



Cite this: *Biomater. Sci.*, 2022, **10**, 5081

Current progress in bionanomaterials to modulate the epigenome

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Recent advances in genomics during the 1990s have made it possible to study and identify genetic and epigenetic responses of cells and tissues to various drugs and environmental factors. This has accelerated the number of targets available to treat a range of diseases from cancer to wound healing disorders. Equally interesting is the understanding of how bio- and nanomaterials alter gene expression through epigenetic mechanisms, and whether they have the potential to elicit a positive therapeutic response without requiring additional biomolecule delivery. In fact, from a cell's perspective, a biomaterial is nothing more than an environmental factor, and so it has the power to epigenetically modulate gene expression of cells in contact with it. Understanding these epigenetic interactions between biomaterials and cells will open new avenues in the development of technologies that can not only provide biological signals (*i.e.* drugs, growth factors) necessary for therapy and regeneration, but also intimately interact with cells to promote the expression of genes of interest. This review article aims to summarise the current state-of-the-art and progress on the development of bio- and nanomaterials to modulate the epigenome.

Received 1st July 2022,
Accepted 15th July 2022

DOI: 10.1039/d2bm01027e

rsc.li/biomaterials-science

Introduction

Biomaterials possess the ability to modulate gene expression and hence, regenerate tissues at a molecular level.¹ For example, biomaterials may be used as depots for the delivery of biological molecules such as growth factors that promote cell growth and differentiation.^{2–4} However, the power of biomaterials extends far beyond their ability to simply deliver drugs or other biomolecules. Mechanical properties, physico-chemical cues, and biological stimuli that cells experience when in contact with a biomaterial all contribute to a cell's epigenetic state and ultimately cell function.⁵ The biomaterials community has exploited this phenomenon to explore how these cues, particularly topography,⁶ surface chemistry,⁷ matrix elasticity,^{8,9} and other mechanical stimuli, influence primarily stem cell differentiation but also other outputs, including cell reprogramming, proliferation, migration, and nuclear deformation.¹⁰ These changes in cell function are mediated by biophysical forces that are mechanotransduced from the extracellular space to the nucleus, resulting in the regulation of epigenetic modifier activity and chromatin or nuclear lamina remodelling.⁵ However, these epigenetic phenomena that govern observed changes in expression have

remained relatively underexplored,¹¹ especially in differentiated cell types that establish pathological milieus. Therefore, further elucidation of the nature of epigenetic biomaterial-tissue interactions will enable the development of a new class of therapeutic biomaterials that can reprogram expression of diseased tissues to healthy ones. In this review, we discuss key findings that illuminate the interface between biomaterials and epigenetics (Fig. 1) with implications in the areas of cancer modelling and therapy, tissue engineering, and regenerative medicine.

Epigenetic regulation

While the basic mechanisms by which biological systems activate genes of interest have been well characterised, a complete understanding of how biomaterials may modulate the expression of genes is still being unravelled. Regulation of the epigenome is required for normal gene expression patterns and development, and for the prevention of inappropriate activation/inhibition of signalling pathways and disease progression.¹² For example, various cancers have been associated with aberrant epigenetic signatures. However, unlike genetic mutations, epigenetic marks are generally reversible. For example, DNA methylation or demethylation can be reversed using DNA methyltransferase (DNMT) enzymes and their inhibitors, respectively. Similarly, histone acetyltransferases (HATs) and histone deacetylases (HDACs) can reversibly add or

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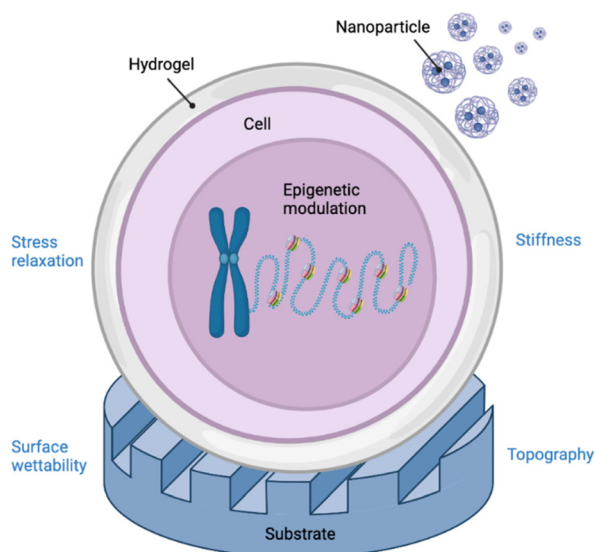


Fig. 1 Graphical abstract depicting the various interactions of bio- and nanomaterials with DNA at an epigenetic level. Created with BioRender.com.

remove acetyl groups from histone proteins, respectively. Moreover, specific microRNAs (miRNAs) can upregulate or downregulate gene expression of different genes by binding to mRNA transcripts. Hence, this versatility and reversibility render epigenetic marks attractive therapeutic targets.¹² For more details describing epigenetic molecular mechanisms, we refer the reader to Allis & Jenuwein.¹³ In this review, we restrict our discussion to the ways in which bionanomaterials' properties can trigger epigenetic cues and signals (DNA methylation, histone modifications, and ncRNAs) to regulate gene expression at the pre- and post-transcriptional levels.

