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Materials as stem cell regulators

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The stem cell/material interface is a complex, dynamic microenvironment in which the cell and the material cooperatively dictate one another's fate: the cell by remodelling its surroundings, and the material through its inherent properties (such as adhesivity, stiffness, nanostructure or degradability). Stem cells in contact with materials are able to sense their properties, integrate cues via signal propagation and ultimately translate parallel signalling information into cell fate decisions. However, discovering the mechanisms by which stem cells respond to inherent material characteristics is challenging because of the highly complex, multicomponent signalling milieu present in the stem cell environment. In this Review, we discuss recent evidence that shows that inherent material properties may be engineered to dictate stem cell fate decisions, and overview a subset of the operative signal transduction mechanisms that have begun to emerge. Further developments in stem cell engineering and mechanotransduction are poised to have substantial implications for stem cell biology and regenerative medicine.

Protocols used to induce stem cell differentiation have historically relied on biochemical supplements, such as animal products, recombinant growth factors or nucleic acids. However, it is increasingly clear that inherent factors always present in the environment of the cell — whether they are intentionally controlled or not — have a substantial influence on stem cell phenotype. These inherent factors are characteristic attributes of the materials in the cell's environment, and developments in the past few years have emphasized that they can influence stem cell behaviour with a potency that rivals that of biochemical supplements. Indeed, recent studies have advanced the hypothesis that the inherent properties of synthetic materials can influence, and perhaps even induce, lineage-specific stem cell differentiation by virtue of their inherent stiffness, molecular flexibility, nanotopography, cell adhesiveness, binding affinity, chemical functionality, degradability and/or degradation by-products (Fig. 1). The diversity of inherent material properties known to influence stem cell fate represents a tremendous opportunity for stem cell biologists and materials scientists to work collaboratively. There is also a critical need to more rigorously characterize the signalling pathways by which inherent material properties are transduced by cells to refine their use in directing cell fate specification.

Defining material properties

The physical and chemical properties of materials in the cellular environment are increasingly appreciated as key players in stem cell fate decisions. For example, recent studies have implicated various solid-phase material properties — presented to stem cells at the outset of cell culture — as critical elements of the stem cell environment (Fig. 2). Substrate mechanical stiffness[1,2], nanometre-scale topography[3–5] and simple chemical functionality[6,7] each impact human mesenchymal stem cell (hMSC) differentiation (Box 1). In the examples shown in Fig. 2, each of these factors has been tailored to promote hMSC differentiation into osteoblasts; however, they can be tailored to a variety of lineages. Other studies emphasize the cell's ability to redefine its own environment after the onset of cell culture (Fig. 3), including the ability to adhere within a defined cell area[8], occupy a defined cell shape[2,8,9], cluster tethered cell adhesion ligands[10], modulate extracellular matrix (ECM) protein organization[11], or degrade the material surrounding the cell and thereby exert traction forces[12]. These studies clearly show that materials can provide an environment that supports specific stem cell fate decisions in the presence of other co-factors, such as serum-containing cell culture medium or biochemical supplements. Importantly, these studies also demonstrate the intriguing concept that inherent factors that always exist during cell culture can be the determining factor as a cell undergoes a shift in phenotype. This is in contrast to the traditional view that these inherent factors simply provide a permissive context in which biochemical supplements (such as growth factors) do the heavy lifting of induced differentiation. Still, whereas a number of studies demonstrate that materials influence cell fate decisions, few examples so far have observed material-induced stem cell self-renewal or differentiation in completely chemically defined cell culture environments. The limited examples that have been shown so far feature pluripotent stem cell expansion on defined substrates[13–15]. Our ability to routinely control stem cell behaviour in chemically defined conditions using only inherent material properties will depend on a greater in-depth understanding of how, when and why materials influence stem cell behaviour.

Material dynamics

The properties of the stem cell/material interface are not static, and there are important stem cell regulators that arise from the inherent 'give and take' between cells and materials (Fig. 4). Materials can 'give' new signals to stem cells in the form of degradation by-products, which traditionally have been largely ignored, or viewed as contextual background components whose effects must be neutralized. The concept that a degradation by-product can productively influence stem cell function has begun to emerge in the biomaterials literature. Indeed, recent studies indicate that ions (such as calcium, magnesium, fluoride and strontium) released from dissolving inorganic minerals can influence stem cell phenotype[16–19]. The identity of degradable biomaterials (for example, agarose, gelatin or poly(lactide-co-glycolide)) strategically placed within a pluripotent stem cell aggregate can also influence differentiation[20], which further suggests a role for degradation by-products. Degradation of naturally derived ECM molecules, such as collagen and hyaluronic acid, has a long history of influencing cell behaviour, in some cases dependent on the molecular weight range[21,22] or the specific amino acid sequence[23] of the degradation by-product. Although previous

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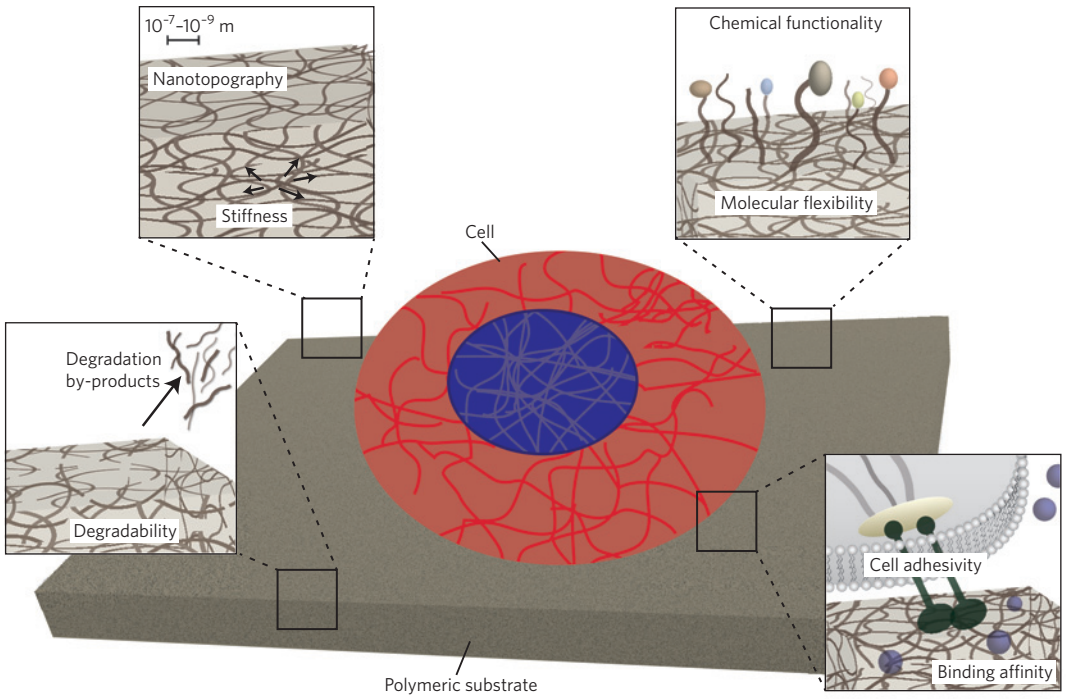

