhesion, hESCs clearly aligned in the direction of the grooves and stained positive for a number of neural markers.[89] Moreover, nanotopography-induced differentiation required no additional factors or the formation of EBs prior to seeding, indicating that adhesion to these nanotopography surfaces and removal of FGF from culture media was sufficient to induce neural differentiation.[89] However, within the ESC field, it is argued that neural differentiation may be the default differentiation model of ESCs cultured without extrinsic cues to differentiate toward other lineages.[90,91] In keeping with this model, non-neural lineage differentiation would require alternative topographical features or a combined approach of nanotopography and chemical induction.

Surface Stiffness and Mesenchymal Directed Differentiation. Engler *et al.* reported that a tight or loose attachment of multipotent stem cells, depending on the surface stiffness, can influence the lineage toward which stem cells differentiate.[25] In an investigation using polyelectrolyte films, ESCs were also shown to respond to stiffness.[92] Multilayered films of poly(L-lysine) and hyaluronan that hold fewer cross-links (and thus less stiff) were demonstrated to correlate with reduced mESC proliferation compared to that observed for either highly cross-linked nanofilms or typical culture on gelatin-coated tissue culture plastic.[92] Similarly, stiffer polydimethylsiloxane substrates of 1.9−2.7 MPa yielded greater numbers of mESCs after 6 days than less stiff polydimethylsiloxane substrates of 0.041 and 0.26 MPa.[93]

The expression of mESC markers Nanog, *Sox2*, and *Oct-4* were unaffected by the surface stiffness of poly(L-lysine) and hyaluronan.[92] However, a reduction in the expression of ICM marker genes *Rex1*, Stella, and *Tbx3* was observed on all nanofilms in comparison to suspension culture, indicating a switch akin to the epiblast stage. In terms of ESC differentiation, mesodermal markers Brachyury and Goosecoid expression levels were observed to be enhanced by culture of mESCs on nanofilms compared to either culture on non-cross-linked nanofilm or suspension culture.[92] Similarly, culture of mESCs on stiffer polydimethylsiloxane substrates enhanced the expression of primitive streak and mesendodermal markers, Brachyury, Mixl1, and Eomes.[93] Consistent with the expression of early differentiation markers, adhesion to stiff nanofilms prior to EB formation resulted in impaired EB differentiation potential. Hayashi and colleagues suggested that poly(L-lysine) and hyaluronan nanofilms directed mESCs toward an epiblast fate, a developmental progression from ICM, between which mESCs are thought to cycle in culture,[94] although a low level of poly(L-lysine) and hyaluronan was detected to be released from nanofilms into the culture media. Furthermore, when free poly(L-lysine) or free poly(L-lysine)/hyaluronan complexes were added to cultures on gelatin-coated plates, mESCs showed a down-regulation of Brachyury expression, indicating a chemical influence of poly(L-lysine) on retaining a stem cell state.[92] By investigating the stiffness of cells through application of stress to the cell surface and assessment of cell deformation, Chowdhury *et al.* demonstrated that undifferentiated mESCs were softer and exhibited greater spreading in response to local cyclic stress compared to differentiated mESCs.[95] Furthermore, cyclic stress resulted in reduced expression of Oct-3/4 in the targeted cell. In a subsequent study, Chowdhury and co-workers report that matching the softness of the substrate to mESC softness promoted self-renewal in the absence of LIF for up to 5 days.[96] In contrast, hESCs bound and formed more robust colonies that remained attached when presented with stiffer (10 kPa) hydrogels, functionalized with glycosaminoglycans or

Matrigel, compared to less stiff equivalents (0.7 and 3kPA).[97]

Using EB formation to induce differentiation of mESCs, Garreta *et al.* demonstrated enhanced osteogenic differentiation as determined by enhanced osteopontin expression and mineralization of EB-derived cells on a 3D nanofiber peptide scaffold in the presence of osteogenic media.[98] In addition, modification of this 3D self-assembled peptide scaffold, generated by forming a composite material with hydroxyapatite and encapsulation of EB-derived cells, further enhanced mineralization compared to non-composite peptide scaffolds.[99] Consistent with this, nanofibrous scaffolds of poly(L-lactic acid) were demonstrated to induce mouse EB-derived cells to differentiate with enhanced expression of osteogenic markers and increased calcium mineralization compared to culture on thin film scaffolds of the same material (Figure 3D).[100] It should be noted that each of these studies employed EB formation to induce ESC differentiation prior to seeding. An alternative approach to induce differentiation employed electrical stimulation of hESCs *via* a fibronectin-coated gold nanoparticle layer on tissue culture plastic with a resultant loss of stem cell marker OCT-4 expression and enhanced expression of osteogenic markers collagen type I and Cbfa1 in contrast to unstimulated cells.[101] Following initial adhesion, mESCs seeded onto a 3D poly(L-lactic acid) nanofiber matrix scaffold displayed greater interaction with the scaffold *via* extended processes than mESCs on a 2D poly(L-lactic acid) nanofiber film. Following differentiation on these nanomaterials, in the presence of osteogenic media, enhanced expression of osteogenic markers was detected in cells cultured on 3D nanofiber scaffolds compared to 2D nanofiber films or gelatin-coated tissue culture plastic. Furthermore, cells grown on 3D nanofiber scaffolds displayed reduced expression of neural markers, suggesting that differentiation was directed toward an osteogenic lineage as opposed to general differentiation toward nonspecific lineages.[102] Similarly, Farzaneh *et al.* reported enhanced functionality of hESC-derived hepatocyte cells when hESCs were differentiated on Ultra-Web with stepwise introduction of chemical differentiation factors.[103] Endodermal differentiation was also enhanced in mouse iPSCs cultured on graphene-oxide-coated glass surfaces in the absence of LIF, although expression of ectodermal and mesodermal markers was also detected.[69] Interestingly, Chen and co-workers reported mouse iPSCs to exhibit rapid proliferation on graphene oxide with outgrowth of colonies in contrast to graphene-coated glass or uncoated glass surfaces.[69] In contrast, a reduced capacity for endodermal differentiation was reported following incubation of mESCs on a surface formed from 400 nm diameter silica colloidal crystal microspheres in comparison to mESCs on flat glass or EB differentiation was reported.[68]

Recently, we have described a polycarbonate nanotopography surface that promotes hESC differentiation toward cell types with both expression and epigenetic characteristics akin to fetal skeletal stem cells (Figure 4).[104,105] Markers of skeletal stem cells (STRO-1, CD44, CD63, ALCAM) were detectable in differentiated ESCs following culture on nanotopographical surfaces displaying a near square arrangement of 120 nm diameter nanopits (300 nm center−center spacing randomly displaced by <50 nm on *x* and *y* axes) (Figure 4). Moreover, neural markers were undetectable at either the RNA or protein level, providing sufficient evidence of efficient directed differentiation