Bionanomaterials and epigenetics in stem cells

The defining hallmarks of stem cells (perpetual self-renewal and the ability to differentiate into at least one specialised cell type) make them invaluable resources in tissue engineering and regenerative medicine. However, their biology must be fully understood and characterised before their complete therapeutic potential can be realised.¹⁴ As a result, researchers have focused their efforts on how various biochemical, biophysical, and material cues influence stem cell behaviour. Arguably, studies have focused primarily on how differentiation is affected, but these cues can also regulate cell migration, morphology, proliferation, and reprogramming.^{10,15} For example, many groups have demonstrated that substrate stiffness can determine stem cell lineage specification *via* mimicking native tissue stiffness. A seminal publication by Engler *et al.*⁸ showed that human mesenchymal stem cells (hMSCs) cultured on softer substrates (elastic modulus, $E = 0.1\text{--}1$ kPa) preferentially differentiated into cells which exhibited neuronal morphology, whereas hMSCs cultured on inter-

mediate ($E = 8\text{--}17$ kPa) and stiff substrates ($E = 25\text{--}40$ kPa) tended to express myogenic and osteogenic markers, respectively.⁸ At present, researchers are focusing on decoding the mechanisms that result in these phenomena, including characterising the interplay between material properties and epigenetic regulation. Recent work has demonstrated that the biophysical features of biomaterials, such as stiffness, surface roughness, patterning, and wetting characteristics,¹⁶ may alter a cell's epigenetic state through several epigenetic regulatory mechanisms, including chromatin remodelling and DNA methylation. Table 1 summarises the genes, proteins, and pathways found to have been affected by biomaterials *via* epigenetic modulation.

Reprogramming

Although reprogramming technology has advanced significantly since 2006, induced pluripotent stem cell (iPSC) generation still relies heavily on viral-induced transcription factor (TF) overexpression, which introduces safety risks such as unexpected genomic integration and insertional mutagenesis.¹⁶ In addition, the reprogramming process is naturally inefficient due to epigenetic barriers that stabilise gene expression in terminally differentiated cells.¹⁷ Therefore, several studies have investigated the addition of factors such as silencing miRNAs, mRNAs, gene-editing machinery such as CRISPR-Cas9, and epigenetic 'signals' in an effort to identify an alternative strategy that could replace viral methods and increase efficiency in the future.¹⁶ We focus our discussion on how material properties can induce epigenetic modifications that lead to enhanced reprogramming.

Yoo *et al.*¹⁸ investigated the capability of a graphene monolayer to induce reprogramming of mouse fibroblasts to an embryonic-like state. Interestingly, they found that pluripotency master regulators, such as *OCT4*, *NANOG*, and *SOX2*, were markedly upregulated in graphene-coated substrate cultures compared with uncoated cultures. They hypothesised that this observed difference was due to the graphene substrate enhancing induction of the mesenchymal-to-epithelial transition (MET), an essential process for reprogramming.^{19–21} To investigate this theory, they measured enrichment of histone modifications at various transcription start sites (TSSs) to determine the extent of chromatin remodelling. Indeed, they found that the TSSs of *OCT4* and *NANOG* were enriched for H3K4me3, a histone mark associated with gene activation and MET.²² In addition, cells cultured on graphene substrates exhibited significantly increased expression of epithelial markers, including *E-CAD*. These results demonstrate the MET-promoting potential of graphene and its ability to increase reprogramming efficiency through an epigenetic mechanism.¹⁸

Another study conducted by Downing *et al.*²¹ explored the effects of microgrooved (10, 20, and 40 μm spacing) polydimethylsiloxane (PDMS) substrates on cell reprogramming. The authors initially observed that microgrooves elicited significant upregulation in fibroblast-derived *NANOG*+ iPSC colonies compared with flat surfaces, and subsequently hypothesised that



Table 1 Summary of biomaterial-induced epigenetics changes and affected downstream genes, proteins, and signalling pathways in the discussed papers

