Materials control of the epigenetics underlying cell plasticity

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Abstract | The dynamic epigenetic landscape directs gene expression patterns that dictate cellular form and function, and drive the assembly of cells into tissues. The high degree of plasticity in the epigenetic landscape of mammalian cells is directed by materials, which provide the context in which cells receive and integrate multivariate signals to programme the chromatin state towards specific functional outcomes. In this Review, we explore how materials guide the cellular epigenetic landscape and discuss how engineered materials target cell plasticity, particularly through dynamic changes in histone methylation and acetylation. After discussing findings in developmental biology and cancer research that link materials parameters to chromatin state, we highlight how cell culture materials that control ligand presentation, mechanics, topography and geometry have shown how materials cues and context influence chromatin state through mechanotransduction. Finally, we describe how tissue fabrication can control cellular plasticity to drive meaningful biological activities that may facilitate the assembly of cells and tissues into functional architectures.

The cells of the early embryo all have the same developmental potential and differentiate into the three germ layers (that is, ectoderm, mesoderm and endoderm) with guidance from the extracellular environment[1–5]. As development progresses, the genomic landscape is progressively restricted through epigenetic modifications, which dictate gene expression patterns that correspond to discrete, lineage-specific activities[6]. Epigenetics control gene expression independently of alterations in the primary DNA sequence and have clear roles in processes spanning development and disease[7–13]. The microenvironment regulates epigenetics by influencing non-coding RNA expression, DNA methylation and histone modifications, including histone methylation and acetylation[14].

Cell plasticity describes this ability of cells to transform into another state in response to intrinsic or extrinsic cues. These cues include multivariate signals that propagate biophysical and biochemical information from the extracellular environment to the nuclear compartment, either directly or through signal transduction cascades that converge on chromatin to regulate gene expression programmes and, thus, cellular activity. Multiple complementary and opposing processes, such as transduction of soluble and insoluble signals in the microenvironment, regulate the epigenome (that is, all of the chemical modifications to DNA and histone proteins that regulate gene expression) and define a specific cellular outcome. Furthermore, a cell's epigenetic state and gene expression profile are dynamic and constantly shift in response to the environment, which includes biomaterials (such as dynamic biopolymers and mineralized matrices) that engage the membrane through physical and chemical means. This mechanochemical signalling propagates through many of the pathways used by cytokines, metabolites, ions and other small molecules, all of which influence cell plasticity.

Indeed, signal transduction cascades influence cell plasticity by propagating signals from the extracellular environment to the nuclear envelope, where the balance of chromatin-modifying enzymes may be perturbed[15–17]. 'Outside-in' signalling cascades involve factors including soluble cytokines and growth factors, insoluble matrix proteins, matrix mechanics, topography, shear stress, osmotic pressure and more. A striking aspect of epigenetic regulation that is often overlooked is the contribution that the materials surrounding cells and tissue make. After the formation of the three germ layers in the early embryo as a result of epigenetic changes[18–20], the materials microenvironment that surrounds each layer differs and, thereby, guides epigenetics and gene expression, which, in turn, influences the synthesis and deposition of materials[21–23]. This feedback mechanism presumably augments a specific outcome, guiding cell state to the appropriate valley on the 'epigenetic landscape'. Although this 'dynamic reciprocity' between cells and their surrounding matrix is at the heart of tissue form and function (see below), much work is needed to elucidate the precise epigenetic mechanisms underlying development, homeostasis and disease.

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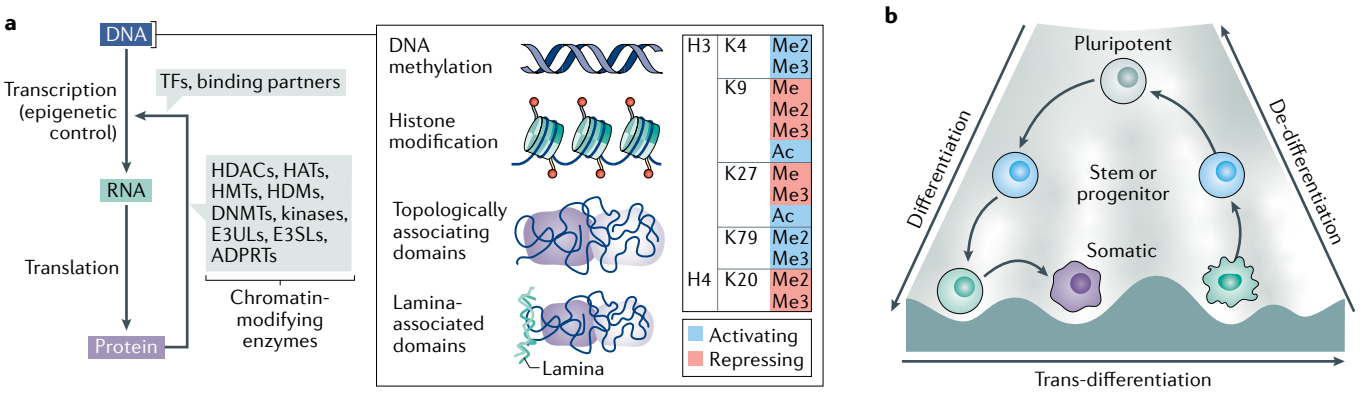

Fig. 1 | **Chromatin modification and the epigenetic landscape in a materials context. a** | The central dogma of, and stages associated with, epigenetic modulation and transcription regulation are shown besides the common activating (light blue boxes) and repressing (red boxes) histone methylation (Me) and acetylation (Ac) marks that occur on the indicated lysine (K) residues of histone H3 and H4. Enzymes involved in epigenetic modification include: histone deacetylases (HDACs), histone acetyltransferases (HATs), histone methyltransferases (HMTs), histone demethylases (HDMs), DNA methyltransferases (DNMTs), kinases, E3 ubiquitin ligases (E3ULs), E3 small ubiquitin-like modifier ligases (E3SLs) and ADP-ribosyl transferases (ADPRTs). **b** | The concept of an epigenetic landscape, in which a cell that is initially pluripotent (that is, a stem or progenitor cell) traverses hills and valleys during differentiation to a somatic cell, and then during reprogramming to a less differentiated state (de-differentiation) or to a different cell type (trans-differentiation) is depicted. TFs, transcription factors.

A major hurdle in understanding how materials guide cellular plasticity is the difficulty of studying cells in living tissue. However, in recent years, cell and tissue culture has evolved from the use of chemically treated plastic to that of well-defined hydrogel biopolymers and bioreactors that mimic physiological environments[24]. This change has allowed the role of materials in guiding signal transduction, epigenetics and gene expression to be systematically queried and 'matrix structure–cell function' relationships to be proposed. For instance, several seminal papers in the past decade demonstrated the importance of matrix mechanics in directing lineage specification in stem cells[25–27] and pathogenesis in cancer[28], leading to many subsequent studies into the role of stiffness in guiding cellular activities[29,30]. Indeed, materials-centric studies confirmed the importance of biophysical and biochemical parameters, including shear, viscoelasticity, matrix composition, topography and geometry, in driving cellular decisions[31–33]. Furthermore, several studies in the past 5 years have demonstrated how designer cell culture materials influence morphogenesis[34,35] and regulate epigenetic marks to enhance reprogramming[36]. Nevertheless, precise molecular mechanisms relating materials properties to epigenome regulation is only now gaining focus by the community.