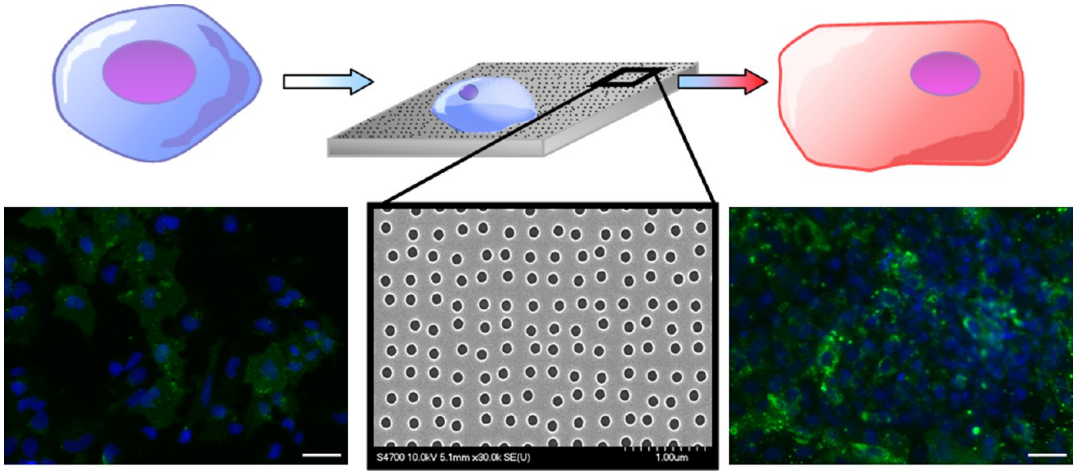

Figure 4. Nanoscale surface topography directs stem cell differentiation. In a basal media lacking soluble differentiation-directing factors, hESC seeded onto polycarbonate substrates displaying 120 nm diameter nanopits arranged in a near square geometry (300 nm center−center spacing displaced by 50 nm on *x* and *y* axes) differentiate toward a mesodemal lineage with enhanced expression of skeletal stem cell marker STRO-1 (green). Cells are counterstained with nuclear stain DAPI (blue) (scale bar 50 μm). Reprinted from ref 104. Copyright 2013 Wiley-VCH.

by a defined nanotopography surface[104,105] and with potential applications therein in orthopedic tissue engineering and regenerative medicine.

In Vivo Nanomaterial Scaffold. Typical approaches to investigate ESC differentiation-inducing nanomaterials have investigated *in vitro* scaffold-type induction. However, it is also appropriate to consider nanomaterials that can be co-transplanted with ESCs for therapeutic use. *In vivo* use of nanomaterials was elegantly demonstrated by Davis *et al.*, who reported the co-injection of self-assembling peptide nanofibers and mESCs into the left ventricle of adult mice. The mESCs were shown to survive and spontaneously differentiate within this environment.[106]

These studies reveal the efficacy of nanomaterial scaffolds in the induction of ESC differentiation to a variety of cell types. However, comparability to terminally differentiated cell types, as evidenced by full functionality (including *in vivo* functionality following transplant), as opposed to marker expression and long-term viability remains to be demonstrated. Additionally, and critically for future clinical applications, the absence of residual undifferentiated cell types is scarcely described. While the few cell types reported to be derived from ESCs using nanomaterials are clinically relevant, with over 200 distinct cell types known to comprise the human body, significant challenges lay ahead before every adult human cell can be made available for regenerative medicine applications.

Future Directions—Challenges and Opportunities. Given the self-renewal potential of ESCs and iPSCs and a need to obtain sufficient differentiated populations for transplantation, it is evident that strategies maintaining an undifferentiated stem cell state during large-scale expansion (without spontaneous differentiation) will be crucial. Such approaches, alongside the potential of nanotopography to modulate ESC aggregation and to mimic the ESC niche, remain to be investigated in depth. Within this niche, ESCs may form a microenvironment supportive of self-renewal, a process which has been shown to favor the maintenance of pluripotency of mESCs and hESCs.[107,108] Coupled with a growing understanding of the stem cell nano-niche, it is possible that nanoscaffolds could be developed to scale-up hESC expansion, thus providing a potentially unlimited source of ESCs, although maintenance of self-renewal and retention of pluripotency throughout such a mass expansion procedure will need to be confirmed and monitored.

Currently, there is a paucity of information as to how ESCs sense their nanoenvironments and the mechanisms by which this alters cell fate. The nanomechanical properties of tissues can be investigated by measuring hardness and elastic modulus using nanoindentation techniques. Charitidis compared the nanomechanical properties of bone and cartilage and highlighted that nanoindentation investigations are largely hampered by the water content of tissues, cells, and the *ex vivo* environment.[109] In addition, the reproducibility of results was reported to be variable and dependent on the geometry and composition of the indentation tip selected, on the depth of indentation, and on the calibration materials implemented. However, nanoscale mechanical changes are known to be an important factor in protein biology, in particular, the tensile properties of intermediate filaments. Qin *et al.* reported that vimentin undergoes an α-helix to a β-sheet conformational change under strain and suggested that this may offer a protective mechanism to the cell cytoplasm in resisting large deformation.[110] However, Rho-associated kinase phosphorylation of vimentin is reported to regulate agonist-mediated neurite retraction,[111] coupling vimentin with cell morphology response. Integrin-mediated focal adhesions and E-cadherin (which binds components of the cytoskeleton) were reported to regulate cellular sensing of topographical cues in hESCs.[75] Consistent with these observations, Sun and colleagues describe roles for focal adhesion formation and E-cadherin in mechanoresponsive differentiation and cytoskeleton contractility.[31] (Mechanotransduction and mechanical control of stem cell fate is reviewed by Sun *et al.*[32] and references therein.)

It is conceivable that tension would be altered by cell adhesion to a topographical surface. Tensile forces provided by cell-cortex tension in zebrafish embryos have been shown to direct progenitor cell sorting in a manner that is both actomyosin-dependent and nodal/transforming growth factor β (TGF-β) signaling-dependent.[112] To this end, relatively little information is available regarding the signaling pathways activated or inactivated and translational mechanisms following ESC/iPSC attachment to nanomaterials. Our recently published work on bone-marrow-derived adult skeletal stem cells may offer an insight into stem cell nano-niche sensing. We reported that disruption of actin−myosin interaction and resulting loss of tension disrupted the ability of a square arrangement of nanopits to support adult skeletal stem cell multipotency and induced adipogenic differentiation.[30] ERK signaling was also implicated in nanotopographical maintenance and directed differentiation of adult skeletal stem cells[30] Moreover, ERK signaling has previously been implicated in mESC differentiation[63,64,113,114] in hESC self-renewal[115] and in mechanotransduction.[116] We postulated a role for small untranslated RNAs, which can modulate mRNA transcripts, thus yielding post-translational control as a mechanism for nanotopography-induced regulation of cell fate. Specifically, we observed an up-regulation of C/D box snoRNAs (SNORDs) in adult skeletal stem cells seeded on a square arrangement of nanopits in comparison to a near square (displaced by 50 nm) arrangement.[30] These small RNA have been implicated in stem cell pluripotency and differentiation.[117,118]