Material description	Epigenetic modulation	Downstream gene expression modulation	Ref.
Monolayer of graphene on glass substrate	H3K4me3 enrichment in pluripotency regulator promoters (<i>OCT4</i> , <i>NANOG</i>) in mouse fibroblasts	Upregulation in pluripotency regulator (<i>OCT4</i> , <i>NANOG</i> , <i>SOX2</i>) expression and epithelial (<i>E-CAD</i> , <i>EP-CAM</i> , <i>CLDN3</i>) markers; downregulation in mesenchymal (<i>N-CAD</i> , <i>SLUG</i> , <i>ZEB1</i>) markers	18
Microgrooved PDMS substrate	Ach3 enrichment in pluripotency regulator promoters (<i>OCT4</i> , <i>NANOG</i> , <i>SOX2</i>) caused by HDAC2 downregulation; H3K4me2 and H3K4me3 enrichment in mouse fibroblasts	Upregulation in pluripotency (<i>OCT4</i> , <i>NANOG</i> , <i>SOX2</i>) and epithelial markers (<i>E-CAD</i> , <i>EP-CAM</i> , <i>KRT8</i> , <i>OCLN</i> , <i>CLDN3</i>); downregulation in mesenchymal markers (<i>TGFBR1</i> , <i>SNAI1</i> , <i>SNAI2</i> , <i>VIM</i> , <i>ITGB1</i>)	21
PDMS substrate stiffness	Softer substrates ($E = 1.5$, 15 kPa) induced increased euchromatin and decreased heterochromatin contents in human mesenchymal stem cells	Upregulation in pluripotency markers (<i>OCT3/4</i> , <i>SOX2</i> , <i>NANOG</i>)	23
Microgrooved collagen-coated PDMS membranes	Microgrooves elicited histone hyperacetylation and Ach3 enrichment in human cardiac progenitor cells	Upregulation of mature cardiac marker (sarcomeric MyHC, ACTC1, NPPA) expression	28
Microgrooved Matrigel-coated PDMS substrates	Microgrooves elicited Ach3 enrichment in mouse fibroblasts	Upregulation of mature cardiac transcription factor (<i>GATA4</i> , <i>MEF2C</i> , <i>TBX5</i> , <i>NKX 2.5</i> , <i>HAND2</i>) expression, mature cardiac marker (α -SMA, cTnT, <i>TNNT2</i> , <i>ACTN2</i>) expression, and MKL1 expression	31
PDMS substrate stiffness	Stiff substrates ($E = 140$ kPa) induced downregulation of H3K9-methylating genes and inhibited nuclear peripheral organization of H3K9me3-marked chromatin in cardiomyocytes	Downregulation of cardiac transcription factor (<i>GATA4</i> , <i>HAND2</i> , <i>MEF2C</i> , <i>NKX2-5</i>), structural protein (<i>ATP2A2</i> , <i>MYH6</i> , <i>SCN5A</i> , <i>TNNI3</i>), and histone methyltransferase (<i>KDM6A</i> , <i>EED</i> , <i>EHMT1</i> , <i>EHMT2</i> , <i>SETDB1</i>) expression	34
Polyacrylamide hydrogel stiffness	Stiff substrates ($E = 50$ kPa) induced downregulated miR-6740-5p expression in HUVECs	Suppressed miR-6740-5p led to increased ET-1 production	38
Nanogrooved Matrigel-coated PUA substrates	Nanogrooves induced enrichment of H3K4me3 marks in mouse embryonic fibroblasts	Upregulation of dopaminergic (<i>TH</i> , <i>DAT</i>) and epithelial (<i>E-CAD</i> , <i>CLDN3</i> , <i>EP-CAM</i>) expression; no trend observed with mesenchymal (<i>ZEB1</i> , <i>N-CAD</i> , <i>SLUG</i>) expression	43
Gelatin, gelatin-hydroxyapatite, gelatin-hydroxyapatite-pig brain substrates	Gelatin biomaterials induced upregulation of <i>HDAC3</i> expression in mouse embryonic fibroblasts	Upregulation of astrocytic transcription factor (<i>NFIA</i> , <i>NFIB</i> , <i>SOX9</i>) expression	44
Free nano-hydroxyapatite	Osteogenic (ALP) expression in bone marrow stromal cells	Downregulation in ALP expression	50
TSA-laden PLLA aligned fiber scaffold	Increased Ach3 and Ach4 enrichment and downregulation of HDAC1 activity	Enhanced tenogenic (<i>SCX</i> , <i>MKX</i> , <i>EYAI</i> , <i>HOXA11</i> , <i>DCN</i>) expression	54
G1, G3, and G5 polyamidoamine dendrimer surfaces	Distinct differences in H3K9me3, H3K27me3, and H3K9ac levels on G1, G3, and G5 surfaces	Differentiation to smooth, skeletal, and cardiac muscle lineages	59
Silver (AgNP), gold (AuNP), and superparamagnetic iron oxide nanoparticles (SPIONs)	No differences in DNA methylation as a result of treatment with nanoparticles in HepG2 hepatocarcinoma cells	Significant changes in nine pro- and anti-tumour miRNAs expression	63
Collagen-alginate interpenetrating network (IPN)-based matrices to soften the ECM	Soft matrices ($E = 0.5$ –1 kPa) induced recruitment of DNA methylation inhibitors TET2, GRHL2, and KMT2A in gastric cancer cells	Decrease of YAP overexpression; YAP overexpression is linked to aggravation of cell migration, invasion and promotion of a malignant phenotype	65
Alginate IPN hydrogel stiffness	Stiff matrices ($E = 2$ kPa) led to increased HDAC3 and HDAC8 activity compared with soft matrices ($E = 100$ Pa) in breast cancer epithelial cells	Increased accessibility of transcription factor Sp1 target genes	66
Collagen-Matrigel gel stiffness	Softer substrates ($E = 0.25$ kPa) added activating histone marks to CD133 promoter and repressing histone marks to THBS2 promoter in liver cancer stem cells	Upregulation of CD133 expression and downregulation of THBS2 expression	70
Fibrin gel stiffness	Stiff gels induced increased Tet2 enzyme activity in murine melanoma cells	Upregulation of genes that inhibit cell cycle progression	72
PDMS substrate stiffness	No significant differences in DNA methylation in human mesenchymal stem cells	No significant differences in senescence associated β -galactosidase	76
PEG-crosslinked collagen hydrogel stiffness	Stiff hydrogels ($E = 5$ –6 kPa) decreased accessibility of genes in bivalent chromatin domains in hair follicle stem cells	Softer hydrogels ($E = 1$ –3 kPa) restored expression of key HFSC self-renewal (<i>CCND2</i> , <i>CCNE1</i> , <i>CDC34</i> , <i>CDKL1</i> , <i>HMG2A</i>) and differentiation (<i>LEF1</i> , <i>RSPO3</i> , <i>WNT2B</i> , <i>WNT7A</i>) genes	78
Carbon dots of different surface charge	mRNA and miRNA expression and DNA methylation of human embryonic lung fibroblasts after exposure to carbon dots	Positive dots influenced pathways related to dysregulation of immune response processes, tumorigenesis, and cell cycle regulation; negative dots induced changes in cell proliferation, apoptosis, oxidative stress, and cycle regulation	80