In this Review, we discuss the role of materials in the epigenetic regulation of cell plasticity, highlighting how materials properties guide the integration of multivariate signals during cell fate decisions spanning physiological and pathological processes. We focus on the cell–material interface and how the engagement of cell surface receptors facilitates signal transduction from the outside-in, leading to distinct chromatin marks that drive specific gene expression programmes. We restrict our discussion to histone modifications, a key nuclear feature that is often altered in response to biomaterials. However, DNA methylation and non-coding RNAs also play important roles in regulating the epigenome in response to biomaterials, and this area warrants further investigation. Studies to date highlight the central role of histone modifications in dictating plasticity in response to materials properties, and the subsequent regulation of specific gene expression patterns; understanding how materials guide cells in navigating the epigenetic landscape, through defined programming and reprogramming events, is of critical importance for the design of emerging cell culture systems and biofabrication approaches.

General principles of epigenetics

In the nucleus, chromatin is organized into distinct regulatory regions, called topologically associated domains (FIG. 1a). Within topologically associated domains, DNA wraps around nucleosomes in an open, transcriptionally active conformation or in a condensed, inactive form[37,38]. DNA and histone modifications can enhance the accessibility of chromatin in promoter regions, enabling an open, euchromatin structure that permits gene activation, or fostering heterochromatin-like structures with closed, inactive states[39,40]. Lamina-associated domains (LADs) are primarily heterochromatin regions that interact with the nuclear lamina. In response to stimuli, LADs can be decondensed and partitioned into regions with high transcriptional activity, a process that has been implicated in mechanotransduction cascades that coordinate gene transcription associated with lineage specification and commitment[41].

Nucleosomes are comprised of four histone proteins (H2A, H2B, H3 and H4), with DNA wrapping around two tetramers of histone proteins, and these histone proteins are subjected to covalent post-translational modifications, including methylation, phosphorylation, acetylation, ubiquitylation and sumoylation[42].

These modifications alter histone structure and, thereby, the accessibility of bound chromatin DNA to transcription factors and co-repressors, resulting in transcriptional activation or repression and changes in cell behaviour. For instance, histone H3 lysine 4 methylation (H3K4me) and histone H4 acetylation (H4ac) are associated with gene activation[43], whereas histone H3 lysine 27 methylation (H3K27me) and histone H3 lysine 9 methylation (H3K9me) are associated with gene repression[44] (FIG. 1a). Histone methylations (added by histone methyltransferases) and acetylations (added by histone acetyltransferases) are dynamic and removed by histone demethylases and histone deacetylases (HDACs), respectively[45].

Epigenetic changes tend to be either stable or dynamic; the dynamic aspects of epigenetics result from stimulus-induced changes to the chromatin, for example, physical and chemical perturbations that persist for a set time frame. This aspect of epigenetic persistence is termed 'memory', and the timescales associated with persistence vary depending on the modification[46]. For instance, DNA methylation states are often heritable, owing to the continued recruitment of DNA methyltransferases and maintenance of DNA methylation, whereas histone modifications are more dynamic and not always heritable[47]. There are different levels of epigenetic memory, including cellular memory, heritable transcriptional states and the persistent response to stimuli through 'remembered' epigenetic states. These levels are believed to be mediated by protein complexes that persist through cell divisions to maintain a defined epigenetic state[48]. For instance, multiprotein complexes containing chromatin-modifying enzymes mediate H3K4me to promote gene activation, and others repress genes by promoting H3K27me[49]. These dynamic marks can last for a single cell cycle or for considerably longer; in *Caenorhabditis elegans*, exposure to high temperature stimulated the persistence of histone H3 lysine 9 trimethylation (H3K9me3) through 14 generations of organisms[50].

Epigenetics in development and disease

The epigenetic regulation of lineage specification was classically depicted by Waddington as a landscape with ridges and valleys, in which a valley corresponded to a cell's developmental pathway or 'fate' (REF.[51]) (FIG. 1b). This epigenetic landscape is analogous to a classical energy landscape or reaction coordinate, in which reaction conditions, thermodynamics and kinetics define the path towards a minimum or valley. In chemical reactions, jumping from one valley to another requires energy, often in the form of heat or the use of a catalyst, to surmount the ridges. During the epigenetic regulation of lineage specification, jumping between valleys will similarly require some functional activity to rewire the epigenome[52]. The propensity of a somatic cell to cross an epigenetic barrier can be defined as its plasticity, or as the tendency for an epigenetic state to be rewired in response to microenvironmental factors. This definition applies to the classical differentiation of a pluripotent or multipotent cell and also to more recently identified transformations, including trans-differentiation (that is, when cells cross germ layers) and de-differentiation (that is, when cells are reprogrammed to an earlier developmental state). Indeed, cells can convert between states if they can overcome the 'epigenetic hurdle'.

We now know that epigenetics governs gene expression patterns during development and morphogenesis and is, therefore, central to a molecular understanding of all processes associated with development and disease. After fertilization, the single-cell zygote rapidly proliferates; over time, the cells in the embryo specialize and become more developmentally restricted through epigenetic control[6]. Indeed, DNA methylation, histone modifications and chromosome organization vary widely throughout embryonic development. During the preimplantation stages of embryogenesis and the first differentiation events, DNA methylation is dynamic and dominates the epigenetic mechanisms that orchestrate formation of the three germ layers, with methylation-silencing genes associated with pluripotency[53]. However, histone modifications also play a dynamic role in embryogenesis, regulating the expression of lineage-specific genes through all stages of development following gastrulation[54]. For instance, the pluripotency genes *Oct4* and *Nanog* are silenced through H3K9me[55]. Furthermore, HDACs play a key role in embryonic and postnatal angiogenesis (that is, the development of new blood vessels from those laid down during vasculogenesis, one of the first morphogenetic processes in development), with biophysical aspects of the microenvironment, including shear stress and hypoxia, contributing to this regulation[56].

As tissues develop their form and function, common and distinct epigenetic mechanisms control lineage-specific gene expression. For instance, cardiac development is governed by complex patterns of DNA methylation and histone modifications that regulate the accessibility of DNA to transcription factors[57]. A consequence of epigenetic regulation during cardiac development is the transition of cardiomyocytes from proliferative embryonic cells capable of regeneration to quiescent adult cells as a result of chromatin condensation[58]. Targeted approaches to reverse epigenetic states that arrest cells in quiescence have the potential to facilitate regeneration in adult hearts[59]. Epigenetic states are dynamic during development and homeostasis, where cells experience diverse biophysical microenvironments, including the central nervous system[60], morphogenesis and homeostasis in the lung[61] and during bone development[62]. For instance, epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET) are hallmarks of morphogenesis that occur during normal and pathological processes. Transitioning between these diverse phenotypes and associated microenvironments influences cell adhesion, morphology and mechanotransduction, leading to epigenetic alterations that modulate chromatin accessibility and regulate gene expression associated with morphogenesis and lineage specification (FIG. 2).

Cell plasticity provides opportunities for higher-order physiological function through navigation of the epigenetic landscape. Unfortunately, plasticity also gives rise