Fine tuning of the nanomaterial or nanotopography may be guided by current knowledge of ESC biology since intracellular signaling pathways, proteomics, and genomics underlying the maintenance of self-renewal, lineage commitment, and directed differentiation under typical culture conditions or soluble factor-induced differentiation are, in comparison, well-defined for both mESCs and hESCs. Our recent work offers possible clues as to the pattern order and disorder within a biomiometic physiological context. We have previously highlighted differential control of cell fate from a nonbiomimetic state of absolute order to a biological level of nano-order (*i.e.*, displacement of nanopits from an ordered square to a near square arrangement by displacement of 50 nm) that results in a switch from maintenance of the adult skeletal stem cell state to directed osteogenic differentiation.[30,119] Whether the same phenomenon exists in pluripotent cells is currently under investigation within our laboratory. Furthermore, there exists an untapped potential to generate scaffolds with select nanotopographical cues for directed differentiation. This would build on the studies of Unadkat *et al.*,[120] providing improved surfaces with better differentiation directing efficiency of ESCs and iPSCs for tissue formation both *ex vivo* and *in vivo*. Moreover, future developments in cell deformation and cytoskeletal component nanomechanics may assist in expanding our understanding of how cells sense their nano-niche.

While ESCs provide a satisfactory alternative to adult stem cells, patient-specific iPSC-derived cell types may further overcome ethical and immune rejection issues. However, the challenges faced in bringing iPSC-derived cells and patient-specific strategies using iPSC-derived cells into clinical use are likely to be similar to those for using hESC-derived cell types as therapies with the additional challenge of optimizing a high-throughput method for efficient, homogeneous reprogramming of a patient's somatic cell sample.[17,18] Since iPSCs have a growing number of reported differences to ESCs, it is important that iPSCs continue to be investigated alongside ESCs. If these differences are not detrimental to therapeutic potential, iPSCs could indeed be used to generate patient-specific regenerated tissues for therapy.[16] High-efficiency, viral-free methods for gene transfer are extremely desirable for both cell-based therapies and reprogramming of somatic cells to iPSCs. A number of reports claim to have improved the transfer of genetic material into cells using nanoparticles; however, the full extent of the long-term effects, accumulation, and genotoxicity of nanoparticles in transplanted cells (and systemically in patients) remains to be clarified. Furthermore, other nanomaterials may pose toxicity risks. This has already been shown for multiwalled carbon nanotubes. DNA damage was reported in mESCs, in the form of enhanced p53 expression and increased expression of double-strand break repair proteins, detectable within 4 h of exposure.[121] In addition, single-walled carbon nanotubes were reported to induce early miscarriages and fetal malformations following exposure in female mice together with indications of embryotoxicity in mESCs.[122] However, more focus has recently been placed on improving the biocompatibility and reducing the toxicity of nanotubes for medical applications (reviewed by Bianco and colleagues in ref 123 and references therein).

Efficient production of functionally stable ESC/iPSC-derived cell types remains a significant challenge to regenerative medicine. Moreover, for transplantation, ESC/iPSC-derived cell types need to be comparable to their endogenous counterparts. Recently, a differential capacity for ESC-derived osteogenic cells and human bone-marrow-derived osteogenic cells to deposit mature bone tissue *in vivo* was highlighted.[124]

ESC lines are known to display differences, as a consequence of culture conditions, in genetic and epigenetic stability and genetic variation.[125] This diversity may also apply to the manner in which, and the extent to which, nanomaterials may be able to manipulate the fate of different ESC lines. Furthermore, iPSC research may reveal a similar problem in both patient-to-patient variability as well as the somatic cell type used to form iPSCs, which could in turn alter their response to manipulation with nanomaterials. Undoubtedly, ethical implications are likely to arise as the medical use of nanotechnology and ESCs in combination is realized.[126] To date, reports defining the finer moral and ethical details of this emerging area of therapy remain limited.

CONCLUSION

Innovative nanotechnology platforms and novel stem cell sources continue to enhance interest within this area, in particular, the use of nanomaterials for the investigation and medical applications of ESCs. Regenerative medicine as a whole faces a significant challenge with multiple complexities given the diverse range of tissue types within the human body: hard, soft, contractible, electrically active, 2D layered, and 3D architecture. Fortunately, nanomaterials offer the possibility to form novel scaffolds, medical device surfaces, and/or particles that can influence stem cell fate in an already demonstrated diverse manner. Conceivably, an ECM-mimicking approach, as has been used to fabricate nanomaterials for adult stem or somatic cell studies, will also prove useful for cellular expansion of self-renewing, pluripotent ESCs. Designing a nanoscaffold that creates an environment recapitulating *in vivo* developmental progression or the transfer of genetic material by nanoparticles, guided by our current understanding of ESC biology, may prove to be intuitive approaches for efficient directed differentiation. Looking to the future, a fully collaborative approach

between the disciplines of regenerative medicine, stem cell biology, and nanotechnology will be most beneficial in deriving strategies for tissue augmentation and, ultimately, clinical benefit.

Conflict of Interest: The authors declare no competing financial interest.