Figure 1 | Inherent material properties. Stem cell fate decisions can be affected by properties inherent to materials (exemplified by a two-dimensional polymeric substrate in this schematic) near the cell/material interface, such as nanotopography, stiffness (pictured as force vectors), chemical functionality (represented by coloured beads), molecular flexibility (indicated by the vertical strands sticking out of the substrate), the adhesivity of cells to the material (exemplified by ligand binding to the transmembrane receptor integrin), its binding affinity for soluble factors (pictured as blue spheres), its cell-mediated degradability and its degradation by-products.

studies of natural ECM protein degradation have not focused on identifying an impact on stem cells, their mechanisms of action suggest that by-products of natural ECM degradation may also influence stem cell phenotype. For example, endostatin is a by-product of collagen matrix degradation[23] that has been shown to modulate endothelial cell behaviour by regulating fibroblast growth factor (FGF) signalling[24] and $\alpha_5\beta_1$-integrin activity[25], which are also important molecular regulators of stem cell phenotype. One can therefore speculate that a degradation by-product such as endostatin could prove to be a stem cell regulator. Degradation of materials can also impact cell metabolic mechanisms that influence cell fate decisions, such as in the case of lactic acid by-products of resorbable polyester materials that can scavenge free radicals and thereby modulate the intracellular redox state of multipotent neural precursors[26,27]. Similar arguments can be made for a wide range of other natural and synthetic degradation by-products, which presents a unique opportunity. Next steps in this area will require an ability to screen for the effects of the diverse range of material degradation by-products likely to exist at the stem cell/material interface, as well as a deeper understanding of the mechanisms by which these by-products influence stem cell phenotype. Interestingly, degradation of a surrounding material can also lead to varying stem cell shape[28] and stem cell traction forces[12], which lead to changes in stem cell differentiation. Taken together, these studies may inspire new ways of thinking about material degradation in stem cell culture.

Another way in which materials can 'give' signals to stem cells is via their nanostructural properties, such as surface topographies. In some instances and for experimental purposes, topographic cues can intentionally be well controlled and systematically varied; but for many materials, nanoscale features result from the choice of material(s), bulk formulation methods, subsequent processing steps and/or some combination of these factors. Transcription and metabolic changes of MSCs cultured on nanotopographies have been related to chromosome organization and lamin morphology[29]. Also, force-dependent changes in zyxin expression of MSCs on grated topographic materials affected remodelling of focal adhesions that are responsible for changes in cell shape and differentiation potential[30]. Furthermore, reversible switching of substrate features between topographically patterned and flat surfaces affected MSC alignment and organization[31]. The size, order and timing of topographic cues can also impact pluripotent stem cell fate decisions[32,33]. To dissect the complex functional relationships between various possible topographies and stem cell fate decisions, large libraries of unbiased, random nanoscale surface features need to be generated and correlated accurately to stem cell responses using computational analysis tools, similar to what has recently been reported[34].

Materials can also 'take' cell-secreted signals from the stem cell microenvironment by means of molecular sequestering, and thereby regulate stem cell activity. Many stem cell types secrete inductive growth factors during their differentiation process. For example, hMSCs secrete FGF during proliferation[35], bone morphogenetic protein-2 (BMP-2) during osteogenic differentiation[36,37] and transforming growth factor-β (TGF-β) during chondrogenic differentiation[38,39]. Therefore, one can envisage an autocatalytic process in which stem cells can amplify their own change in phenotype by secreting inductive growth factors (Fig. 5). Recent work indicates that it is possible to use materials to harness cell-secreted factors to amplify stem cell expansion and differentiation[40–42]. Materials intentionally designed to bind to soluble growth factors can be used to upregulate[40,41,43] or downregulate[42] growth factor signalling in stem cell culture. In addition to these explicit demonstrations of sequestering, there are a range of other reports in which sequestering may be an important lurking variable. For example, the recently observed effects of simple organic functional groups[6,7], synthetic biomaterial libraries[44] and inorganic materials[45] on stem cell behaviour may be attributable to molecular sequestering as one