Table 1 (Contd.)

Material description	Epigenetic modulation	Downstream gene expression modulation	Ref.
TiO ₂ nanoparticles	Global DNA methylation and hydroxymethylation alteration in mice lungs following nasal inhalation of TiO ₂ nanoparticles	Alteration of methylation in promoter of TNF- α and Thy-1, key in the inflammatory response and fibration	81
PEG hydrogel	Long-term culture on stiff ($E = 4.7$ kPa) hydrogels induces global chromatin condensation in fibroblasts	Chromatin condensation is permanent, and contributes to persistent fibroblast activation	82

the topography increased the prevalence of histone H3 acetylation (AcH3) marks, which are known to be involved with reprogramming. Surprisingly, they found that the microgrooves increased global AcH3 marks within the promoter regions of *OCT4*, *SOX2*, and *NANOG* in mouse fibroblasts even in the absence of reprogramming TFs, which was subsequently found to be due to downregulation of HDAC2. In addition, there was increased methylation of H3K4me2 and H3K4me3, marks that are also critical for reprogramming. Most notably, the microgrooves elicited analogous histone modifications compared to the sole introduction of *OCT4*, *SOX2*, *KLF4*, and *MYC* (OSKM) or small-molecule epigenetic modifiers, such as valproic acid (VPA) and tranylcypromine hydrochloride (TCP).²¹ Therefore, material cues may have the potential to entirely replace conventional virus-induced TF overexpression-based methods, circumventing safety concerns. However, expansion of the iPSC-like colonies was limited, suggesting that microgrooved surfaces only activate the early phase of reprogramming. In concert, the activity of several genes associated with both epithelial and mesenchymal phenotypes were monitored. All investigated epithelial markers including *E-CAD* were upregulated, whereas all mesenchymal markers including *TGFBR1* were downregulated. There was also an increase in H3K4me2 in the *E-CAD* promoter, again emphasising the role of MET.²¹

Downing *et al.*²¹ additionally sought to elucidate how important cytoskeletal reorganization was in causing the epigenetic changes associated with cell reprogramming. As expected, cells cultured on the microgrooves exhibited greater alignment and nuclear elongation. To investigate the role of alignment, they used nanofibers in both aligned and random orientations. Indeed, aligned nanofibers globally increased AcH3, H3K4me2, and H3K4me3 marks, and significantly upregulated *NANOG* expression compared with random fibres. To determine the importance of elongated cell morphology, they used micropatterning techniques to closely control cell shape on flat surfaces. They found that elongated cells displayed significantly higher levels of nuclear AcH3, H3K4me2, and H3K4me3, compared with circular cells. Together, these results illustrate the key roles of cell alignment and morphology, parameters that can be controlled using biomaterials, in overcoming epigenetic barriers, *i.e.* chromatin remodelling, to achieve cell reprogramming.²¹

Several groups have also investigated the effect of substrate stiffness on reprogramming efficiency. Gerardo *et al.*²³ investigated this phenomenon when reprogramming hMSCs to iPSCs on PDMS substrates of varying stiffness. Previous studies have

identified a negative correlation between 'stemness' and nuclear stiffness – multipotent and terminally differentiated cells possess a higher cellular stiffness compared with pluripotent stem cells.²⁴ Considering this, they hypothesised that culturing hMSCs on soft substrates would induce reprogramming to iPSCs *via* promoting nuclear relaxation and softening the cell as a whole. They found that the hMSCs cultured on softer substrates ($E = 1.5$ and 15 kPa) exhibited increased euchromatin, *i.e.* enriched H4K16ac (transcriptionally permissive, relaxed chromatin) and decreased heterochromatin (transcriptionally restrictive, condensed chromatin) contents compared with hMSCs cultured on stiff GPa-range substrates. Pluripotent cells are characterised by high levels of euchromatin, while gradual enrichment of heterochromatic regions ensues during differentiation.²⁵ This dynamic epigenomic structure blocks transcriptional machinery and TFs from physically accessing irrelevant developmental programmes that are not necessary once a cell has 'chosen' its fate.²³ These results show that by providing a mechanical environment that facilitates decreased cellular stiffness, reprogramming to a pluripotent state may be enhanced.