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REFERENCES AND NOTES

1. Huebsch, N.; Mooney, D. J. Inspiration and Application in the Evolution of Biomaterials. *Nature* **2009**, *462*, 426–432.
2. Evans, M.; Kaufman, M. Establishment in Culture of Pluripotential Cells from Mouse Embryos. *Nature* **1981**, *292*, 154–156.
3. Thomson, J.; Itskovitz-Eldor, J.; Shapiro, S.; Waknitz, M.; Swiergiel, J.; Marshall, V. S.; Jones, J. M. Embryonic Stem Cell Lines Derived from Human Blastocysts. *Science* **1998**, *282*, 1145–1147.
4. Martin, G. Isolation of a Pluripotent Cell Line from Early Mouse Embryos Cultured in Medium Conditioned by Teratocarcinoma Stem Cells. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 7634–7638.
5. Bradley, A.; Evans, M.; Kaufman, M. H.; Robertson, E. Formation of Germ-Line Chimaeras from Embryo-Derived Teratocarcinoma Cell Lines. *Nature* **1984**, *309*, 255–256.
6. Drukker, M.; Katz, G.; Urbach, A.; Schuldiner, M.; Markel, G.; Itskovitz-Eldor, J.; Reubinoff, B.; Mandelboim, O.; Benvenisty, N. Characterization of the Expression of MHC Proteins in Human Embryonic Stem Cells. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 9864–9869.
7. Fändrich, F.; Lin, X.; Chai, G.; Schulze, M.; Ganten, D.; Bader, M.; Holle, J.; Huang, D. S.; Parwaresch, R.; Zavazava, N.; *et al.* Preimplantation-Stage Stem Cells Induce Long-Term Allogeneic Graft Acceptance without Supplementary Host Conditioning. *Nat. Med.* **2002**, *8*, 171–178.
8. Li, L.; Baroja, M.; Majumdar, A.; Chadwick, K.; Rouleau, A.; Gallacher, L.; Ferber, I.; Lebkowski, J.; Martin, T.; Madrenas, J.; *et al.* Human Embryonic Stem Cells Possess Immune-Privileged Properties. *Stem Cells* **2004**, *22*, 448–456.
9. Yen, B.; Chang, C.; Liu, K.-J.; Chen, Y.; Hu, H.-I.; Bai, C.-H.; Yen, M.-L. Brief Report—Human Embryonic Stem Cell-Derived Mesenchymal Progenitors Possess Strong Immunosuppressive Effects toward Natural Killer Cells as Well as T Lymphocytes. *Stem Cells* **2009**, *27*, 451–456.
10. Ghannam, S.; Bouffi, C.; Djouad, F.; Jorgensen, C.; Noël, D. Immunosuppression by Mesenchymal Stem Cells: Mechanisms and Clinical Applications. *Stem Cell Res. Ther.* **2010**, *1*, 1–7.
11. Takahashi, K.; Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell* **2006**, *126*, 663–676.
12. Okita, K.; Ichisaka, T.; Yamanaka, S. Generation of Germline-Competent Induced Pluripotent Stem Cells. *Nature* **2007**, *448*, 313–317.
13. Nakagawa, M.; Koyanagi, M.; Tanabe, K.; Takahashi, K.; Ichisaka, T.; Aoi, T.; Okita, K.; Mochiduki, Y.; Takizawa, N.; Yamanaka, S. Generation of Induced Pluripotent Stem Cells without Myc from Mouse and Human Fibroblasts. *Nat. Biotechnol.* **2008**, *26*, 101–106.
14. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell* **2007**, *131*, 861–872.
15. O'Malley, J.; Woltjen, K.; Kaji, K. New Strategies To Generate Induced Pluripotent Stem Cells. *Curr. Opin. Biotechnol.* **2009**, *20*, 516–521.
16. Condic, M.; Rao, M. Regulatory Issues for Personalized Pluripotent Cells. *Stem Cells* **2008**, *26*, 2753–2758.
17. Amabile, G.; Meissner, A. Induced Pluripotent Stem Cells: Current Progress and Potential for Regenerative Medicine. *Trends Mol. Med.* **2009**, *15*, 59–68.
18. Lee, H.; Park, J.; Forget, B.; Gaines, P. Induced Pluripotent Stem Cells in Regenerative Medicine: An Argument for Continued Research on Human Embryonic Stem Cells. *Regen. Med.* **2009**, *4*, 759–769.
19. Mimeault, M.; Hauke, R.; Batra, S. Stem Cells: A Revolution in Therapeutics—Recent Advances in Stem Cell Biology and Their Therapeutic Applications in Regenerative Medicine and Cancer Therapies. *Clin. Pharmacol. Ther.* **2007**, *82*, 252–264.
20. Keller, G. Embryonic Stem Cell Differentiation: Emergence of a New Era in Biology and Medicine. *Genes Dev.* **2005**, *19*, 1129–1155.
21. Stewart, M.; Bendall, S.; Bhatia, M. Deconstructing Human Embryonic Stem Cell Cultures: Niche Regulation of Self-Renewal and Pluripotency. *J. Mol. Med.* **2008**, *86*, 875–886.
22. Bendall, S.; Stewart, M.; Menendez, P.; George, D.; Vijayaragavan, K.; Werbowetski-Ogilvie, T.; Ramos-Mejia, V.; Rouleau, A.; Yang, J.; Bossé, M.; *et al.* IGF and FGF Cooperatively Establish the Regulatory Stem Cell Niche of Pluripotent Human Cells *in Vitro*. *Nature* **2007**, *448*, 1015–1021.
23. Dalby, M.; Gadegaard, N.; Curtis, A.; Oreffo, R. Nanotopographical Control of Human Osteoprogenitor Differentiation. *Curr. Stem Cell Res. Ther.* **2007**, *2*, 129–138.
24. Discher, D.; Mooney, D.; Zandstra, P. Growth Factors, Matrices, and Forces Combine and Control Stem Cells. *Science* **2009**, *324*, 1673–1677.
25. Engler, A.; Sen, S.; Sweeney, H.; Discher, D. Matrix Elasticity Directs Stem Cell Lineage Specification. *Cell* **2006**, *126*, 677–689.
26. McBeath, R.; Pirone, D.; Nelson, C.; Bhadriraju, K.; Chen, C. Cell Shape, Cytoskeletal Tension, and RhoA Regulate Stem Cell Lineage Commitment. *Dev. Cell* **2004**, *6*, 483–495.
27. Kilian, K. A.; Bugarija, B.; Lahn, B. T.; Mrksich, M. Geometric Cues for Directing the Differentiation of Mesenchymal Stem Cells. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, *107*, 4872–4877.
28. Benoit, D. S.; Schwartz, M. P.; Durney, A. R.; Anseth, K. S. Small Functional Groups for Controlled Differentiation of Hydrogel-Encapsulated Human Mesenchymal Stem Cells. *Nat. Mater.* **2008**, *7*, 816–823.
29. Curran, J. M.; Chen, R.; Hunt, J. A. The Guidance of Human Mesenchymal Stem Cell Differentiation *in Vitro* by Controlled Modifications to the Cell Substrate. *Biomaterials* **2006**, *27*, 4783–4793.
30. McMurray, R. J.; Gadegaard, N.; Tsimbouri, P. M.; Burgess, K. V.; McNamara, L. E.; Tare, R.; Murawski, K.; Kingham, E.; Oreffo, R. O.; Dalby, M. J. Nanoscale Surfaces for the Long-Term Maintenance of Mesenchymal Stem Cell Phenotype and Multipotency. *Nat. Mater.* **2011**, *10*, 637–644.
31. Sun, Y.; Villa-Diaz, L. G.; Lam, R. H.; Chen, W.; Krebsbach, P. H.; Fu, J. Mechanics Regulates Fate Decisions of Human Embryonic Stem Cells. *PLoS One* **2012**, *7*, e37178.
32. Sun, Y.; Chen, C. S.; Fu, J. Forcing Stem Cells To Behave: A Biophysical Perspective of the Cellular Microenvironment. *Annu. Rev. Biophys.* **2012**, *41*, 519–542.
33. Trounson, A. The Production and Directed Differentiation of Human Embryonic Stem Cells. *Endocr. Rev.* **2006**, *27*, 208–219.
34. Grinnell, F.; Bennett, M. Ultrastructural Studies of Cell–Collagen Interactions. *Methods Enzymol.* **1982**, *82*, 535–544.
35. Domogatskaya, A.; Rodin, S.; Boutaud, A.; Tryggvason, K. Laminin-511 but Not -332, -111, or -411 Enables Mouse Embryonic Stem Cell Self-Renewal *in Vitro*. *Stem Cells* **2008**, *26*, 2800–2909.
36. Li, S.; Edgar, D.; Fässler, R.; Wadsworth, W.; Yurchenco, P. The Role of Laminin in Embryonic Cell Polarization and Tissue Organization. *Dev. Cell* **2003**, *4*, 613–624.