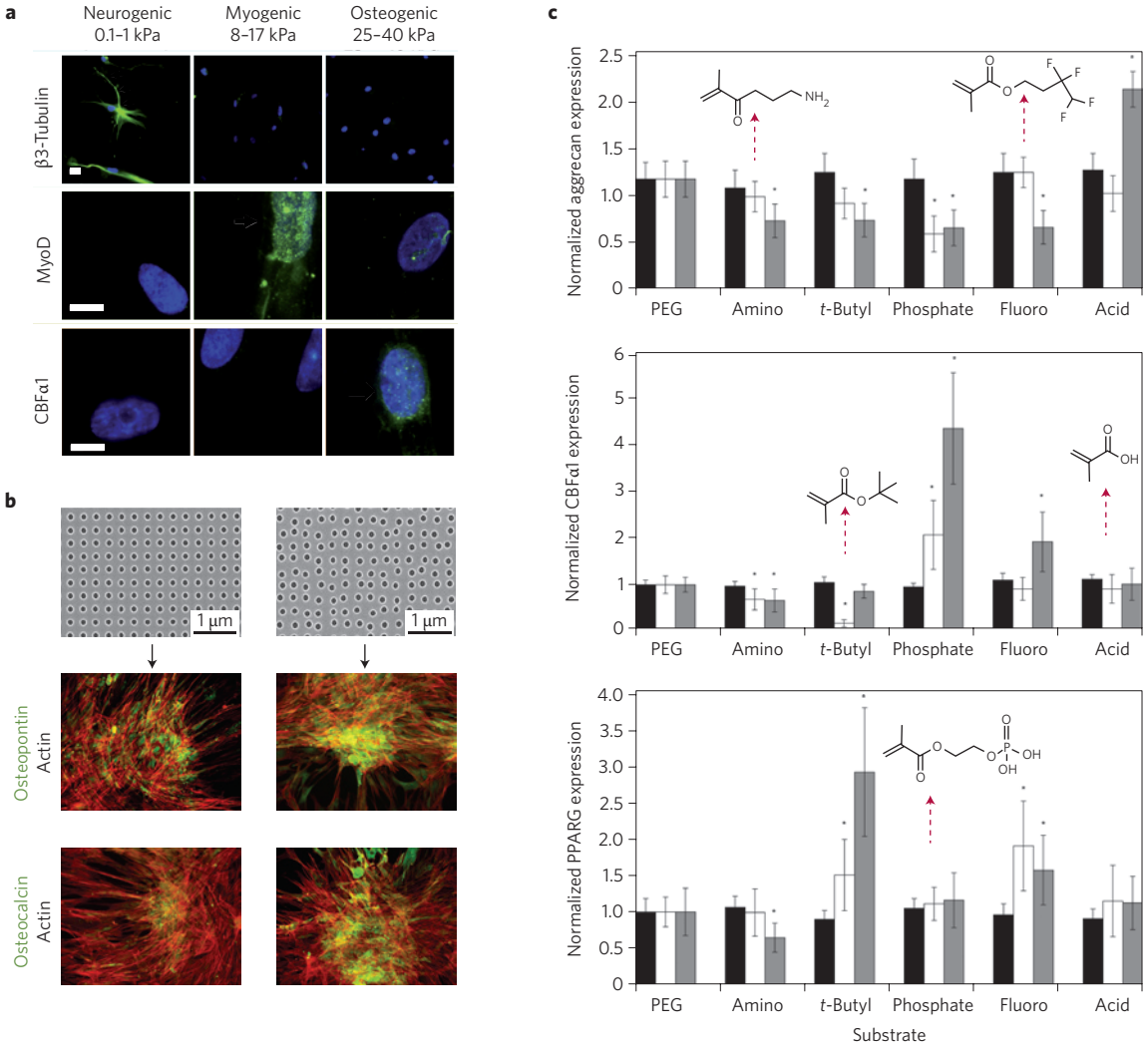

Figure 2 | Stiffness, nanotopography and chemical functionality influence the behaviour of human mesenchymal stem cells. a, The modulus of poly(acrylamide) substrates influences lineage-specific (neurogenic, myogenic or osteogenic) differentiation, as indicated by immunostaining for the appropriate markers (β3-tubulin, MyoD and CBFα1, respectively, shown in green; cell nucleus in blue)[1]. Scale bars, 5 μm. **b**, Substrates with asymmetrically organized nanopits (top row) stimulate osteogenesis (middle and bottom rows), as indicated by immunostaining for bone-specific extracellular-matrix proteins (osteopontin and osteocalcin, green)[3]. **c**, Poly(ethylene glycol) (PEG) substrates modified with 50 mM of simple functional groups (insets) influence gene expression associated with chondrogenesis (top), osteogenesis (middle) and adipogenesis (bottom), as indicated by the normalized expression of appropriate markers (aggrecan, CBFα1 and PPARG, respectively) at days 0 (black bars), 4 (white bars) and 10 (grey bars) of culture[6]. Gene expression was normalized by the expression of β-actin in cells cultured on PEG. Error bars, standard deviation. Asterisks denote statistical significance with respect to PEG ($p < 0.05$). Figures reproduced with permission from: **a**, ref. 1, © 2006 Elsevier; **b**, ref. 3, 2007 NPG; **c**, ref. 6, 2008 NPG.

modus operandi. An illustrative example was provided in a recent study in which phosphate-functionalized hydrogels were shown to sequester ECM proteins and thereby promote osteogenic differentiation of hMSCs[46]. Chemically defined experimental platforms and an ability to track cell-secreted products in real time will be necessary to gain more detailed insights into molecular sequestering effects. Sequestering may be a particularly impactful phenomenon in stem cell bioprocessing and biomanufacturing, in which recombinant human growth factor supplements can represent over half of the cost of goods[47]. Decreasing the cost and the need for exogenous-factor addition by encouraging cells to produce their own inductive factors may be an attractive alternative approach. Furthermore, sequestering of stem cell-secreted growth factors could be a particularly common occurrence in standard cell culture, as the extracellular milieu commonly includes proteoglycans and glycoproteins that bind a range of growth factors with high affinity. For instance, the 12th–14th type III repeats of fibronectin were recently found to bind to over 20 distinct growth factors with strikingly high affinity[48].

An important tool that may provide insights into the 'give and take' in stem cell culture is the ability to perturb material properties in predictable and controllable ways in the midst of an experiment. Dynamic materials recently developed using clever synthetic chemistries are poised to enable investigators to probe time-dependent phenomena at the stem cell/material interface[49,50]. The ability to dynamically vary cell–material adhesiveness[51,52], stiffness[53,54] and ECM degradability[12] in a user-defined manner has recently induced readily observable changes in cell behaviour. For example, light-induced switching of permissive (degradable) hydrogels into restrictive (non-degradable) hydrogels was used to show that cell-traction forces are crucial for osteogenic differentiation of hMSCs in a three-dimensional context[12]. Another recent example used dynamic hydrogels to show that hMSCs change their activation of critical

intracellular mechanical rheostats when cultured on soft versus stiff substrates, demonstrating that stem cells can possess 'mechanical memory'[55]. A series of other questions are also ripe for exploration using dynamic materials. For instance, whereas it is certain that substrate stiffness influences stem cell phenotype, it is not yet clear to what extent 'switching' material stiffness can lead to broad phenotypic changes, or how the rate of change in material stiffness can influence cell behaviour. Emerging, dynamic materials chemistries may rapidly increase our mechanistic insights regarding stem cell/material 'give and take'.

Box 1 | Features of stem cell substrate materials.

Where does one start when designing a material with appropriate inherent properties for stem cell applications? A common approach involves drawing inspiration from nature. Indeed, material properties have often been designed to mimic physiologically relevant ECM stiffness, topography, and adhesion-ligand type, density and affinity. Furthermore, materials have been designed to systematically vary material properties over wide ranges in a hypothesis-driven manner. For example, an analysis of the influence of nanotopography on stem cell fate is more meaningful if feature size can be varied over a wide range, and feature size therefore becomes a valuable independent variable. Taken together, clever materials synthesis strategies have enabled investigators to identify ranges of inherent material properties that influence stem cell behaviour, and to systematically address the hypothesis that inherent material properties can regulate stem cell fate.