Differentiation

Multi- or unipotent stem cells are present in almost all adult tissues. They serve to facilitate repair and rejuvenation of damaged, diseased, or aged tissues *via* differentiation to replace the specialised cells that were lost.²⁶ For example, following injury to the skin, basal epidermal stem cells differentiate and migrate towards the surface to close the wound.²⁷ However, some organs and tissues have limited intrinsic regenerative potential²⁸ due to a paucity of tissue-resident stem cells.

Cardiac differentiation. The heart is an example of an organ with limited innate regenerative capacity. Various pathologies, including myocardial infarction, lead to cardiomyocyte (CM) death and diminished cardiac pump function. These effects are essentially irreversible due to the restricted ability of the heart to replace the lost cells.²⁸ To this end, researchers have proposed multiple strategies to replace lost cardiomyocytes, which include isolating cardiac progenitor cells (CPCs) or their derivatives, subsequent *ex vivo* expansion and transplantation, or direct reprogramming of other cell types to cardiomyocytes.²⁹

Although induced cardiomyocytes (iCMs) have been successfully generated both *in vitro* and *in vivo* from cardiac fibroblasts *via* direct reprogramming TFs (*GATA4*, *MEF2C*, and *TBX5*), the efficiency remains extremely low (10–15%). Another



limitation is that iCMs do not reach complete maturation *in vitro* due to the lack of an *in vivo*-like structural organisation.³⁰ A study by Morez *et al.*²⁸ sought to increase the efficiency of *de novo* cardiomyocyte generation from CPCs by using topographical cues that offer enhanced recapitulation of the 3D cardiac environment and circumvent epigenetic barriers associated with direct lineage reprogramming.²⁸ For example, a high degree of chromatin condensation or histone deacetylation, typical of differentiated cells, prevents transcriptional regulators from accessing DNA binding/recognition sites.¹⁷ They found that microgrooves elicited histone hyperacetylation in a baseline-dependent manner, unwinding the chromatin. They also identified a significant positive correlation between nuclear elongation and AcH3 levels. Furthermore, they concluded that histone acetylation directly resulted in increased reprogramming efficiency. This finding suggests that material-induced histone acetylation not only improves reprogramming cells to iPSCs but also enhances direct conversion of cells to highly differentiated states. In addition, the combination of their established cardiac differentiation protocol and the microgrooves proved to be synergistic, resulting in a two-fold increase in cells that were positive for sarcomeric MyHC, ACTC1, and NPPA compared with flat surfaces,²⁸ likely owing to the relaxed chromatin structure.

Sia *et al.*³¹ also questioned the augmenting effect of microtopography as well as substrate stiffness on direct conversion efficiency of mouse fibroblasts to CMs.³¹ Cells cultured on microgrooved substrates (3 and 5 μm spacing) yielded a higher proportion of α -SMA- and cTnT-positive cells (both of which are markers of contractility)^{32,33} compared to flat substrates. They concluded that the microgrooves increased conversion efficiency through increased AcH3 marks. Interestingly, there were no significant differences in the yield of α -SMA-positive cells comparing substrates which had stiffnesses of 1, 21 and 62 kPa.³¹

In contrast, the work of Seelbinder *et al.*³⁴ suggested that there is a correlation between stiffness and chromatin structure, which is important for cardiac development and tissue maintenance. It is known that perturbations to the mechanical properties of the heart, due to genetic disorders and chronic conditions, lead to onset of disease states as a result of diminished CM gene expression and consequent attenuated cardiac function.³⁵ Therefore, they sought to further elucidate the role of environmental mechanics in CM de-differentiation. They observed that culturing CMs on stiff substrates ($E = 140$ kPa) elicited downregulation of histone proteins that are replication-dependent, suggesting a reduction in cell proliferation. There was also downregulation of H1, H2a, and H3 histone variants, which are involved in cell differentiation, implying considerable de-differentiation. Overall, these results show that stiff environments result in acquisition of an epigenetic profile that impairs CM function. In contrast, culturing CMs on a substrate that mimicked tissue stiffness ($E = 13$ kPa) supported normal spatial chromatin organisation and gene expression characteristic of healthy CMs.³⁴

Stiffness has also been shown to be an important biophysical cue for endothelial cells, which line the inner wall of blood vessels.³⁶ Vascular stiffening has been linked to endothelial dysfunction, which is an early indicator of cardiovascular disease.³⁷ To further probe how stiffness regulates endothelial function, Song *et al.*³⁸ cultured human umbilical vein endothelial cells (HUVECs) on polyacrylamide hydrogels with several stiffnesses ($E = 4, 25, \text{ and } 50$ kPa), and performed a miRNA array analysis to identify mechanosensitive miRNAs. Normal stiffness of the basement membrane has been reported to be in the range of $E = 2.5\text{--}8$ kPa.³⁹ They found that miR-6740-5p was significantly downregulated on the stiffest substrate ($E = 50$ kPa), compared with the softest substrate ($E = 4$ kPa). Using a bioinformatics approach, they identified *EDN1* as a target of miR-6740-5p. *EDN1* mRNA codes for ET-1, which is a powerful vasoconstrictor – its upregulation has been associated with high blood pressure and the development of atherosclerotic plaques. Ultimately, this result shows that increased stiffness leads to the establishment of a vascular environment that favours hypertension through an epigenetic mechanism.³⁸