37. Amit, M.; Shariki, C.; Margulets, V.; Itskovitz-Eldor, J. Feeder Layer- and Serum-Free Culture of Human Embryonic Stem Cells. *Biol. Reprod.* **2004**, *70*, 837–845.
38. Xu, C.; Inokuma, M.; Denham, J.; Golds, K.; Kundu, P.; Gold, J.; Carpenter, M. K. Feeder-Free Growth of Undifferentiated Human Embryonic Stem Cells. *Nat. Biotechnol.* **2001**, *19*, 971–974.
39. Philp, D.; Chen, S.; Fitzgerald, W.; Orenstein, J.; Margolis, L.; Kleinman, H. Complex Extracellular Matrices Promote Tissue-Specific Stem Cell Differentiation. *Stem Cells* **2005**, *23*, 288–296.
40. Wei, G.; Ma, P. Nanostructured Biomaterials for Regeneration. *Adv. Funct. Mater.* **2008**, *18*, 3566–3582.
41. Eiges, R.; Schuldiner, M.; Drukker, M.; Yanuka, O.; Itskovitz-Eldor, J.; Benvenisty, N. Establishment of Human Embryonic Stem Cell-Transfected Clones Carrying a Marker for Undifferentiated Cells. *Curr. Biol.* **2001**, *11*, 514–518.
42. Ferreira, L.; Squier, T.; Park, H.; Choe, H.; Kohane, D.; Langer, R. Human Embryoid Bodies Containing Nano- and Microparticulate Delivery Vehicles. *Adv. Mater.* **2008**, *20*, 2285–2291.
43. 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.* **2006**, *350*, 514–520.
44. Kutsuzawa, K.; Akaike, T.; Chowdhury, E. 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* **2008**, *29*, 370–376.
45. Kutsuzawa, K.; Maruyama, K.; Akiyama, Y.; Akaike, T.; Chowdhury, E. Efficient Transfection of Mouse Embryonic Stem Cells with Cell-Adhesive Protein-Embedded Inorganic Nanocarrier. *Anal. Biochem.* **2008**, *372*, 122–124.
46. Green, J.; Zhou, B.; Mitalipova, M.; Beard, C.; Langer, R.; Jaenisch, R.; Anderson, D. G. Nanoparticles for Gene Transfer to Human Embryonic Stem Cell Colonies. *Nano Lett.* **2008**, *8*, 3126–3130.
47. Green, J. J.; Zugates, G. T.; Tedford, N. C.; Huang, Y.-H.; Griffith, L. G.; Lauffenburger, D. A.; Sawicki, J. A.; Langer, R.; Anderson, D. G. Combinatorial Modification of Degradable Polymers Enables Transfection of Human Cells Comparable to Adenovirus. *Adv. Mater.* **2007**, *19*, 2836–2842.
48. Ruan, J.; Shen, J.; Wang, Z.; Ji, J.; Song, H.; Wang, K.; Liu, B.; Li, J.; Cui, D. Efficient Preparation and Labeling of Human Induced Pluripotent Stem Cells by Nanotechnology. *Int. J. Nanomed.* **2011**, *6*, 425–435.
49. Lee, C. H.; Kim, J. H.; Lee, H. J.; Jeon, K.; Lim, H.; Choi, H.; Lee, E. R.; Park, S. H.; Park, J. Y.; Hong, S.; *et al.* The Generation of iPS Cells Using Non-viral Magnetic Nanoparticle Based Transfection. *Biomaterials* **2011**, *32*, 6683–6691.
50. Hoehn, M.; Küstermann, E.; Blunk, J.; Wiedermann, D.; Trapp, T.; Wecker, S.; Föcking, M.; Arnold, H.; Hescheler, J.; Fleischmann, B. K.; *et al.* Monitoring of Implanted Stem Cell Migration *in Vivo*: A Highly Resolved *In Vivo* Magnetic Resonance Imaging Investigation of Experimental Stroke in Rat. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16267–16272.
51. Arai, T.; Kofidis, T.; Bulte, J. W.; de Bruin, J.; Venook, R. D.; Berry, G. J.; Mcconnell, M. V.; Quertermous, T.; Robbins, R. C.; Yang, P. C. Dual *In Vivo* Magnetic Resonance Evaluation of Magnetically Labeled Mouse Embryonic Stem Cells and Cardiac Function at 1.5 t. *Magn. Reson. Med.* **2006**, *55*, 203–209.
52. Au, K. W.; Liao, S. Y.; Lee, Y. K.; Lai, W. H.; Ng, K. M.; Chan, Y. C.; Yip, M. C.; Ho, C. Y.; Wu, E. X.; Li, R. A.; *et al.* Effects of Iron Oxide Nanoparticles on Cardiac Differentiation of Embryonic Stem Cells. *Biochem. Biophys. Res. Commun.* **2009**, *379*, 898–903.
53. Ahamed, M.; Karns, M.; Goodson, M.; Rowe, J.; Hussain, S. M.; Schlager, J. J.; Hong, Y. DNA Damage Response to Different Surface Chemistry of Silver Nanoparticles in Mammalian Cells. *Toxicol. Appl. Pharmacol.* **2008**, *233*, 404–410.
54. Park, M. V.; Annema, W.; Salvati, A.; Lesniak, A.; Elsaesser, A.; Barnes, C.; McKerr, G.; Howard, C. V.; Lynch, I.; Dawson, K. A.; *et al. In Vitro* Developmental Toxicity Test Detects Inhibition of Stem Cell Differentiation by Silica Nanoparticles. *Toxicol. Appl. Pharmacol.* **2009**, *240*, 108–116.
55. Tran, D.; Ota, L.; Jacobson, J.; Patton, W.; Chan, P. Influence of Nanoparticles on Morphological Differentiation of Mouse Embryonic Stem Cells. *Fertil. Steril.* **2007**, *87*, 965–970.
56. Donaldson, K.; Stone, V.; Tran, C.; Kreyling, W.; Borm, P. Nanotoxicology. *Occup. Environ. Med.* **2004**, *61*, 727–728.
57. Seaton, A.; Donaldson, K. Nanoscience, Nanotoxicology, and the Need To Think Small. *Lancet* **2005**, *365*, 923–924.
58. Savolainen, K.; Alenius, H.; Norppa, H.; Pylkkänen, L.; Tuomi, T.; Kasper, G. Risk Assessment of Engineered Nanomaterials and Nanotechnologies—A Review. *Toxicology* **2010**, *269*, 92–104.
59. Ferreira, L. Nanoparticles as Tools To Study and Control Stem Cells. *J. Cell. Biochem.* **2009**, *108*, 746–752.
60. Lin, S.; Xie, X.; Patel, M.; Yang, Y.; Li, Z.; Cao, F.; Gheysens, O.; Zhang, Y.; Gambhir, S. S.; Rao, J. H.; *et al.* Quantum Dot Imaging for Embryonic Stem Cells. *BMC Biotechnol.* **2007**, *7*, 67.
61. Nagesha, D.; Laevsky, G.; Lampton, P.; Banyal, R.; Warner, C.; DiMarzio, C.; Sridhar, S. *In Vitro* Imaging of Embryonic Stem Cells Using Multiphoton Luminescence of Gold Nanoparticles. *Int. J. Nanomed.* **2007**, *2*, 813–819.
62. Nur-E-Kamal, A.; Ahmed, I.; Kamal, J.; Schindler, M.; Meiners, S. Three-Dimensional Nanofibrillar Surfaces Promote Self-Renewal in Mouse Embryonic Stem Cells. *Stem Cells* **2006**, *24*, 426–433.
63. Kingham, E.; Welham, M. Distinct Roles for Isoforms of the Catalytic Subunit of Class-IA PI3K in the Regulation of Behaviour of Murine Embryonic Stem Cells. *J. Cell Sci.* **2009**, *122*, 2311–2321.
64. Paling, N.; Wheadon, H.; Bone, H.; Welham, M. Regulation of Embryonic Stem Cell Self-Renewal by Phosphoinositide 3-Kinase-Dependent Signaling. *J. Biol. Chem.* **2004**, *279*, 48063–48070.
65. Storm, M.; Bone, H.; Beck, C.; Bourillot, P.; Schreiber, V.; Damiano, T.; Nelson, A.; Savatier, P.; Welham, M. J. Regulation of Nanog Expression by Phosphoinositide 3-Kinase-Dependent Signaling in Murine Embryonic Stem Cells. *J. Biol. Chem.* **2007**, *282*, 6265–6273.
66. Sun, H.; Lesche, R.; Li, D.; Liliental, J.; Zhang, H.; Gao, J.; Gavrilova, N.; Mueller, B.; Liu, X.; Wu, H. PTEN Modulates Cell Cycle Progression and Cell Survival by Regulating Phosphatidylinositol 3,4,5,-trisphosphate and Akt/Protein Kinase B Signaling Pathway. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 6199–6204.
67. Jirmanova, L.; Afanassieff, M.; Gobert-Gosse, S.; Markossian, S.; Savatier, P. Differential Contributions of ERK and PI3-Kinase to the Regulation of Cyclin D1 Expression and to the Control of the G1/S Transition in Mouse Embryonic Stem Cells. *Oncogene* **2002**, *21*, 5515–5528.
68. Ji, L.; LaPointe, V. L.; Evans, N. D.; Stevens, M. M. Changes in Embryonic Stem Cell Colony Morphology and Early Differentiation Markers Driven by Colloidal Crystal Topographical Cues. *Eur. Cell Mater.* **2012**, *23*, 135–146.
69. Chen, G.; Pang, D.; Hwang, S.; Tuan, H.; Hu, Y. A Graphene-Based Platform For Induced Pluripotent Stem Cells Culture and Differentiation. *Biomaterials* **2012**, *33*, 418–427.
70. Levenstein, M.; Ludwig, T.; Xu, R.; Llanas, R.; VanDenHeuvel-Kramer, K.; Manning, D.; Thomson, J. A. Basic Fibroblast Growth Factor Support of Human Embryonic Stem Cell Self-Renewal. *Stem Cells* **2006**, *24*, 568–574.
71. Wang, L.; Li, L.; Menendez, P.; Cerdan, C.; Bhatia, M. Human Embryonic Stem Cells Maintained in the Absence of Mouse Embryonic Fibroblasts or Conditioned