Stiffness. Elastomeric polymer networks, such as hydrogels, have been commonly used, as their physicochemical properties can be controlled by simply adapting the density of crosslinks in the network. Hydrogels can span the range from very soft (<1 kPa in elastic modulus, similar in consistency to a viscous fluid such as honey) to rather stiff (~500 kPa, similar to silicone rubber). Poly(acrylamide) (PAAm) hydrogels have been a common base material, as their elastic modulus can range from 0.1 kPa (similar to soft neural tissue[110]) to 40 kPa (similar to non-mineralized bone tissue[111]) by simply varying the degree of crosslinking with bisacrylamide. Although PAAm hydrogels provide simple and adaptable control over stiffness, they do not directly control the means by which cells adhere to the material, as cell adhesion to PAAm is often mediated by adsorbed or covalently linked ECM proteins, such as type 1 collagen[1,11]. One can combine controllable stiffness with controllable cell adhesion by using hydrogels that resist cell and protein interactions, and derivatizing them with integrin-binding cell adhesion peptides. Examples include peptide-derivatized versions of hyaluronic acid hydrogels (4–100 kPa; ref. 12) and alginate hydrogels (1–160 kPa; ref. 10), or PAAm hydrogels with covalently coupled glucamine groups to resist nonspecific cell interactions[112]. Interestingly, poly(ethylene glycol) (PEG) and alginate hydrogels have also enabled investigators to study the role of stiffness in three-dimensional stem cell culture[10,12], where similar ranges of stiffness influence stem cell differentiation when compared with two-dimensional culture. Researchers are now starting to look beyond the elastic modulus of hydrogel materials, and also beginning to probe more complex viscoelastic behaviour, such as dynamic changes in stiffness and frequency-dependent stress responses.

Nanotopography. Various nanolithography strategies have been used to control the size, shape, spacing and organizational symmetry of nanometre-scale features in a variety of materials. Electrospinning can form nanofibrous substrates from natural or synthetic polymer precursors[113], and nanolithography methods common in the microelectronics industry (such as electron-beam lithography, block-copolymer assembly, selective etching or nanoimprint lithography) can generate nanopits[3,4], nanopillars[114] or nanochannels[115] on various surfaces. The diameter and spacing of electrospun nanofibres or nanochannels can be varied in ranges that approach the dimensions of natural basement-membrane fibre sizes (5–200 nm) and pore sizes (3–80 nm; ref. 116). For example, electrospinning has been used to generate fibrous materials with fibre and pore diameters on the order of ~20 nm and ~100 nm, respectively[117]. Channels and pillars formed via lithographic techniques can also be varied in ranges that mimic the porosity of natural ECM. For example, nanopillars 30 nm in diameter arranged in hexagonal or honeycomb arrangements supported human embryonic stem cell (hESC) self-renewal[114], and nanopits 120 nm in diameter with 300 nm centre-to-centre spacing could support hMSC expansion in a symmetric configuration[4], and osteogenesis when organized asymmetrically[3] (Fig. 2b). Parallel microgrooves (10 μM groove width) on the surface of cell-adhesive substrates were recently used to replace the effects of small-molecule epigenetic modifiers and significantly improve the efficiency of cellular reprogramming[118]. Other noteworthy strategies have attempted to control the nanometre-scale organization of cell-adhesive areas by patterning cell-adhesive gold nanodots[119], or using nanopatterned block copolymer stencils to form synthetic hydrogels[120]. An interesting future direction could involve dynamically varying the nanometre-scale topography or cell-adhesive regions on a substrate, and then observing responsive changes in stem cell phenotype[31].

Chemistry. The chemistries commonly used to link cell-adhesion peptides to defined cell culture substrates can similarly be used to link other chemicals (for instance, specific functional groups). Monomethacrylated functional groups can be incorporated into PEG hydrogels during photopolymerization[6] (Fig. 2c), whereas vinyl-containing monomers can be bound to the surface of poly(ethersulfone) membranes by means of high-throughput photoinduced graft polymerization[121]. Collectively, these studies demonstrate that simple, covalently immobilized chemical groups (such as phosphate or *t*-butyl) in PEG hydrogels influence MSC phenotype at 50 mM concentration in hydrogels[6], and that chemical groups on two-dimensional poly(ethersulfone) membranes (such as *N*-(3-(dimethylamino)propyl)methacrylamide) influence ESC self-renewal[121]. In each case, the mechanisms for these effects remain unclear, but have been associated with adsorption and sequestering of signalling molecules (such as growth factors or glycoproteins)[46]. Similarly, simple chemical groups released during degradation of a material have not been well studied in stem cell culture, but recent studies indicate that released calcium and phosphate ions can influence osteogenic differentiation through mechanisms that involve c-Fos signalling[16] and adenosine signalling[102], respectively. The use of simple chemistries to induce stem cell behaviours is an emerging area, and much remains to be learned about the underlying mechanisms. Interestingly, the observed impact of simple chemistries in early studies has opened up the possibility of high-throughput screening, and libraries of organic polymers[44,101] or inorganic materials[17,98] have been screened to optimize stem cell expansion, differentiation and transfection.

Emerging material-screening strategies will allow for the individual categories of inherent material properties described here to be co-varied simultaneously to further understand the relationship between the effects of distinct inherent material properties.

Illuminating the mechanisms

Regardless of the material, its inherent properties and the phenomenological observations that result from its interaction with cells — be it as simple as cell adhesion or as complex as stem cell differentiation — the biological signals induced by materials-directed behaviour have remained an open challenge for much of the past decade[56]. Very recently, however, several types of material-mediated mechanism that could convert material stimuli into biochemical signals have been identified in stem cells. Stem cells integrate these stimuli together through time and space to guide transcription factor expression that regulates cell fate and differentiation by means of several distinct mechanisms.

Although there are many mechanisms at play at the cell/material interface, the fundamental interaction that all cells must have is a link between the cytoskeleton and the material. The consequences of this interaction include a cascade of events in the cell, all of which are initiated by the cytoskeleton or by structures that link it to the material. One of the broadest of these mechanisms is the generation of contractile forces by cells against a substrate (Fig. 6, mechanism 1). To accomplish this, the cytoskeletal protein actin and its molecular motor myosin II bind and slide past one another to contract the cell. This mechanism is highly organized in muscle, yet it is present in all adherent cell types, and in stem cells it enables them to 'feel' the stiffness[1,57] and topography[4,58] of their environment, as well as to control their size, shape and polarity[8,9]. Although such inherent properties of the material may seem disparate, they are united by a common contractility-based mechanism that directs stem cells towards specific lineages based on the degree of activation. Conversely, inhibition of the binding activity of myosin II renders cells unable to respond to any of these material effects[1,59].