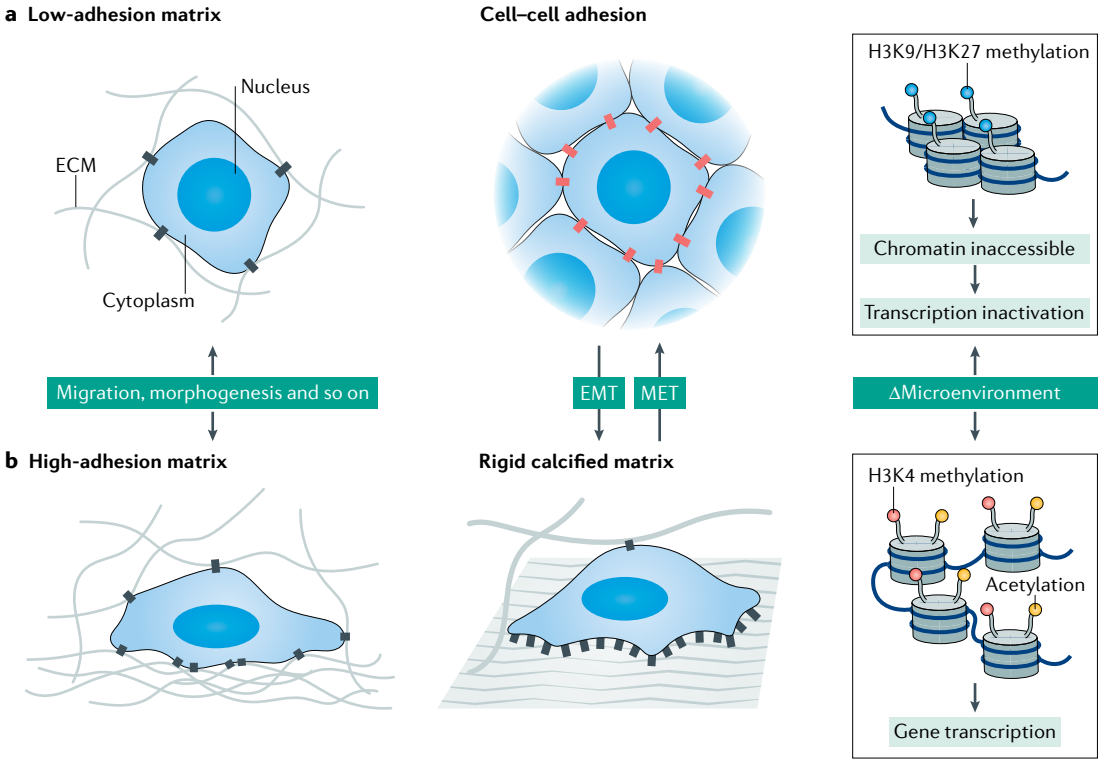

Fig. 2 | **The tissue microenvironment can dictate the epigenetic state. a** | A cell with low adhesion within a biopolymeric matrix or with low adhesion to adjacent cells can acquire histone H3 lysine 9 (H3K9) and histone H3 lysine 27 (H3K27) methylation, which results in chromatin condensation and the inactivation of transcription. **b** | By contrast, a cell with high adhesion within a rigid biopolymeric matrix or with high adhesion within a rigid calcified matrix can have increased chromatin accessibility and transcription as a result of the induction of histone acetylation and/or histone H3 lysine 4 (H3K4) methylation. Cell migration and morphogenetic processes like epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET)(arrows) will lead to microenvironments with different biophysical and biochemical properties (ΔMicroenvironment), which, in turn, will modify the epigenetic state of the cell. ECM, extracellular matrix.

to pathogenesis, where signal imbalances can augment disease states[63]. Considering that the dynamic epigenome regulates gene expression, it is not surprising that epigenetic modifiers are central to signals underlying pathogenic phenotypes. Of particular note is the role of epigenetics in cancer, in which the microenvironment, including matrix mechanics, topography and geometry, influences gene expression. There is also considerable interplay between the epigenome and DNA mutations, where DNA repair genes may be epigenetically silenced, thereby preserving or enhancing malignant-mutation-related phenotypes[63,64]. Thus, it is not only important to consider how materials guide plasticity through the epigenome but also how DNA mutations may influence these effects.

Finally, although much literature is associated with cell plasticity in developmental and disease biology[65,66], the seminal reports by Yamanaka and colleagues[67,68] and by Thomson and colleagues[69] — showing that somatic cells could be reprogrammed into pluripotent cells by exogenous transcription factors — highlighted that plasticity can be harnessed for regenerative medicine. Although during reprogramming the genetic material is initially modified directly through viral transduction, epigenetic changes drive these transformations and initiate key morphogenic events (for example, MET) that overcome the epigenetic barrier to the pluripotent state[70–78]. Pathways that enable somatic cells to overcome the epigenetic barrier to reprogramming can be stimulated by internal and external factors, including the use of multipotent cells[79], alternative genetic techniques[67,80], the delivery of recombinant proteins[81], mRNA[82] or microRNAs (miRNAs), and the use of small molecules that target chromatin-modifying enzymes and other signalling pathways[70–78,83–85].

Given that cellular plasticity is regulated by microenvironment-induced changes in the epigenome, extracellular materials play a major role in guiding processes governing development and disease.

Materials and the epigenome

To understand how materials influence cell plasticity, the dynamic architecture of the cell–material interface must be explored. Cells receive cues from the surrounding environment that travel to the nucleus, and study of the signalling cascades that transmit these cues highlights how materials can influence the environment at the level of the extracellular matrix (ECM) and of chromatin. Indeed, by directing epigenetics and plasticity, materials can cause changes in cellular form and function.

Mechanotransduction and dynamic reciprocity. The cell–material interface interacts at multiple points. In animal tissue, the ECM is composed of a rich assortment of insoluble proteins and proteoglycans, as well as sequestered soluble signalling molecules, and it is a dynamic network with differences in biochemical and biophysical properties across all tissues and across developmental and disease contexts[86]. As an interface, this natural biomaterial presents a variable surface energy that contrasts with that of neighbouring cells or fluid compartments, and can orchestrate complex biomolecular and cellular interactions[87].

Cells adhere to each other through junctional proteins called cadherins and to the ECM through cell surface receptors such as integrins[88]. The affinity of integrins for distinct ligands in the ECM catalyses a conformational change in the receptor that initiates a complex signal transduction cascade that can involve the recruitment of >100 cytosolic proteins, some of which constitute the core focal complex and others that propagate signals into the cytoplasm[89]. The mechanical properties of the ECM are converted to biological activities through the formation of these multiprotein plaques, which range from nanoscale focal contacts to microscale focal adhesions and mature fibrillar adhesions. These plaques tether the external matrix to the cell surface and nucleate the formation of cytoskeletal filaments, including filamentous actin, intermediate filaments and microtubules, which regulate cytoskeletal tension and are connected to the nucleus (FIG. 3). Forces from the microenvironment are propagated through integrins to the network of cytoskeletal filaments, which can be tethered to the nuclear membrane[90]. Ion channels and G-protein–G-protein coupled receptor complexes also respond to membrane tension and ECM dynamics[90–92], where complementary and/or opposing activities will feed into signal transduction cascades. Downstream signal transduction is, therefore, intimately linked to the viscoelastic properties of the engaged ECM. A stiffer ECM invariably leads to larger adhesions and increased cytoskeletal tension, which, in turn, can influence the activity of mechanosensitive ion channels[93], and, thereby, drive numerous downstream cascades to dictate cellular processes, including adhesion, proliferation, migration and differentiation[94–96].