Media are Capable of Hematopoietic Development. *Blood* **2005**, *105*, 4598–45603.
72. Nur-E-Kamal, A.; Ahmed, I.; Kamal, J.; Babu, A.; Schindler, M.; Meiners, S. Covalently Attached FGF-2 to Three-Dimensional Polyamide Nanofibrillar Surfaces Demonstrates Enhanced Biological Stability and Activity. *Mol. Cell. Biochem.* **2008**, *309*, 157–166.
73. Gerecht, S.; Bettinger, C.; Zhang, Z.; Borenstein, J.; Vunjak-Novakovic, G.; Langer, R. The Effect of Actin Disrupting Agents on Contact Guidance of Human Embryonic Stem Cells. *Biomaterials* **2007**, *28*, 4068–4077.
74. Hakala, H.; Rajala, K.; Ojala, M.; Panula, S.; Areva, S.; Kellomäki, M.; Suuronen, R.; Skottman, H. Comparison of Biomaterials and Extracellular Matrices as a Culture Platform for Multiple, Independently Derived Human Embryonic Stem Cell Lines. *Tissue Eng., Part A* **2009**, *15*, 1775–1785.
75. Chen, W.; Villa-Diaz, L. G.; Sun, Y.; Weng, S.; Kim, J. K.; Lam, R. H.; Han, L.; Fan, R.; Krebsbach, P. H.; Fu, J. Nanotopography Influences Adhesion, Spreading, and Self-Renewal of Human Embryonic Stem Cells. *ACS Nano* **2012**, *6*, 4094–4103.
76. Villa-Diaz, L. G.; Nandivada, H.; Ding, J.; Nogueira-de-Souza, N. C.; Krebsbach, P. H.; O'Shea, K. S.; O'Shea, K. S.; Lahann, J.; Smith, G. D. Synthetic Polymer Coatings for Long-Term Growth of Human Embryonic Stem Cells. *Nat. Biotechnol.* **2010**, *28*, 581–583.
77. Klim, J. R.; Li, L.; Wrighton, P. J.; Piekarczyk, M. S.; Kiessling, L. L. A Defined Glycosaminoglycan-Binding Substratum for Human Pluripotent Stem Cells. *Nat. Methods* **2010**, *7*, 989–994.
78. Melkoumian, Z.; Weber, J. L.; Weber, D. M.; Fadeev, A. D.; Zhou, Y.; Dolley-Sonneville, P.; Yang, J.; Qiu, L.; Priest, C. A.; Shogbon, C.; *et al.* Synthetic Peptide-Acrylate Surfaces for Long-Term Self-Renewal and Cardiomyocyte Differentiation of Human Embryonic Stem Cells. *Nat. Biotechnol.* **2010**, *26*, 606–610.
79. Rosenberg, M. Cell Guidance by Alterations in Monomolecular Films. *Science* **1963**, *139*, 411–412.
80. Curtis, A.; Varde, M. Control of Cell Behaviours: Topographical Factors. *J. Natl. Cancer Inst.* **1964**, *33*, 15–26.
81. Bain, G.; Kitchens, D.; Yao, M.; Huettner, J.; Gottlieb, D. Embryonic Stem Cells Express Neuronal Properties *in Vitro*. *Dev. Biol.* **1995**, *168*, 342–357.
82. Fraichard, A.; Chassande, O.; Bilbaut, G.; Dehay, C.; Savatier, P.; Samarut, J. *In Vitro* Differentiation of Embryonic Stem Cells into Glial Cells and Functional Neurons. *J. Cell Sci.* **1995**, *108*, 3181–3188.
83. Strübing, C.; Ahnert-Hilger, G.; Shan, J.; Wiedenmann, B.; Hescheler, J.; Wobus, A. Differentiation of Pluripotent Embryonic Stem Cells into the Neuronal Lineage *in Vitro* Gives Rise to Mature Inhibitory and Excitatory Neurons. *Mech. Dev.* **1995**, *53*, 275–287.
84. Xie, J.; Willerth, S.; Li, X.; Macewan, M.; Rader, A.; Sakiyama-Elbert, S.; Xia, Y. The Differentiation of Embryonic Stem Cells Seeded on Electrospun Nanofibers into Neural Lineages. *Biomaterials* **2009**, *30*, 354–362.
85. Kabiri, M.; Soleimani, M.; Shabani, I.; Futrega, K.; Ghaemi, N.; Ahvaz, H. H.; Elahi, E.; Doran, M. R. Neural Differentiation of Mouse Embryonic Stem Cells on Conductive Nanofiber Scaffolds. *Biotechnol. Lett.* **2012**, *34*, 1357–1365.
86. Chao, T.; Xiang, S.; Chen, C.; Chin, W.; Nelson, A.; Wang, C.; Lu, J. Carbon Nanotubes Promote Neuron Differentiation from Human Embryonic Stem Cells. *Biochem. Biophys. Res. Commun.* **2009**, *384*, 426–430.
87. Chen, C. S.; Soni, S.; Le, C.; Biasca, M.; Farr, E.; Chen, E. Y.; Chin, W. C. Human Stem Cell Neuronal Differentiation on Silk-Carbon Nanotube Composite. *Nanoscale Res Lett.* **2012**, *7*, 126.
88. Sridharan, I.; Kim, T.; Wang, R. Adapting Collaged/CNT Matrix in Directing hESC Differentiation. *Biochem. Biophys. Res. Commun.* **2009**, *381*, 508–512.