Certain transcription factors — which serve as the 'molecular switches' that start to convert a stem cell into a mature cell — have been identified whose expression is sensitive to material properties. For example, cell contraction against a 40-kPa substrate in two dimensions[60] or shape changes in a three-dimensional matrix[61] induce nuclear localization of the transcription factor YAP/TAZ, causing cells to adopt an osteogenic fate. However, contraction is far upstream from a stem cell maturing into an adult cell type, and cells must first bind to the protein or peptide grafted to the material through integrins — these are part of the structures called focal adhesions that bind to actin — before contracting against it. Indeed, recent evidence shows that both ligand type[62] and the way in which

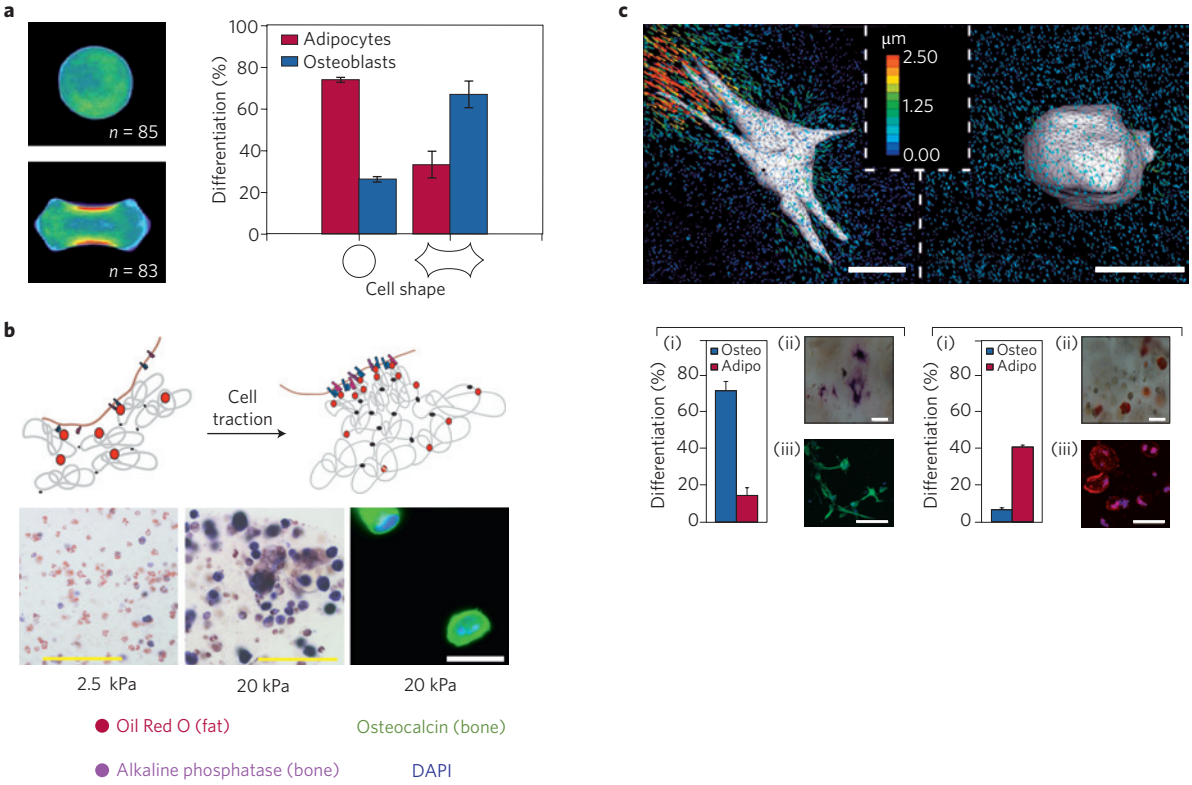

Figure 3 | Cell–material interactions established at the outset but evolving during the course of cell culture regulate the behaviour of mesenchymal stem cells (MSCs). a, Substrates patterned with fibronectin in the shape of circles or holly leaves of the same area control human MSC (hMSC) shape on adhesion and spreading (left; colours from blue (low) to red (high) represent the levels of myosin IIa immunofluorescence). In turn, cell shape influences their fate after exposure to mixed media (right)[9]. Error bars, standard deviation of *n* samples. **b**, Cells encapsulated in an alginate hydrogel actively cluster cell-adhesion ligands covalently linked to the hydrogel, whose stiffness dictates the ability of the cells to exert traction forces and cluster the ligands. After 1 week of culture in hydrogels of different stiffness but identical ligand density, mouse MSCs differentiated into fat (bone) for hydrogels with low (high) stiffness, as indicated by the staining of cryosectioned samples[10]. Scale bars, 100 μm (yellow) and 20 μm (white). **c**, Differences in hydrogel degradability create either a permissive (left) or a restrictive (right) environment that leads hMSCs to undergo osteogenesis or adipogenesis, respectively, as indicated by three-dimensional traction-force microscopy images (top) of hMSCs (grey) within hyaluronic acid hydrogels with embedded beads (punctate spots throughout; bead displacement, a proxy for hydrogel degradability, is indicated by the colour map), as well as by alkaline phosphatase staining (purple; top bright-field images in bottom panels), and osteocalcin (green; bottom bright-field image in left bottom panel) and fatty acid-binding protein (red; bottom bright-field image in right bottom panel) immunostaining and associated quantification of stained cells (bar graphs)[12]. Error bars, standard error of the mean. Scale bars, 10 μm (top), 25 μm (bottom, (ii)) and 20 μm (bottom, (iii)). Figures reproduced with permission from: **a**, ref. 9, © 2010 NAS; **b**, ref. 10, 2010 NPG; **c**, ref. 12, 2013 NPG.

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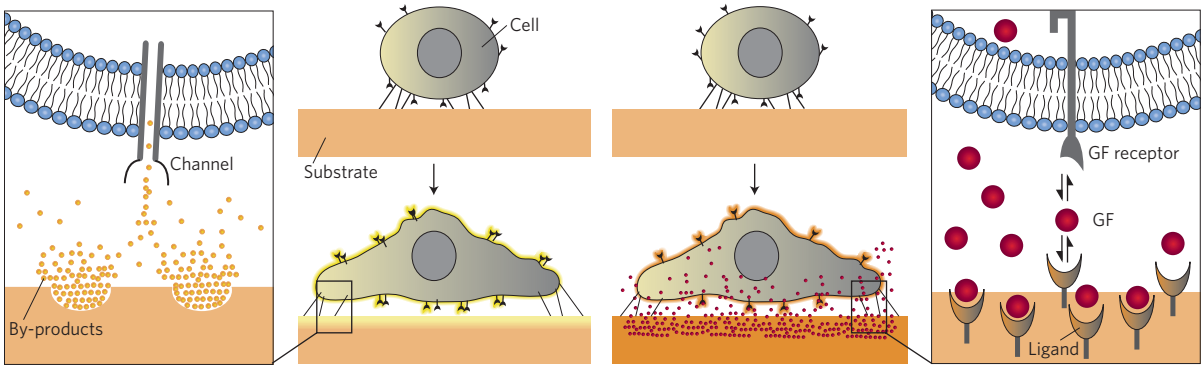

Figure 4 | 'Give and take' at the stem cell/material interface. Materials can give signals to cells in the form of degradation by-products, here exemplified by the translocation of material-released by-products to the cell's cytosol through a transmembrane channel (left), and also take signals from cells through molecular sequestering, as in the binding and unbinding of a growth factor (GF) from a material-associated ligand (right).