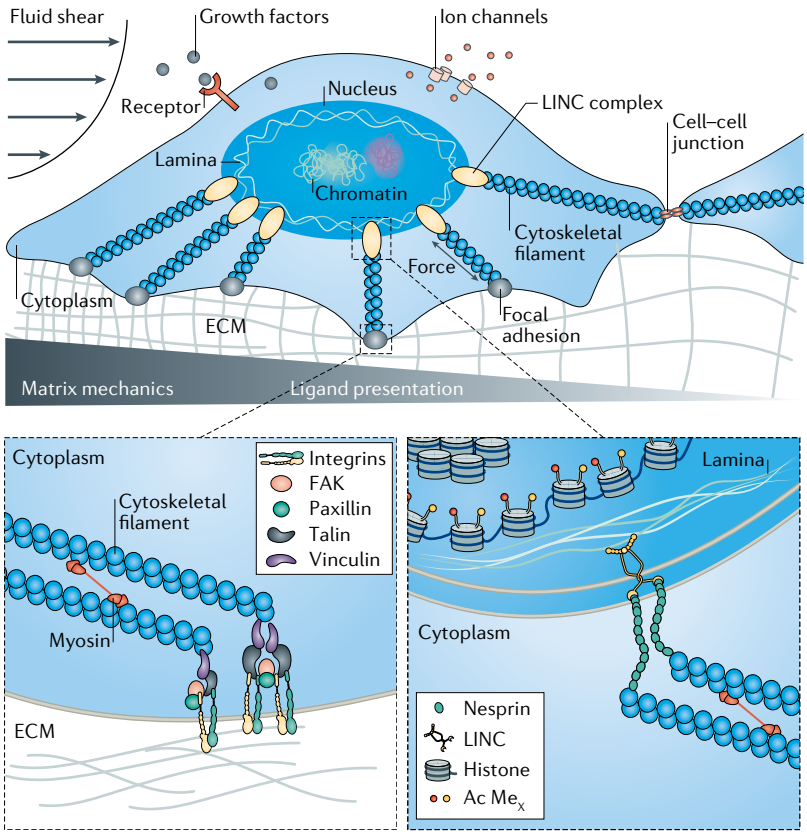

Fig. 3 | **Mechanotransduction at the biomaterials interface.** A cell adherent to the extracellular matrix (ECM) with gradient mechanical properties and ligand presentation is shown. Cells adhere to ECM ligands via cell surface integrins that nucleate focal adhesions to sense and transduce the viscoelastic properties of the matrix and propagate this information through cytoskeletal filaments (comprising actin, microtubules and intermediate filaments) to the nuclear membrane, where the linker of nucleoskeleton and cytoskeleton (LINC) complex will convey these signals to nuclear lamina and associated chromatin. Other signals at the membrane, including growth factors and ion channel activity, are similarly processed through the cytoplasm to the nucleus, often using the same downstream effectors. How integrin adhesion and coupling to cytoskeletal filaments (bottom-left panel) and the propagation of filaments to the nuclear membrane (bottom-right panel) transmit materials properties and forces to regulate chromatin state and, subsequently, gene expression, is shown. Note that filamentous actin is depicted as a key filament for force transduction; similar structures linking the cytoplasm to the nucleus are observed with microtubules and intermediate filaments. Ac, acetylation; FAK, focal adhesion kinase; Me_x, methylation where $_x$ indicates any number of methyl groups.

Once cells have interpreted the ECM through this process of mechanotransduction, forces and mechanical information in the cellular microenvironment are converted to biochemical signals. In the past several decades, many biophysical studies have investigated force-induced changes in the molecular landscape at the cell–material interface and the subsequent biochemical activities that influence cell behaviour[97]. Mechanical signals are translated to biochemical activities through conformational changes in membrane proteins, the activation of stretch-gated ion channels, force-induced unfolding of ECM proteins to reveal cryptic binding sites and changes in protein phosphorylation[98,99]. Furthermore, signal propagation often results in the production of secondary signals, such as ions, cAMP and other small molecules, which modulate cellular activities, such as the contraction of actomyosin, a complex of actin and myosin motors that regulate cytoskeletal tension, thereby shaping the form and function of cells and tissues during development[100].

Mechanotransduction from the ECM to the cytoplasm has been well studied[101], but how extracellular materials parameters and forces regulate chromatin has only recently taken centre stage. Nearly 40 years ago, Bissell and colleagues proposed that mechanotransduction and signal propagation were reciprocal between cells and the ECM[102]. This concept of dynamic reciprocity postulates a continuum between the matrix, cytoskeleton and nucleus that translates biophysical information encoded in the ECM. It, thus, provides a framework for the nucleus responding to forces and matrix parameters by initiating corresponding gene expression programmes that, in turn, model or remodel themselves and their surrounding matrix.

Dynamic reciprocity and the crosstalk between cells and their environment are exemplified in vivo throughout development and disease, including during reproduction and embryogenesis[103], wound healing[104], mammary morphogenesis[105] and other instances of branching morphogenesis[106]. An interesting outcome of this reciprocity is that the ECM, adhesive structures, membrane, cytoskeleton, nuclear envelope and chromatin all influence the dynamic interplay between cells and their surrounding environment.

Mechanotransduction at the nuclear membrane. Mechanotransduction influences chromatin architecture and the epigenetic state by propagating diffusion-based signals initiated by cell surface receptor–matrix engagement. For instance, focal adhesion and cytoskeletal tension enhance various opposing cellular activities, such as the activity of phosphatases and kinases; the phosphorylation state of membrane-bound proteins modulates signal transduction in the cytoplasm, and the phosphorylation state of cytosolic proteins can dictate nuclear translocation events. The phosphorylation of focal adhesion kinase and extracellular related kinase (cytoplasmic proteins with a role in mechanotransduction) leads to their nuclear translocation and regulates transcription[107,108]. The transcriptional regulators YAP and TAZ also translocate to the nucleus in response to matrix stiffening through integrin-mediated mechanotransduction and the downstream phosphorylation of YAP and TAZ, with evidence for enhancement via direct tension at the nucleus to facilitate pore opening[109–114]. Indeed, model systems using microfluidics have demonstrated how confined microenvironments will lead to partial rupture of the nuclear membrane, thereby aiding translocation of cytosolic proteins[115]. As well as sensing the viscoelasticity of materials, YAP and TAZ signalling helps propagate signals from growth factors, metabolites, G-proteins and inflammatory pathways, as reviewed recently[116].

The nucleus itself also acts as a mechanosensor[117,118]. Perceived forces are transmitted from cytoskeletal filaments to nucleoskeletal anchors called lamins through the linker of nucleoskeleton and cytoskeleton (LINC) complex[109] (FIG. 3). Indeed, lamins, which are nuclear intermediate filaments that can be separated into A, B and C lamins, are primary drivers of nuclear mechanosensing[117–119]. Lamins associate with transcriptional regulators, as well as directly with chromatin in LADs, to link nucleosomes at the membrane to the cytoskeleton, thereby facilitating mechanical communication between the ECM and DNA. The nucleus is directly connected to the cytoskeleton through the LINC complex, in which SUN and KASH proteins connect the nuclear lamina to cytoskeletal filaments. Forces acting on a cell can deform the nucleus[120–122], and work over the past 10 years has demonstrated how force applied to the cell membrane can alter chromatin organization[123]. A link between lamin A composition and nuclear stiffness, which scaled with bulk tissue stiffness and collagen content, has also been demonstrated[124]. Increased tissue stiffness increases lamin A levels, thereby stabilizing the chromatin state to drive specific gene expression programmes. This observation suggests that there is feedback between signals that are conveyed through mechanotransduction and the restructuring of the lamin network and, thus, dynamic reciprocity[125,126]. A mechanosensory complex containing emerin, non-muscle myosin IIA and actin attenuates nuclear lamina through force-mediated defective heterochromatin anchoring and increased histone methylation[127]. A relationship between nuclear stabilization and histone methylation state was demonstrated in 2018, where nucleoskeleton softening correlates with the activity of WD repeat domain 5 (WDR5), a component of H3K4 methyltransferases, thereby increasing H3K4me to facilitate cell migration in constrained 3D environments[109].

Finally, a network of actin filaments in the perinuclear space, referred to as the 'actin cap', directly links cytoskeletal filaments to the nuclear membrane through KASH proteins. These actin cap filaments can transmit force to the nuclear membrane to reorganize chromatin[125,126,128,129]. Taken together, it is believed that cytoskeletal filaments transduce materials properties from the ECM to the nuclear lamins to influence chromatin architecture and the positioning of genetic loci to coordinate transcription. We now explore how engineered cell culture materials have paved the way to understanding the interplay between extracellular parameters and the epigenetic control of gene expression underlying cell plasticity.