Neural differentiation. Neurodegenerative disorders, such as Parkinson's and amyotrophic lateral sclerosis (ALS), that cause the loss or dysfunction of cell populations in the nervous system,⁴⁰ are extremely debilitating and permanent due to the system's limited regenerative capacity.⁴¹ Therefore, the ability to generate induced neurons (iN) has significant implications for treatment of these conditions. Pioneering work by Vierbuchen *et al.*⁴² showed that fibroblasts can be directly converted to functional iN but with relatively low efficiency (19.5%).⁴² Several subsequent studies have focused on improving efficiency using various material cues. Yoo *et al.*⁴³ demonstrated that the synergistic combination of nanoscale topographical cues and ectopic expression of pro-neural TFs promoted the direct conversion of mouse embryonic fibroblasts (MEFs) into functional induced dopaminergic (iDA) neurons.⁴³ Like microgrooves, nanogrooves also result in greater (and superior) cell alignment. Indeed, they found that the iDA neurons on the nanogrooved (400 nm spacing) substrate had higher expression of dopaminergic genes such as TH and DAT and stronger electrophysical properties characteristic of neurons relative to microgrooved (1.2 μm) and flat substrates. They found that the nanoscale topography elicited higher levels of H3K4me3 compared to the flat substrate as a result of amplified MET induction,⁴³ which ultimately led to enhanced conversion.

Kantawong *et al.*⁴⁴ explored the potential of gelatin-hydroxyapatite-pig brain biomaterials in generating induced neural stem cells (iNSCs) from MEFs. iNSCs are not only capable of differentiating into neurons, but also into glial cells including astrocytes and oligodendrocytes.⁴⁵ They found that cells cultured on the gelatin biomaterials had increased *HDAC3* expression. HDACs have been shown to repress miR-124,⁴⁶ whose target is PTBP1 – this TF has been implicated in suppressing neuronal differentiation whilst activating astrocytic differentiation.⁴⁷ They postulated that decreased miR-124 expression could have caused increased PTBP1 expression,



leading to a preference to differentiate into the astrocytic lineage. Indeed, culture on the gelatin materials upregulated astrocytic TF (*NFIA*, *NFIB*, *SOX9*) expression even without viral TF infection.⁴⁴ Currently, astrocyte generation is slow *in vitro*,⁴⁸ which is a considerable limiting factor for studying neurodegenerative disease. Future studies could focus on further optimising gelatin-based substrates to shorten the differentiation period.

Mesenchymal differentiation. Bone regeneration is a complex, physiological process and is naturally fulfilled by the body, but it can be disrupted by trauma and skeletal diseases such as osteoporosis.⁴⁹ In these cases, medical intervention is required to replace damaged or diseased tissue. Despite advances in medical implant fabrication techniques and polymer chemistry, there are still challenges in eliciting a pro-osteogenic response in guided bone regeneration. Ha *et al.*⁵⁰ investigated the effect of free nano-hydroxyapatite (HA) on osteoinduction in various cell lines within the osteoblast lineage. HA is known to play a critical role in the early stages of osteoblast differentiation. Unexpectedly, the expression of genes required for bone formation and early osteoblast differentiation, such as ALP, decreased in the long term in bone marrow stromal cells (BMSCs), even after treatment was removed. This effect was less pronounced in more highly differentiated cell types, such as pre-osteoblasts and fully differentiated osteoblasts. They attributed the decrease in ALP expression in the BMSCs to an increase in DNA methylation in its promoter, as a result of the nano-HA treatment. They postulated that this decrease in expression serves as a safety mechanism to promote plastic cells to become terminally differentiated once they have sufficiently mineralised the surrounding bone matrix. Ultimately, this regulatory mechanism prevents inappropriate acquisition of increased proliferation capacity and onset of a cancer phenotype.⁵⁰ Importantly, this study provides proof-of-concept results that tuneable nanomaterial properties, as opposed to the genetic cargo they typically carry and deliver, may be utilised to regulate gene expression and cell function through promoter DNA methylation.