the adhesive ligand is attached[11] may influence the extent to which each inherent property may stimulate cells and induce signals. Regardless of the material stimulus, however, the inputs directly influence integrins and the associated signalling proteins, such as Ras homolog gene family member A (RhoA) GTPases, which regulate cell fate and behaviour. RhoA, for example, modulates myosin contraction through myosin light chain kinase (MLC) and rho kinase (ROCK). Their activity is correlated with material stiffness and elevated force production[63], leading to spreading and shape changes[8,9], migration[64], and ultimately differentiation into more contractile lineages, such as the osteogenic lineage[8]. Instead, softer substrates have been shown to maintain self-renewal in embryonic stem cells (ESCs)[65] and muscle satellite cells[66]. Cell shape can also directly regulate these processes via ROCK-dependent contractility: well spread, polarized shapes versus small, rounded shapes have high and low ROCK activity, respectively[8], suggesting contractility again as the common mechanism that can cause stem cells to become bone and fat, respectively. This theme has been further advanced in three-dimensional culture: whereas cells confined to a nondegradable matrix, no matter how soft or stiff, cannot exert sufficient tractions[12], a more labile matrix permits tractions in a stiffness-mediated manner[10]. Thus, contractility clearly correlates with stem cell phenotype in multiple *in vitro* contexts.

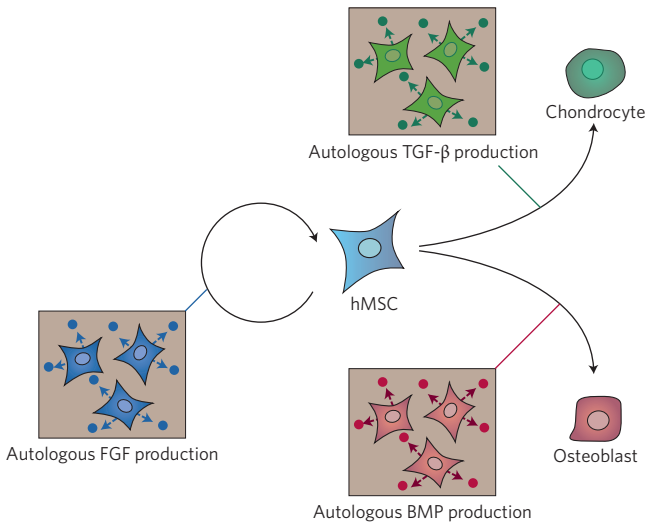

Figure 5 | Autocatalytic processes in stem cell culture. Human mesenchymal stem cells (hMSCs) secrete key inductive growth factors (dots) during proliferation (for instance, autologous production of fibroblast growth factor (FGF); left) and differentiation (for example, the production of transforming growth factor-β (TGF-β) or bone morphogenetic protein (BMP), which can induce differentiation into cartilage or bone tissue, respectively; right), and these factors can be harnessed by material-mediated sequestering.

In addition to contractile-regulated RhoA and ROCK signalling, focal adhesions are rich in other proposed sensors and sensor types, such as stretch-sensitive proteins[65] (sometimes referred to as 'molecular strain gauges'[56]; Fig. 6, mechanism 2). Being in series with actin–myosin complexes and integrins, these proteins have been proposed to change configuration with increasing cell-mediated force, exposing cryptic sites and inducing new signalling that would not otherwise be present; many sensors could exist, each tuned to respond to a different set of inherent matrix properties. For example, when placed under sufficient force, vinculin — which has been shown to undergo stretching both *in vitro*[67] and *in situ*[68] — has recently been shown to initiate stiffness-sensitive mitogen-activated protein kinase 1 (MAPK1) signalling that causes hMSCs to become muscle[69]; however, softer or stiffer matrices produce too little or too much force to engage this sensor[56], which suggests that there may be lineage-specific sensors, each with their own 'set point.' p130CAS, which undergoes phosphorylation by Src family kinases under stretch[70], and focal adhesion kinase (FAK), which undergoes tyrosine phosphorylation under force[71], could also be sensors of this type (Fig. 6, orange stars). More specifically, p130CAS overexpression has been recently shown to activate c-Kit signalling to differentiate mammary epithelial precursors[72], whereas stiffness-mediated FAK upregulation is required for osteogenesis[73]. However, 250-nm-wide nanogratings induce FAK phosphorylation, which is required for topography-induced MSC myogenesis[74]. These seemingly disparate findings illustrate the fact that the exact mechanism of mechanical signalling for these other sensors, and whether they have an optimal set point, remain unknown, especially in the context of stem cells and the specific inherent material property that is inducing the response.

It should also be noted that although some proteins with kinase sites do indeed regulate fate[69], this may not be the rule. For example, the tyrosine phosphatase Shp-2, β1-integrin and talin-1 all seem to regulate stiffness-mediated fibroblast spreading, but receptor-like protein tyrosine phosphatase-α only does this on collagen IV — not on fibronectin-coated substrates[75]. These data suggest that these specific focal adhesion proteins may not play a role in all types of material-directed stem cell differentiation. Despite this caveat, focal adhesion-based sensors are highly probable and may be influential in converting material cues into biochemical signals. Part of the reason for this high likelihood is the shear number of proteins within these structures that could exhibit material sensitivity. Recent

estimates indicate that as many as 200 proteins may be within a focal adhesion[76], and that the dimensionality[77] and ligand spacing[78] within the matrix could influence adhesion composition. This would change the signals that could be induced and ultimately cause a material to alter how stem cells differentiate.

Regardless of mechanism, gene transcription (Fig. 6, yellow star) must be activated to induce expression of lineage-specific cell markers, and perinuclear and intranuclear mechanisms that are activated as a result of inherent material properties may be indispensable in regulating cell fate. Within the nucleus, DNA is condensed around histones to improve packing, forming structures known as heterochromatin, and burying many signalling sites that regulate stem cell fate (Fig. 6, mechanism 3). When histones undergo acetylation via histone acetyltransferase, the positive charge on histones is removed, decreasing DNA–histone association and converting the heterochromatin to euchromatin. Many previously inaccessible binding sites then become accessible and permit lineage commitment. Conversely, histone deacetylase (HDAC) restores the charge and thus the tight DNA–histone association. MSCs cultured on microgrooves align with the pattern, and when compressed or stretched perpendicular to the microgrooves show decreased HDAC activity and increased histone acetylation[79]. Thus, the DNA is more accessible and the stem cells are capable of differentiating into mature cell types. However, these material-based signals must be transduced from the cytoskeleton to the nucleus. Cells have complex nuclear cytoskeletons, known as nuclear lamins, which anchor transnuclear membrane proteins (such as SUN and LINK proteins) that connect chromatin and the actin cytoskeleton[80]. Chromatin condenses[81] and the nuclear lamina stiffen[82] once the stem cell has differentiated. Reducing lamin expression abolishes strain-induced changes in HDAC activity and thus differentiation[79]. Very recently, lamin A/C was also found to both scale its expression with tissue stiffness (low for soft fat and high for stiff bone) and regulate stem cell differentiation by stabilizing and transmitting forces across the nucleus[83]. Collectively, these data strongly suggest that there is positive regulation of heterochromatin within stem cells, which under the appropriate material conditions leads to differentiation.