Engineered materials and cell plasticity

Cells in tissues are surrounded by a dynamic, composite material composed of protein and carbohydrate biopolymers, often with integrated mineral phases. The tissue-specific variations in chemistry, mechanics and diffusional properties are vast, having evolved to guide the differentiation of cells into over 200 cell types, so that they can form complex functional associations and structures. Although there has been immense progress in the development of techniques to study cell signalling within living tissue[130,131], these techniques do not always relate biological materials properties to signal transduction and epigenome dynamics.

Overview of materials cues and epigenetic plasticity. In the last several decades, the assembly of synthetic and natural materials into biomimetic architectures has enabled researchers to simplify complex biosystems and deconstruct the multivariate signals that coordinate cell behaviour[132–134]. Capitalizing on the infrastructure from the semiconductor-manufacturing sector and advances in additive manufacturing, biomaterials have been engineered to precisely control the cell and tissue environment from the molecular level to the macroscale. Thereby, researchers can precisely tune and articulate the direct contact of the material to the cellular membrane, with exquisite control over ligand density and presentation, mechanical properties and multiscale topography.

Synthetic biomaterials have been developed that leverage biophysical cues to influence a broad range of behaviours, from cell adhesion and morphology[135] to cell proliferation and differentiation[136,137]. Some of the most widely used platforms to investigate how

materials influence cell plasticity include microstructured and nanostructured interfaces created through electrospinning, printing and lithographic means, planar surfaces that present adhesions in a controlled manner and matrix-mimetic-hydrogel networks that vary cell-binding sites, mechanical properties, dimensionality and dynamic compression and tension. Evidence suggests that each of these materials parameters will influence the epigenetic regulatory machinery via mechanochemical signalling (FIG. 4). Although there are many cell-specific and context-specific variations in histone modifications that coordinate gene activation and silencing, in general, materials that catalyse histone H3 lysine 4 trimethylation (H3K4me3) and histone H3 acetylation (H3ac) are associated with gene activation[138], whereas materials that promote trimethylation of lysine 27 on histone 3 (H3K27me3), which marks active cis-regulatory elements, are associated with gene inactivation[139] and epigenetic programming and reprogramming[140,141] (TABLE 1).

Cells and materials first make contact through interactions at their interface; cells directly contact soluble (liquid) and/or insoluble (matrix) matter. In the case of soluble matter, fluid shear stress can influence the regulation of histone-modifying enzymes in endothelial cells[142,143]. For instance, the force applied at the surface

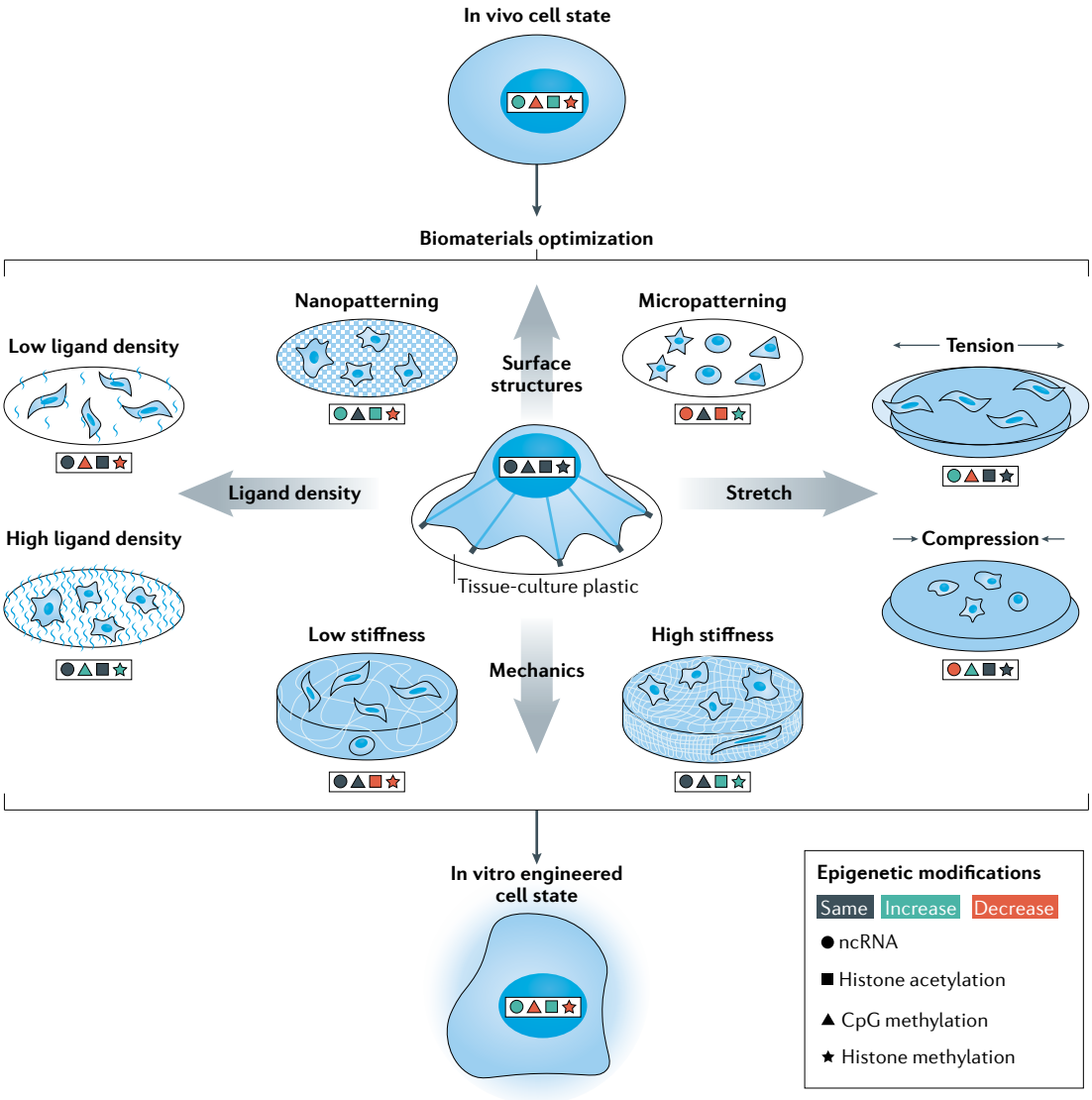

Fig. 4 | **Engineering epigenetics using defined materials.** The epigenetic state of cells cultured on tissue culture plastic varies from the native epigenetic state of cells in vivo. Engineered biomaterials can be used to present multivariate signals to cells in culture to recreate in vivo-like microenvironments and, thus, reconstitute native epigenetic states; these states direct phenotypes that permit development and disease to be modelled. Specifically, nanopatterning and micropatterning, deformable substrates, dynamic mechanics and ligand density can be harnessed in vitro to recreate the 'in vitro engineered cell state' (bottom) that emulates the desired 'in vivo cell state' (top) far better than culture on tissue culture plastic. Epigenetic modifications, including those imposed by non-coding RNAs (ncRNAs), DNA CpG methylation and histone acetylation and methylation, are numerous and varied, depending on context, cell type, tissue and many other factors. Modifications are presented as an illustration only and are not intended to show actual changes associated with primary research findings.