Tendon is another tissue with poor regenerative capacity that often requires clinical intervention.⁵¹ The conventional treatments for tendon injuries present detrimental side effects including donor site morbidity and risk of disease transmission,⁵² as well as being relatively short-term solutions.⁵³ An alternative approach that holds promise is tendon tissue engineering, which uses human tendon stem/progenitor cells (hTSPCs) seeded in scaffolds that replicate the physiological array of parallel collagen fibres in the tendon microenvironment to direct tenogenesis.⁵⁴ Zhang *et al.*⁵⁴ used the synergistic combination of electrospun poly(L-lactic acid) (PLLA) fibres and an HDAC inhibitor (TSA) to promote tendon differentiation. The aligned PLLA fibres resulted in elongated cell morphology, provided remarkable mechanical properties, as well as sustained drug release, which was due to a higher degree of polymer crystallinity because of TSA incorporation. SCX, a marker of tendon differentiation was approximately 2-fold higher on parallel aligned TSA fibres compared with just

aligned fibres with no active HDAC inhibitor and randomly aligned TSA fibres. They confirmed that the increased differentiation efficiency was in fact due to chromatin remodelling and increased AcH3 and AcH4 marks, as well as a decrease in HDAC1.⁵⁴

hMSCs are another cell type of interest to both clinicians and researchers for their potential to differentiate into many different tissues, including cartilage, bone, fat, muscle, and skin.⁵⁵ Endogenous hMSCs are a key component enabling both tissue development and long-term maintenance, and as such, the depletion of the hMSC reservoirs in tissues results in degenerative or age-related disease onset.⁵⁶ As well as their ability to directly differentiate, hMSCs have the potential to regenerate tissues by promoting normal tissue remodelling in a paracrine manner. To date, a number of studies have investigated the effect of polyamidoamine (PAMAM) dendrimer generation number on cell fate within the mesenchymal lineage.^{57,58} Ayuningtyas *et al.*⁵⁹ explored the mechanism governing muscle lineage switching of hMSCs through migration and cellular and nuclear deformation on dendrimer surfaces. Cells cultured on G3 and G5 surfaces exhibited more dynamic temporal stretching and contraction, which is representative of active migration, compared with cells grown on a G1 surface. Interestingly, on G5 surfaces, the cells developed rounder shapes and spreading was inhibited across the surface, leading to formation of spherical aggregates. Accompanying these migratory behaviours were substantial conformational changes of the cytoskeleton and nuclear lamina deformation, which in turn led to chromatin reorganisation. Overall, they found H3K9me3 levels were consistently decreased on the G3 and G5 surfaces, whereas both H3K27me3 and H3K9ac levels were decreased in the short-term but increased in the long-term. In contrast, nuclear lamina deformation was comparatively lower in G1 cells and was correlated with the upregulation of H3K9ac in the short-term, and upregulation of H3K9me3 in the long-term, suppressing hMSC differentiation. It is thought that initial downregulation of H3K27me3 in the short-term causes unwinding of chromatin and increased gene transcription, initiating the differentiation process,⁶⁰ whereas at later stages, the increase in H3K27me3 suppresses genetic programs of alternative specific cell fate programs not within the muscle lineages. Importantly, this material-induced spectrum of histone modification resulted in diverse differentiation patterns; differentiation for G1, G3, and G5 cells tended towards smooth muscle, skeletal muscle and cardiac muscle lineages, respectively.⁵⁹

Bionanomaterials and epigenetics in cancer cells

As stated above, various cancers have been associated with aberrant epigenetic signatures. Hence, it is not surprising that the scientific community has placed a large effort on delivering drugs and other therapeutics to modulate epigenetics and overall gene expression in cancer cells (beyond the scope of



this review).^{61,62} For example, nanoparticles and nano-carriers (NCs) have been used for decades to address the challenges associated with delivery of drugs with poor stability or bio-availability. However, only recently has it been suggested that the ability of nanoparticles to modify cells' epigenetics extends beyond simply delivering therapeutics. The rapid advance of sequencing technologies has recently revealed the effects of nanocarrier size, surface charge, shape, and the administration dose on epigenetic regulation. Brzóska *et al.*⁶³ studied the change of miRNA expression and DNA methylation status of different genes related to inflammatory processes and apoptosis in HepG2 hepatocarcinoma cells after exposure of mildly cytotoxic doses of silver, gold, and iron oxide nanoparticles. No significant difference was shown in DNA methylation, but the expression of nine miRNAs was significantly altered by treatment using these nanoparticles. Specifically, silver nanoparticles induced more changes in miRNAs expression than gold, followed by iron oxide nanoparticles, thus showing a dependence on the type of material.⁶³

Beyond material type, its mechanical properties are also defining traits to modulate cancer cell epigenetics. Generally, a stiffer ECM is a characteristic of malignant tumours – this environment leads to aggravation of cell migration and invasion, as well as promoting a malignant phenotype in surrounding cells.⁶⁴ Jang *et al.*⁶⁵ showed that softening a stiff ECM (from $E = 7$ to 0.5–1 kPa) in a gastric cancer model could epigenetically reverse elevated YAP protein expression, through recruitment of DNA methylation inhibitors, namely TET2, GRHL2, and, KMT2A. YAP is a mechano-sensitive transcription factor that is associated with metastasis and malignant behaviour. Stowers *et al.*⁶⁶ explored this phenomenon in the context of breast cancer; they showed that stiff interpenetrating networks (IPNs) comprised of alginate and reconstituted basement membrane matrix ($E = 2$ kPa) promoted the development of an invasive phenotype, whereas IPNs with stiffness comparable to native mammary tissue ($E = 100$ Pa) promoted healthy, acinar morphology. Stiff matrices led to increased HDAC3 and HDAC8 activity and increased accessibility of Sp1 target genes; Sp1 is a transcription factor that is upregulated in breast cancer.⁶⁷