Finally, stretch-activated channels (SACs; Fig. 6, mechanism 4) regulate protein–protein interactions contained in all of the aforementioned pathways, especially myosin II binding to actin, which is heavily regulated by the cytoplasmic concentration of calcium[84]. SACs form from integral-membrane proteins that self-assemble into a closed pore structure. Unlike voltage-gated pores, actin–myosin structures pull on focal adhesions and integrins connected to the surrounding material, and this indirect transmission of tension to the plasma membrane causes these channels to stretch open or become more permeable to the flow of a particular ion[56,85,86]. This ensures appropriate phospholipid spacing in the membrane, but also permits the flow of cations down the cytoplasm/extracellular space concentration gradient. SACs were identified over two decades ago, and can influence very basic cell functions including proliferation[87], contractility[84] and the activity of voltage-gated channels[88]. SACs open when piconewton forces are applied to them, and with such a low threshold it is uncertain whether SACs could play a significant enough role in affecting phenotype, or whether they are opened by the typical tension present in stem cells[89]. Simple contact with an atomic force microscope probe has been shown to influence SAC activation, which can be ablated when either contractility or the channels themselves are blocked[90]. In addition to sensitivity, SACs are cation specific; for example, transient receptor potential channels are a calcium SAC known to permit transient calcium influx when cells are stretched[91]. SACs also seem to be matrix-stiffness

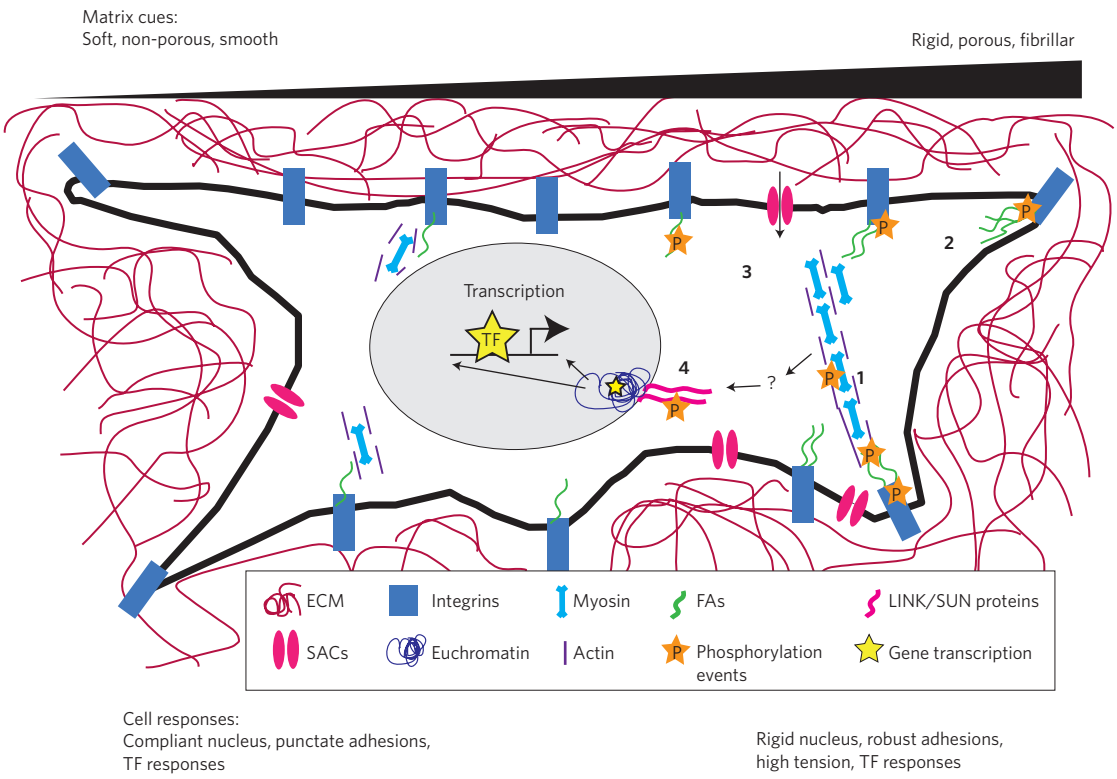

Figure 6 | Materials-based signalling mechanisms within cells. The inherent material properties of an extracellular matrix (ECM) can vary (as indicated by the height of the horizontal bar at the top) and result in cellular responses (specified at the bottom) that are mediated by signals from the ECM: (1) actin–myosin contraction, (2) focal adhesion (FA) signalling, (3) stretch-activated-channel (SAC)-induced ion changes, (4) nuclear-associated-protein signalling and chromatin unfolding. There are tissue- and stem cell-specific transcription factor (TF) responses for both sets of properties. The question mark signifies that it is unclear whether the perinuclear and linking proteins (pink lines) such as LINK and SUN1/2 attach to the actin cytoskeleton.

sensitive, as cells exhibit changes in calcium ion oscillation amplitude that is ablated when treated with gadolinium[92]. In addition to mechanosensitive calcium SACs, the TREK1 potassium channel has more recently been examined as a touch sensor[93]; but as with the calcium-based SACs, these channels are very sensitive. Yet in the context of stem cells, much less is known about the role of SACs in differentiation. Cyclic stretch of compliant substrates has been shown to regulate satellite cell activation, their calcium transients necessary for contraction and the release of growth factors[94]. When magnetic particles were directly tethered to TREK1 channels and intermittently forced into an open state over 21 days, marrow-derived stromal cells had elevated levels of osteogenic genes[95]. Similar loading schemes over 7 days were also reported to enhance glycosaminoglycan production[96]. Despite these data, SACs remain vastly under-characterized in stem cells; and because of their very low activation-force thresholds[89], it remains to be shown whether they, rather than other channels that they may activate, play a role in stem cell fate.

Although we have discussed these four mechanisms separately, they all together regulate stem cell fate. In fact, the slow progress in identifying specific pathways mitigating these types of mechanism is probably due to our inability to separate them. Further material refinement, including the use of temporally[53,54] or spatially patterned materials[97], may improve our ability to tease apart these signalling mechanisms and understand them more comprehensively.