Table 1 | **Histone marks influenced by materials parameters**

Histone mark	Level	Cell source	Materials interface	Outcome	Ref.
H3ac	↑	Human mesenchymal stem cells	Microgrooves on PDMS	Nuclear elongation and decreased HDAC activity	187
		Primary murine fibroblasts	Microgrooves on PDMS	Improved reprogramming to IPS cells	186
		Human mammary epithelial cells	3D stiff (~2 kPa) interpenetrating network of basement membrane proteins and alginate	Tumour formation	166
	↓	Human mammary epithelial cells	3D laminin-rich ECM on PDMS	Cell rounding and chromatin condensation	193
		Murine embryonic stem cells	Micropatterned fibronectin on plastic	Nuclear-stiffness-dependent localization of transcription cofactor and target-gene upregulation during differentiation	212
H3K9ac	↑	Murine embryonic fibroblasts	Micropatterned spheroids on fibronectin-coated plastic	Trans-differentiation	196
		Murine embryonic fibroblasts	Micropatterned fibronectin on plastic	Chromatin condensation and changes in gene expression	195
		Murine melanoma	Micropatterned high-perimeter-curvature fibronectin on polyacrylamide hydrogels	Reprogramming to stem-cell-like melanoma-initiating cells	205
H3K14ac	↑	Human umbilical vein endothelial cells	Laminar shear stress in cone-plate apparatus	Changes in gene expression	213
		Marsupial kidney epithelial cells	Stiff polyelectrolyte multilayers[a]	Regulation of DNA replication	174
H3K4me2	↑	Murine melanoma	Micropatterned high-perimeter-curvature fibronectin on polyacrylamide hydrogels	Reprogramming to stem-cell-like melanoma-initiating cells	205
H3K4me2,3	↑	Murine fibroblasts	Microgrooves on PDMS	Improved reprogramming to IPS cells	186
H3K4me3	↑	Human T lymphocytes	3D high-density collagen type I matrix	Nuclear deformability, viscosity and softening	109
		Murine embryonic fibroblasts	Nanogrooved gelatin-coated polyurethane acrylate on glass substrates	Reprogramming into dopaminergic neurons	188
H3K27me3	↑	Murine embryonic fibroblasts	Micropatterned spheroids on fibronectin-coated plastic	Trans-differentiation	196
H3S10p	↑	Human umbilical vein endothelial cells	Laminar shear stress and trichostatin A in cone-plate apparatus	Changes in gene expression	213
H4ac	↑	Primary rabbit mammary cells	Dense collagen	Changes in gene expression	173
	↓	Human mammary epithelial cells	3D stiff (~2 kPa) interpenetrating network of basement membrane proteins and alginate	Tumour formation	166
		Human mammary epithelial cells	Non-adhesive polyHEMA on PDMS substrata	Regulated cell and nuclear shape	193
			Micropatterned collagen type I on PDMS		193

ECM, extracellular matrix; H3ac, histone H3 acetylation; H3K14ac, histone H3 lysine 14 acetylation; H3K27me3, histone H3 lysine 27 trimethylation; H3K4me2, histone H3 lysine 4 dimethylation; H3K4me3, histone H3 lysine 4 trimethylation; H3K9ac, histone H3 lysine 9 acetylation; H3S10p, histone H3 serine phosphorylation; H4ac, histone H4 acetylation; HDAC, histone deacetylase; IPS, induced pluripotent stem; PDMS, polydimethylsiloxane; polyHEMA, poly(2-hydroxyethyl methacrylate). [a]Hyaluronic acid and poly-L-lysine capped with poly(styrene) sulfonate and polyallylamine hydrochloride.

of embryonic stem (ES) cells through shear stress from liquid activates transcription by promoting histone H3 lysine 14 acetylation (H3K14ac) and histone H3 lysine 79 methylation (H3K79me), leading to the expression of genes associated with cardiovascular lineage specification[142]. The interaction of cells with insoluble ECM materials activates many of the signalling pathways in response to shear, such as mechanically gated ion channels and membrane receptors. In addition, shear on the apical cell surface will propagate to the basal side to impact adhesion of the cell to the underlying matrix[144]. To deconstruct the signalling associated with both soluble and insoluble cues, biomaterials design requires control over multiple chemical and physical parameters at the cell–materials interface.

Receptor–ligand engagement at the cell–biomaterials interface. In vivo, cells engage with complex mixtures of proteins through matrix-bound combinations of short biomolecule motifs, often in the form of exposed peptide ligands at the surface of matrix proteins and resident growth factors. A classic example of how signals in a microenvironment can influence cell plasticity comes from the demonstration that embryonic microenvironments attenuate malignant cancer phenotypes[145]. Similarly, matrices constructed from embryonic stem cells have been demonstrated to modulate melanoma cell plasticity through ligand–receptor signals with reversion to a melanocyte phenotype[146,147]. Interestingly, conditioned media from ES cells did not reproduce these changes, suggesting that reprogramming was accomplished through melanoma cell–biomaterial interactions in the local microenvironment, rather than through soluble signals from ES cells. This concept was also demonstrated during the induction of induced pluripotent stem (IPS) cells; treating somatic cells with ES cell extracts during reprogramming increased reprogramming efficiency[148]. Taken together, these studies demonstrate the importance of local extracellular signals in regulating the epigenetic state in both normal and cancer cells.

The interface of synthetic biomaterials can be tuned to present proteins or peptide motifs that influence cellular outcomes similar to those driven by extracellular signals in vivo. However, due to the complexity of native matrices, many researchers have turned to high-throughput techniques aided by high-content imaging to empirically discover immobilized biological materials that promote specific cellular outcomes. For instance, robotics was used to array polyacrylate libraries to direct ES cell adhesion and self-renewal[149]. Presumably, the wide range of polymers show differences in surface energy, which promotes differential adhesion of matrix proteins, thereby providing interfaces to enrich subpopulations of ES cells or promote ES cell maintenance after adhesion. Using fabricated peptide microarrays on self-assembled monolayers to stimulate specific ligand–receptor interactions demonstrated how short peptide ligands can control the plasticity of ES cells[150,151]. These high-throughput approaches have also been used to explore the role of matrix in pathological plasticity. For instance, a protein microarray can be used to identify ECM proteins that foster invasive microenvironments for cancer cells[152]. Indeed, screens for combinations of ECM proteins that mimic those present in the bone, brain and lung revealed distinct signatures that could predict metastatic tropism in panels of cell lines[153]. We have demonstrated how combinations of short peptides derived from the matrix and growth factors can guide stem cell differentiation[154,155] and reprogramme cancer cells to a stem-cell-like state[156]. When a proteoglycan-binding motif is presented with a sequence derived from bone morphogenetic protein 7 (BMP7), melanoma cells adopt a highly invasive tumorigenic phenotype[156].

In addition to peptide and protein identity, how a ligand is presented to cells is important, with the density and affinity of ligand–receptor engagement steering stem-cell-lineage specification[157]. Indeed, a recent report demonstrated the importance of dynamic engagement of integrins by twisting magnetic beads coated with the matrix-derived integrin ligand Arg-Gly-Asp (RGD) at the cell surface; the stress at the cell membrane propagated through integrin receptors, cytoplasm and to the LINC complex, disrupting chromatin and driving transcription[158]. Interestingly, although development and disease outcomes have clear connections to epigenetic regulation, few studies relate extracellular composition and ligand engagement to specific epigenetic states. Nevertheless, considering how engaging the matrix via precise ligands can modulate actomyosin contractility and growth factor signalling, it is reasonable to expect that different matrix-engagement profiles will influence signalling at the nucleus.

Static and dynamic matrix mechanics. Engagement of the matrix through receptor–ligand interactions enables the cell to sense the viscoelastic properties of the matrix and to respond by organizing intracellular-filament networks. Both the density and affinity of cell–matrix adhesions will impact the degree to which a cell can push or pull biological materials[159,160]. Seminal work over the past couple of decades demonstrates the importance of stiffness and viscoelastic properties on cell plasticity in physiological[25,26,161–163] and pathological[28,164] contexts. ECM mechanics directly influence the properties of the cytoskeleton and nucleoskeleton[165], which together control nuclear and chromatin dynamics[127,164].