89. Lee, M.; Kwon, K.; Jung, H.; Kim, H.; Suh, K.; Kim, K.; Kim, K. S. Direct Differentiation of Human Embryonic Stem Cells into Selective Neurons on Nanoscale Ridge/Groove Pattern Arrays. *Biomaterials* **2010**, *31*, 4360–4366.
90. Muñoz-Sanjuán, I.; Brivanlou, A. Neural Induction, the Default Model and Embryonic Stem Cells. *Nat. Rev. Neurosci.* **2002**, *3*, 271–280.
91. Bouhon, I.; Kato, H.; Chandran, S.; Allen, N. Neural Differentiation of Mouse Embryonic Stem Cells in Chemically Defined Medium. *Brain Res. Bull.* **2005**, *68*, 62–75.
92. 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* **2009**, *31*, 1742–1750.
93. Evans, N. D.; Minelli, C.; Gentleman, E.; LaPointe, V.; Patankar, S.; Kallivretaki, M.; Chen, X.; Roberts, C.; Stevens, M. Substrate Stiffness Affects Early Differentiation Events in Embryonic Stem Cells. *Eur. Cells Mater.* **2009**, *18*, 1–13.
94. Hayashi, K.; Lopes, S.; Tang, F.; Surani, M. Dynamic Equilibrium and Heterogeneity of Mouse Pluripotent Stem Cells with Distinct Functional and Epigenetic States. *Cell Stem Cell* **2008**, *3*, 391–401.
95. Chowdhury, F.; Na, S.; Li, D.; Poh, Y.; Tanaka, T.; Wang, F.; Wang, N. Material Properties of the Cell Dictate Stress-Induced Spreading and Differentiation in Embryonic Stem Cells. *Nat. Mater.* **2010**, *9*, 82–88.
96. Chowdhury, F.; Li, Y.; Poh, Y.; Yokohama-Tamaki, T.; Wang, N.; Tanaka, T. Soft Substrates Promote Homogeneous Self-Renewal of Embryonic Stem Cells *via* Downregulating Cell-Matrix Tractions. *PloS One* **2010**, *5*, e15655.
97. Musah, S.; Morin, S. A.; Wrighton, P. J.; Zwick, D. B.; Jin, S.; Kiessling, L. L. Glycosaminoglycan-Binding Hydrogels Enable Mechanical Control of Human Pluripotent Stem Cell Self-Renewal. *ACS Nano* **2012**, *6*, 10168–10177.
98. Garreta, E.; Genové, E.; Borrós, S.; Semino, C. Osteogenic Differentiation of Mouse Embryonic Stem Cells and Mouse Embryonic Fibroblasts in a Three-Dimensional Self-Assembling Peptide Scaffold. *Tissue Eng.* **2006**, *12*, 2215–2227.
99. Garreta, E.; Gasset, D.; Semino, C.; Borrós, S. Fabrication of a Three-Dimensional Nanostructured Biomaterial for Tissue Engineering of Bone. *Biomol. Eng.* **2007**, *24*, 75–80.
100. Smith, L.; Liu, X.; Hu, J.; Ma, P. The Influence of Three-Dimensional Nanofibrous Scaffolds on the Osteogenic Differentiation of Embryonic Stem Cells. *Biomaterials* **2009**, *30*, 2516–2522.
101. Woo, D. G.; Shim, M.-S.; Park, J. S.; Yang, H. N.; Lee, D. R.; Park, K.-H. The Effect of Electrical Stimulation on the Differentiation of hESCs Adhered onto Fibronectin-Coated Gold Nanoparticles. *Biomaterials* **2009**, *30*, 5631–5638.
102. Smith, L.; Liu, X.; Hu, J.; Wang, P.; Ma, P. Enhancing Osteogenic Differentiation of Mouse Embryonic Stem Cells by Nanofibers. *Tissue Eng., Part A* **2009**, *15*, 1855–1864.
103. Farzaneh, Z.; Pournasr, B.; Ebrahimi, M.; Aghdami, N.; Baharvand, H. Enhanced Functions of Human Embryonic Stem Cell-Derived Hepatocyte-like Cells on Three-Dimensional Nanofibrillar Surfaces. *Stem Cell Rev.* **2010**, *6*, 601–610.
104. Kingham, E.; White, K.; Gadegaard, N.; Dalby, M. J.; Oreffo, R. O. C. Nanotopographical Cues Augment Mesenchymal Differentiation of Human Embryonic Stem Cells. *Small* **2013**, doi: 10.1002/smll.201202340.
105. Dawson, J. I.; Kingham, E.; Evans, N. R.; Tayton, E.; Oreffo, R. O. C. Skeletal Regeneration: Application of Nanotopography and Biomaterials for Skeletal Stem Cell Based Bone Repair. *Inflammation Regener.* **2012**, *32*, 72–89.
106. Davis, M. E.; Motion, J. P.; Narmoneva, D. A.; Takahashi, T.; Hakuno, D.; Kamm, R. D.; Zhang, S.; Lee, R. T. Injectable Self-Assembling Peptide Nanofibers Create Intramyocardial Microenvironments for Endothelial Cells. *Circulation* **2005**, *111*, 442–450.
107. Davey, R.; Zandstra, P. Spatial Organization of Embryonic Stem Cell Responsiveness to Autocrine gp130 Ligands