From integration to convergence

Because stem cells are clearly capable of responding to multiple material inputs simultaneously, the array of potentially relevant material properties makes it difficult to precisely control or effectively predict material-induced stem cell responses in complex microenvironments. Therefore, beyond simply allowing stem cells to integrate divergent material parameters, there is a need to move towards directions that feature the targeted activation of signalling pathways that converge on a desired cell phenotype. Next-generation materials for engineering stem cell phenotype(s) may be designed to stimulate expression of specific sets of regulatory genes using 'catalogues' of information that relate material parameters to gene-expression signatures. The optimal conditions for such convergence will require catalogues relating the relative influence of different material properties to resultant gene activation. This type of controllable, systematic tailoring of multiple material properties may result in more efficient material-induced stem cell differentiation protocols. Indeed, efficient biochemically induced differentiation protocols have historically required cocktails of biochemical supplements added to stem cell culture in a specific sequence. We might therefore expect a similar model for material-induced differentiation, where multiple inputs will be delivered in a particular temporal sequence, and where the desired sequence of signals may be informed by the temporal display of similar cues during tissue development and regeneration. It is noteworthy that the delivery of multiple inherent material characteristics to stem cells is a complex proposition, and not simply a linear combination of complementary inputs.

The understanding of how distinct material characteristics combine to regulate stem cell fate is still in its infancy. The systematic tailoring of material properties will require highly efficient experimental platforms, as stem cell/material dynamics are multivariate and highly complex. Towards that end, high-throughput material-screening systems have recently emerged as enabling technologies. Such platforms have recently been used to examine stem cell behaviours in response to complex synthetic libraries of polymeric materials[44], inorganic materials[17,98] and biofunctionalized hydrogels[2,99,100]. Polymer-library screening identified a specific polymer that promoted human ESC (hESC) self-renewal in standard cell culture conditions[44,101]. Inorganic material screening identified mineral-substrate characteristics that optimize hMSC transfection, expansion or differentiation[17,102]. A series of recent studies have used hydrogel-based arrays to probe stem cell/material interactions[2,99,100], and one study showed that systematically co-varying hydrogel stiffness and cell shape allows for more efficient neurogenic and myogenic differentiation of hMSCs[2]. In general, these platforms offer an ability to explore inherent material influences on stem cell phenotype without formulating detailed mechanistic hypotheses a priori — analogous to high-throughput screening of synthetic small-molecule libraries in stem cell culture[103]. Our limited understanding of the mechanisms by which materials influence stem cell behaviour make it difficult to frame clear hypotheses a priori, which makes high-throughput screening platforms uniquely valuable.

Of course, high-throughput material screening platforms become more valuable when they are coupled with high-content characterization tools to analyse stem cell/material interactions[104,105]. Screening outputs so far have largely focused on analysing simple outcomes (such as cell viability or metabolic activity), fixing cells for subsequent analysis of more complex markers, or using stem cell lines engineered to include fluorescent or luminescent reporter constructs. Although reporter lines are a valuable tool, there may be significant differences between these lines and the native stem cell types of interest, which complicates insights into material-dependent stem cell behaviour. Ideal tools would be capable of characterizing cell behaviour in a label-free manner, without interrupting the normal course of stem cell culture. Towards that end, recent studies have rapidly collected data through analysis of stem cell shape and cytoskeletal characteristics to predict differentiated phenotype[104]. There is a need to develop more of these enabling technologies for efficient, label-free analysis of stem cell phenotype.

Another critical set of enabling technologies will involve controllably changing the characteristics of the stem cell/material interface. Engineering a temporal sequence of material-based cues requires either transferring cells from one material environment to another or inducing changes in material properties in a dynamic manner. Cell transfer to another material environment has served as the conventional means of changing exposure to different material properties, but it requires extracting cells physically from their existing environment, thereby severing inter- and extracellular adhesive interactions and disrupting processes underway. In contrast, inducing changes in the material properties requires more sophisticated control of the material. This is now possible using the aforementioned clever synthetic techniques[49], although often dependent on applying a stimulus of some kind. In most cases, external stimuli (for example, light or temperature) have been applied with user-defined timing and amplitude that are largely independent of a cell's inherent properties and phenotypic state information. However, the opportunity to design dynamic material properties that respond to phenotypic changes in cells represents a challenge, which will require not only new bioresponsive materials, but also more detailed information about the secretory and physical properties of cells as they adopt different phenotypes. For example, protease expression dynamics that might accompany cell fate changes could be used to soften materials or release degradable by-products in an on-demand fashion that in turn could further influence differentiation processes in a predictable manner.

Impact of inherent material properties

Material-induced stem cell differentiation in defined conditions remains a critical goal in stem cell engineering to advance cell therapies and diagnostics[106]. Approaches that harness the inductive capabilities of common materials may help to circumvent cost barriers to efficient stem cell expansion and lineage-specific differentiation, and to allow for more efficient regulatory acceptance and clinical adoption of stem cell therapies. The introduction of engineered

materials within stem cell biomanufacturing practices, particularly synthetics that can be mass produced and easily sterilized, may substitute or lessen the need for biochemical reagents and thereby significantly reduce cell bioprocessing expenses.

Efficient stem cell differentiation caused by inherent material properties could have important implications not only for stem cell bioprocessing, but also for medical-device design and endogenous tissue engineering. The surfaces of medical devices could be coated or physically modified not just to encourage tissue integration, but also to target specific endogenous stem cell populations and their function at the material/tissue interface. Surface-mediated stem cell regulation could perhaps lengthen the lifetime of implanted devices by promoting appropriate stem cell responses, which in turn could reduce the incidence or extent of a foreign body reaction to implanted materials. Similarly, for tissue-engineering purposes inherent properties of materials could locally control the expansion and direct differentiation of endogenous stem cells in a sequential manner for *de novo* tissue formation. *In vivo* tissue-engineering strategies will benefit from an enhanced understanding of endogenous stem cell populations and their normal physiology, and of their response(s) to material properties.

Furthermore, although so far observations at the stem cell/material interface have focused on self-renewal and lineage-specific differentiation, materials may serve as inductive components and regulators for a broader range of stem cell behaviours. Recent studies suggest that tissue morphogenesis[107], homing and recruitment[108], and therapeutic cytokine secretion[109] are important stem cell behaviours that could be induced and/or manipulated by inherent material properties. Altogether, the integration of signalling pathways stimulated by inherent material properties will enable convergence on specific stem cells and differentiated phenotypes useful for regenerative medicine applications.

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Additional information

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Competing financial interests

The authors declare no competing financial interests.

ERRATUM

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In the version of this Review Article originally published, Fig. 6 was incorrect; it should have been as shown below. This error has now been corrected in the online versions of the Review Article.