Recent advances in our understanding of how matrix mechanics influence nuclear architecture has led to targeted investigations of the molecular mechanisms underlying cell plasticity. Stiff ECMs promote nuclei wrinkling, enhance chromatin accessibility and upregulate the expression of tumorigenic genes through the action of HDAC3 and HDAC8 in breast cancer cells[166]. Similarly, increasing matrix stiffness will increase nuclear localization of HDAC4 by promoting its phosphorylation, which impedes the fibroblast–myofibroblast transition in embryonic fibroblasts[167]. In addition to nuclear shuttling of epigenetic modifiers, we now know that stiff substrates promote nuclear localization of the mechanosensor YAP with some epigenetic memory; epigenetic changes induced by YAP are still evident after softening the substrate using dynamic hydrogel chemistry[168,169]. Changes in HDAC

and histone acetyltransferase activity and the histone acetylation state adapt dynamically after matrix softening, with evidence for epigenetic memory playing a role in the temporal response[169]. A link between YAP activity and epigenetic regulation was also suggested when polyacrylamide gradient hydrogels were used to study mechanotransduction in stem cells[170]; YAP expression and nuclear localization were dependent on stiffness, with a direct link to lamins and myocardin-related transcription factor A (MRTF-A) through the action of non-coding RNAs, which are believed to exert regulatory control over gene expression but also through pre-transcriptional mechanisms involving protein and DNA binding[12,171] Another study showed that YAP localization does not directly correlate with stiffness in 3D due to the decreased size of the nucleus and the absence of stress fibres in this setting[172]. These studies illustrate the importance of replicating tissue-mimetic stiffness and dimensionality in vitro to accurately study signalling that may occur in a 3D context, and together provide clues as to how messages encoded by dynamic matrix mechanics traverse the cytoplasm to the nucleus.

Epigenetic changes on account of dimensionality have been demonstrated using 3D collagen hydrogel environments alongside tissue-culture-plastic controls[109,173]. WDR5 is directed by actomyosin contractility from the 3D microenvironment to increase histone methylation and decrease chromatin compaction[109]. Furthermore, one group leveraged polyelectrolyte multilayer films to demonstrate and decondensed nuclei in response to variable mechanics[174]. Capitalizing on how stiffness influences these signalling pathways, polyacrylamide hydrogels have also been used to demonstrate that soft microenvironments can enhance MET to accelerate reprogramming-factor-mediated induction of pluripotent stem cells[170].

Although these platforms more closely emulate tissue compared with tissue culture plasticware, the ECM has a viscous component with dynamic tendencies, and often displays stiffening and softening behaviour in response to stress. For instance, stress relaxation in hydrogels can influence stem cell differentiation[175,176] and cancer cell invasive phenotypes[177]. Indeed, the use of a synthetic 3D hydrogel pronounced the influence of temporally controlled stress-stiffening in guiding stem cell differentiation[177,178]. Together, these reports show how the dynamic viscoelastic behaviour of biopolymeric materials can set the context in which cells interpret and transduce mechanical information to regulate functional activity.

Nanotopography and microtopography. A governing aspect of tissue form and function is the connectivity of cells to their neighbours and the ECM, which is driven by the mechanics, composition, topography and intratissue forces of the matrix and, thereby, gives rise to a wide array of cell and tissue shapes. Microengineering and nanoengineering techniques provide a versatile approach to study how geometry and topology influence the way in which cells receive and integrate signals[179]. In contrast to flat culture substrates, membranes in tissue form submicron-sized fibrils, with hierarchical structure providing topographic signals[180]. Nanofabrication enables the formation of nanoscale gratings, posts, pits, aligned fibres and composite isotropic, anisotropic or gradients structures[181].

Controlling the nanotopography under adherent cells will dictate the initial conditions of adhesion and the stability of the cytoskeleton, thereby directing mechanotransduction to the nucleus. Nanoscale ordering at the biomaterials interface guides stem cell differentiation[182,183] by regulating histone modifications and the activity of miRNAs that drive lineage-specific gene expression[184,185]. Microgrooves and nanofibres were designed to induce MET to reprogramme fibroblasts to IPS cells[186]. Reprogramming was enhanced by the decrease in HDAC activity and the upregulation of WDR5 induced by these biophysical cues, which activated the transcription of pluripotency genes through the acetylation and methylation of H3. Microgrooves formed on polydimethylsiloxane of various spacing also increased H3ac, histone 3 lysine 4 dimethylation (H3K4me2) and H3K4me3 compared with non-patterned surfaces[186,187]. Similarly, somatic fibroblasts were reprogrammed into induced dopaminergitic neurons on microgrooved substrates and even more efficiently on nanogrooved substrates[188].

Lithography-based techniques facilitate precise patterning of substrata with matrix molecules, which has allowed fundamental studies into geometry–cell function relationships[189]. Pioneering work[27,190] demonstrated that controlling the shape of single cells influences cell cycle, proliferation and differentiation. Single stem cells confined to microislands directed their cytoskeletal architecture in response to subcellular cues triggered by the microisland area, aspect ratio and perimeter features[191,192]. Changing subcellular cues at the cell periphery from convex to concave curvature increased actomyosin contractility, thereby setting the context for the integration of soluble cues that promote osteogenesis over adipogenesis[191]. The first study to directly look at how cell shape influences chromatin marks showed that cell spreading influenced histone acetylation[193]. Specifically, cell rounding either on a micropatterned substrate or on a 3D hydrogel led to global histone deacetylation, chromatin condensation and decreased global gene expression in 3D versus 2D cultures. Consistent with this report, we found that micropatterning mesenchymal stromal cells leads to a decrease in nuclear area, with a corresponding decrease in histone H3 lysine 9 acetylation (H3K9ac) and H3K9 and H3K36 methylation[194]. In another study, H3K9ac levels increased with increased pattern size and nuclear volume, regardless of cell shape. Additionally, levels of filamentous actin and phosphorylated myosin light chain in cells increased on larger patterns, whereas tumour necrosis factor alpha (TNFα) depolymerized actin and promoted HDAC3 translocation to the nucleus, in turn influencing the epigenetic state[195]. Furthermore, recent work demonstrated that microconfinement can direct cell state, with deacetylation of H3K9ac in fibroblasts and cancer cells in the promoters of mesenchymal genes and acetylation of H3K9ac in the promoters of genes that drive reprogramming to stem-cell-like states[196].

This year, the same group demonstrated how microconfinement can 'rejuvenate' fibroblasts to have open chromatin configurations with increased propensity to synthesize collagen and display contractile phenotypes in 3D hydrogels[197]. These works illustrate how nuclear and chromatin architecture is susceptible to reprogramming through materials properties alone, thereby making materials design critical in ensuring appropriate outcomes.

Micropatterning has also been used to engineer the architecture of small populations of cells, where geometric confinement can emulate structural cues during development and disease, for example, compact tissue structures formed during morphogenesis. For instance, the self-assembling behaviour of ES cells was exploited to form gastruloid mimics[198]. In this work, geometry drove cell fate patterning through WNT, Nodal and BMP signalling, demonstrating that soluble signals can integrate with biophysical parameters. In addition to the patterning of lineage specification, pattern size can influence the differentiation of pluripotent stem cells into multiple lineages with spatiotemporal control[199–202]. We used soft lithography on hydrogel matrices to explore cancer cell state, finding that regions of high-convex curvature at the perimeter of microislands will direct cell and nuclear shape changes, activate mitogen-activated protein kinase (MAPK) signalling and reprogramme melanoma cells to cells with a stem-cell-like phenotype[203], and enhance metastatic potential through mechanotransduction-augmented neovascularization[204]. Both high interfacial curvature at the border and microaggregates with a high interfacial boundary enriched H3K4me2 and H3K9ac in cells adjacent to the boundary, which activated expression of the pluripotency epigenetic modulator PRDM14[205], thereby revealing a stem-cell-like phenotype in melanoma cells. Taken together, these studies demonstrate how controlling matrix parameters on microengineered materials can precisely modulate differentiation and de-differentiation.