Reveals an Autoregulatory Stem Cell Niche. *Stem Cells* **2006**, *24*, 2538–2548.
108. Peerani, R.; Rao, B.; Bauwens, C.; Yin, T.; Wood, G.; Nagy, A.; Kumacheva, E.; Zandstra, P. W. Niche-Mediated Control of Human Embryonic Stem Cell Self-Renewal and Differentiation. *EMBO J.* **2007**, *26*, 4744–4755.
109. Charitidis, C. Nanoscale Deformation and Nanomechanical Properties of Soft Matter Study Cases: Polydimethylsiloxane, Cells and Tissues. *ISRN Nanotechnol.* **2011**, 13.
110. Qin, Z.; Kreplak, L.; Buehler, M. J. Hierarchical Structure Controls Nanomechanical Properties of Vimentin Intermediate Filaments. *PLoS One* **2009**, *4*, e7294.
111. Nakamura, Y.; Hashimoto, R.; Amano, M.; Nagata, K.; Matsumoto, N.; Goto, H.; Fukusho, E.; Mori, H.; Kashiwagi, Y.; Kudo, T.; *et al.* Localized Phosphorylation of Vimentin by Rho-Kinase in Neuroblastoma N2a Cells. *Genes Cells* **2000**, *5*, 823–837.
112. Krieg, M.; Arboleda-Estudillo, Y.; Puech, P.; Käfer, J.; Graner, F.; Müller, D.; Heisenberg, C. P. Tensile Forces Govern Germ-Layer Organization in Zebrafish. *Nat. Cell Biol.* **2008**, *10*, 429–436.
113. Burdon, T.; Stracey, C.; Chambers, I.; Nichols, J.; Smith, A. Suppression of SHP-2 and ERK Signalling Promotes Self-Renewal of Mouse Embryonic Stem Cells. *Dev. Biol.* **1999**, *210*, 30–43.
114. Kunath, T.; Saba-El-Leil, M. K.; Almousailleakh, M.; Wray, J.; Meloche, S.; Smith, A. FGF Stimulation of the Erk1/2 Signalling Cascade Triggers Transition of Pluripotent Embryonic Stem Cells from Self-Renewal to Lineage Commitment. *Development* **2007**, *134*, 2895–2902.
115. Li, J.; Wang, G.; Wang, C.; Zhao, Y.; Zhang, H.; Tan, Z.; Song, Z.; Ding, M.; Deng, H. MEK/ERK Signaling Contributes to the Maintenance of Human Embryonic Stem Cell Self-Renewal. *Differentiation* **2007**, *75*, 299–307.
116. Katsumi, A.; Orr, A. W.; Tzima, E.; Schwartz, M. A. Integrins in Mechanotransduction. *J. Biol. Chem.* **2004**, *279*, 12001–12004.
117. Gangaraju, V. K.; Lin, H. MicroRNAs: Key Regulators of Stem Cells. *Nat. Rev. Mol. Cell. Biol.* **2009**, *10*, 116–125.
118. Stadler, B. M.; Ruohola-Baker, H. Small RNAs: Keeping Stem Cells in Line. *Cell.* **2008**, *132*, 563–566.
119. Dalby, M.; Gadegaard, N.; Tare, R.; Andar, A.; Riehle, M.; Herzyk, P.; Wilkinson, C. D. W.; Oreffo, R. O. C. The Control of Human Mesenchymal Cell Differentiation Using Nanoscale Symmetry and Disorder. *Nat. Mater.* **2007**, *6*, 997–1003.
120. Unadkat, H. V.; Hulsman, M.; Cornelissen, K.; Papenburg, B. J.; Truckenmüller, R. K.; Post, G. F.; Uetz, M.; Reindersb, M. J. T.; Stamatialisd, D.; van Blitterswijka, C. A.; *et al.* An Algorithm-Based Topographical Biomaterials Library To Instruct Cell Fate. *Proc. Natl. Acad. Sci. U.S.A.* **2011**, *108*, 16565–16570.
121. Zhu, L.; Chang, D.; Dai, L.; Hong, Y. DNA Damage Induced by Multiwalled Carbon Nanotubes in Mouse Embryonic Stem Cells. *Nano Lett.* **2007**, *7*, 3592–3597.
122. Pietroiusti, A.; Massimiani, M.; Fenoglio, I.; Colonna, M.; Valentini, F.; Palleschi, G.; Camaioni, A.; Magrini, A.; Siracusa, G.; Bergamaschi, A.; *et al.* Low Doses of Pristine and Oxidized Single-Wall Carbon Nanotubes Affect Mammalian Embryonic Ddevelopment. *ACS Nano* **2011**, *5*, 4624–4633.
123. Bianco, A.; Kostarelos, K.; Prato, M. Making Carbon Nanotubes Biocompatible and Biodegradable. *Chem. Commun.* **2011**, *47*, 10182–10188.
124. Both, S. K.; van Apeldoorn, A. A.; Jukes, J. M.; Englund, M. C.; Hyllner, J.; van Blitterswijk, C. A.; de Boer, J. Differential Bone-Forming Capacity of Osteogenic Cells from Either Embryonic Stem Cells or Bone Marrow-Derived Mesenchymal Stem Cells. *J. Tissue Eng. Regen. Med.* **2011**, *5*, 180–190.
125. Allegrucci, C.; l Young, L. Differences between Human Embryonic Stem Cell Lines. *Hum. Reprod. Update* **2007**, *13*, 103–120.
126. Ebbesen, M.; Jensen, T. Nanomedicine: Techniques, Potentials, and Ethical Implications. *J. Biomed. Biotechnol.* **2006**, 51516.