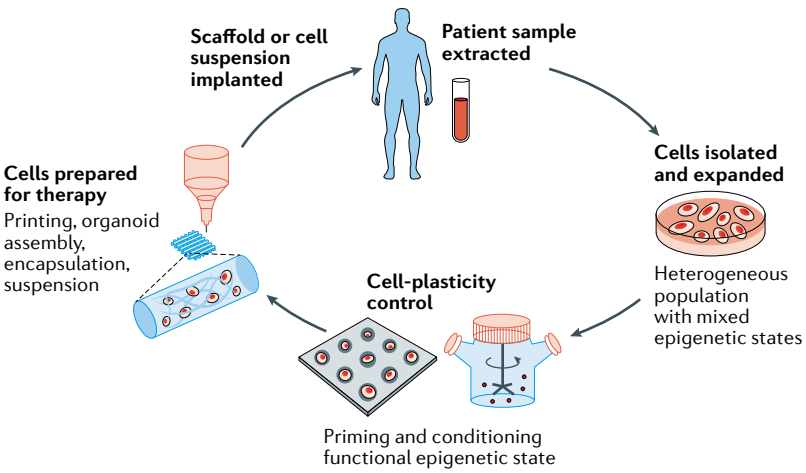

Fig. 5 | **Update to the tissue engineering paradigm.** Cells isolated from patients need to be expanded to ensure sufficient numbers of cells for disease modelling and regenerative therapy. However, this expansion leads to a heterogeneous population of cells, with mixed epigenetic states (and, thus, inappropriate lineage specification), cell senescence and other undesirable outcomes. Materials control of cell plasticity, designer substrates and bioreactors can be harnessed to prime cells towards the appropriate epigenetic state so that they assemble in the desired way, such as into organoids, when integrated with new materials for regenerative therapies.

Conclusions and future perspectives

Evidence from basic cell biology to clinical physiology and biomedical engineering indicates that cells have a high level of plasticity. From differentiation during development to the constantly shifting morphogenetic landscape during homeostasis and pathological progression, the behaviour of cells in tissue is guided by the physical and soluble microenvironment via the dynamic rewiring of the epigenome. It is important to understand how the context in which a cell perceives its surrounding microenvironment guides these activities, in order to gain insight into mammalian development and disease progression.

One challenge facing biologists and biomaterials scientists is ensuring that 2D cell biology studies reflect the signalling inherent to 3D in vivo systems. This challenge is unsurprising, considering how different the environment in 3D in vivo tissue is to that in a rigid 2D plastic dish. Many studies indicate that signalling differs greatly between cells of the same type cultured in 2D versus within an engineered model system (for example, micropatterned culture with defined surface ligands) or a 3D matrix[206,207]. Indeed, histone acetylation is influenced more in cells grown on plastic (these cells have a high level of spreading and cytoskeletal tension) than in lithographically microconfined cells or in rounded cells within a 3D matrix. To ensure biologically meaningful results, researchers must consider how biophysical and biochemical properties, as well as dimensionality and context, may influence cell plasticity, particularly considering the rapid acceleration of tissue fabrication and assembly technologies[208]. FIGURE 5 depicts a modified version of the 'tissue engineering paradigm', in which plasticity control through integrating designer substrates and bioreactors during in vitro cell expansion can ensure a desired cellular assembly and activity during the preparation of cells for therapy by printing, encapsulation and so on.

In addition to tissue-specific somatic cells, stem cells and cancer cells, which are the most studied cell systems in materials-centric epigenetics, the plasticity of many other cell types has important roles in physiology and pathology. For instance, the plasticity of cells like fibroblasts and pericytes dictates the efficiency of steps associated with tissue assembly, morphogenesis and wound healing[209]. Of note, cells in the immune system are implicated in most processes that occur during development and disease progression. Epigenetic changes in T cells in response to environmental conditions can influence their response to foreign antigens and their epigenetic memory, which influences their ability to initiate gene expression programmes involved in functional activities[210]. It is clear that, if the plasticity of one cell type is affected, the response of associated cell types may be changed. Therefore, understanding the interplay and associated plasticity of all cell types involved in a particular biological outcome will be essential to recreating biomimetic processes with designed biomaterials.

Box 1 | **Tools to characterize the epigenome**

As more biomaterials scientists begin to explore how materials parameters influence the epigenetic state of cells, integrating advanced techniques for the quantitative isolation and profiling of chromatin and the multiprotein complexes, enzymes and non-coding RNAs (ncRNAs) that are associated with it will be critical. Techniques like next-generation sequencing[214] are now readily accessible for investigating 'epigenetic state–gene expression' relationships at the single-cell level. Here, we describe current techniques that are useful for querying the epigenome at the level of DNA, histones, enzymes and ncRNAs[215].

The classical technique for analysing DNA methylation involves the isolation of nuclear material, electrophoresis and the use of methylation-specific antibodies in western blot analyses or enzyme-linked immunosorbent assays. Broad patterns of methylation are elucidated through bisulfite sequencing using 5-methylcytosine and analysis through pyrosequencing, methylation-specific polymerase chain reaction, DNA microarrays, next-generation sequencing and mass spectrometry techniques[216]. A popular technique for mapping chromatin accessibility is the assay for transposase-accessible chromatin with sequencing (ATAC-Seq), in which Tn5 transposase is used to cut open chromatin for subsequent sequencing[217,218]. In addition, there have been considerable advances in detecting DNA methylation via bisulfite-free methods, such as those using optical and electrochemical means[4]. These techniques and others provide a host of complementary approaches to assess changes in DNA methylation between conditions.

Chromatin immunoprecipitation (ChIP) involves the use of antibodies to gather chromatin via specific marks for the subsequent analysis of protein–DNA interactions[5]. When coupled with nucleic acid sequencing (ChIP-Seq) or using microarrays (ChIP-chip), genome-wide mapping of histone modifications can probe associated genetic elements and proteins. RNA-Seq or the use of DNA microarrays is performed in tandem to relate protein–DNA interactions to gene expression profiles. Long ncRNAs and microRNAs provide epigenetic regulation without translation into proteins, and are important determinants in chromatin state[12]. These molecules are isolated using similar techniques to mRNA preparations and are evaluated using quantitative polymerase chain reaction, microarrays and sequencing approaches. Antibody-based techniques quantify abundance and not activity, which is particularly important when considering the complementary and opposing activities of chromatin-modifying enzymes. Sequence-specific enzyme substrates with fluorometric or colorimetric readouts are useful reagents for assessing specific enzyme activities[13]. Label-free techniques based on peptide microarrays and mass spectrometry have proved useful for dynamic assessments of multiple chromatin-modifying enzymes in parallel[40].

The integration of advanced biomaterials-fabrication techniques with frontier bioassays for quantification of epigenetic status will help establish increased depth of mechanistic insight to support efforts in fundamental biology and biomaterials science and engineering.

Future studies of biomaterials science and engineering require further interdisciplinary efforts, in which isolated cells are cultured in microenvironments that ensure the epigenetic state and corresponding gene expression programmes are appropriately maintained. Similarly, keeping epigenome regulation front and centre during biofabrication will facilitate a deeper understanding of the biology, while ensuring the desired tissue assembly and functional outcome. This approach will also require the integration of new materials design with frontier bioassays, including single-cell omics, miRNA and non-coding RNA, and next-generation chromatin analysis[211] (BOX 1). These efforts will need to match the sophistication of materials design with biological understanding to ensure that meaningful biological outcomes are gained from fundamental studies and that biomimetic-assembly processes during fabrication faithfully replicate nature's designs.

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