Interestingly, there is evidence that stiffness is actually non-uniform within a tumour,⁶⁸ which has important implications for cancer cell invasiveness and drug resistance. In contrast to the notion of increased bulk stiffness promoting malignancy, some research groups have shown that soft matrices promote the plasticity of cancer stem cell (CSC) populations – CSCs are thought to be the main drivers behind tumorigenesis.⁶⁹ In this sense, Ng *et al.*⁷⁰ investigated the role of matrix stiffness in regulating the 'stemness' of CD133+ liver CSCs with THBS2 deficiency in hepatocellular carcinoma. They observed that the CSCs grown on softer collagen-Matrigel gels ($E = 0.25$ kPa) had more 'aggressive' phenotypes – there was increased CD133 expression and reduced THBS2 production, compared with stiffer gels ($E = 15$ kPa). CD133 is a marker of self-renewal potential,⁷¹ and THBS2 is known to interfere with tumour growth and angiogenesis by suppressing matrix metalloprotei-

nase (MMP) activity.⁷⁰ To further examine the mechanism underlying the changes in protein expression, they looked for differential histone H3 modifications. They discovered that upon culture on softer supports, transcriptionally activating H3K4me3 and H3K9ac marks were enriched within the CD133 promoter, whereas repressing H3K9me3 and H3K27me3 marks were present in the THBS2 promoter. Interestingly, they determined that there was a positive feed-forward loop where induced THBS2 downregulation led to further accumulation of CD133-high CSCs, which enabled the maintenance of localised 'soft' regions in the ECM. This in turn, ultimately confers enhanced metastasis due to decreased ECM presence.⁷⁰ Furthermore, Liu *et al.*⁷² showed that a stiff mechanical environment can induce dormancy in tumorigenic cells *via* an epigenetic mechanism. They cultured murine B16 melanoma tumour-repopulating cells (TRCs) in several fibrin gel formulations ($E = 90, 450, 1050,$ and 2000 Pa); they found that the stiffer gels induced the nuclear translocation of Cdc42, which resulted in activation of a hydroxymethylating enzyme, Tet2. This activation then led to upregulation of genes that inhibit cell cycle progression, including p21 and p27.⁷² In the future, designing bespoke biomaterial scaffolds with heterogeneous stiffness will be important to mimic the diverse mechanical landscape of tumours, and ultimately to conserve the functional phenotypes of distinct cell populations within a tumour.

Bionanomaterials and epigenetics in primary cells

Ageing

All primary cells enter a senescent state after reaching approximately 50 cell divisions, also known as the Hayflick Limit.⁷³ Hallmarks of cellular senescence include flattened morphology and proliferative cessation despite an optimal growth environment with sufficient mitogenic stimuli; the aggregation of senescent cells has been confirmed to provoke cellular aging and dysfunction.⁷⁴ Acquisition of a senescent phenotype has been linked to epigenetic changes; Schellenberg *et al.*⁷⁵ demonstrated that senescent hMSCs had distinct DNA methylation profiles that corresponded with repressive histone marks. They found that senescent hMSCs had significantly differentially methylated CpG sites in genes associated with development, including *DLX5*, *CDKN2B*, and *HOXD10*. This differential methylation was correlated to absence of activating-H3K4me3 and presence of repressing-H3K9me3 marks.⁷⁵ Schellenberg *et al.*⁷⁶ then asked whether substrate stiffness could affect senescence onset. Interestingly, they showed that hMSCs cultured until senescence on elastic substrates did not display the typical flattened morphology characteristic of a senescent cell and remained relatively condensed. Despite this, there was no significant difference in the activity of senescence associated β -galactosidase (SA- β -GAL) or DNA methylation as a function of matrix stiffness.⁷⁶

Another characteristic of cellular aging is decline in stem cell number, resulting in diminished regenerative capacity of



tissues.⁷⁷ Koester *et al.*⁷⁸ sought to investigate if restoration of youthful niche mechanics could rescue aged stem cells using the hair follicle as a model. Initially, they characterised both young and aged hair follicle stem cells (HFSCs) and they discovered that the aged HFSCs showed large-scale reduced chromatin accessibility compared to the young HFSCs, particularly in promoter regions of genes that regulate tissue development and differentiation (Fig. 2A and B). One category of regions with decreased accessibility in the aged HFSCs were promoters with antagonising epigenetic marks, namely activating-H3K4me3 and repressing-H3K27me3 (Fig. 2C). These regions

are known as bivalent chromatin domains. Gene ontology enrichment analyses demonstrated that these bivalent genes were involved with HFSC differentiation and self-renewal, which compromises adequate cell cycle entry and tissue turnover in aged HFSCs. Interestingly, placing the aged HFSCs in a young niche activated previously repressed bivalent genes, leading to a return of self-renewal capacity, equivalent to that of young HFSCs. They later discovered that upregulation of various collagens and laminins that comprise the extracellular matrix (ECM) and the basement membrane (BM) was associated with the aged HFSC phenotype. These alterations led to

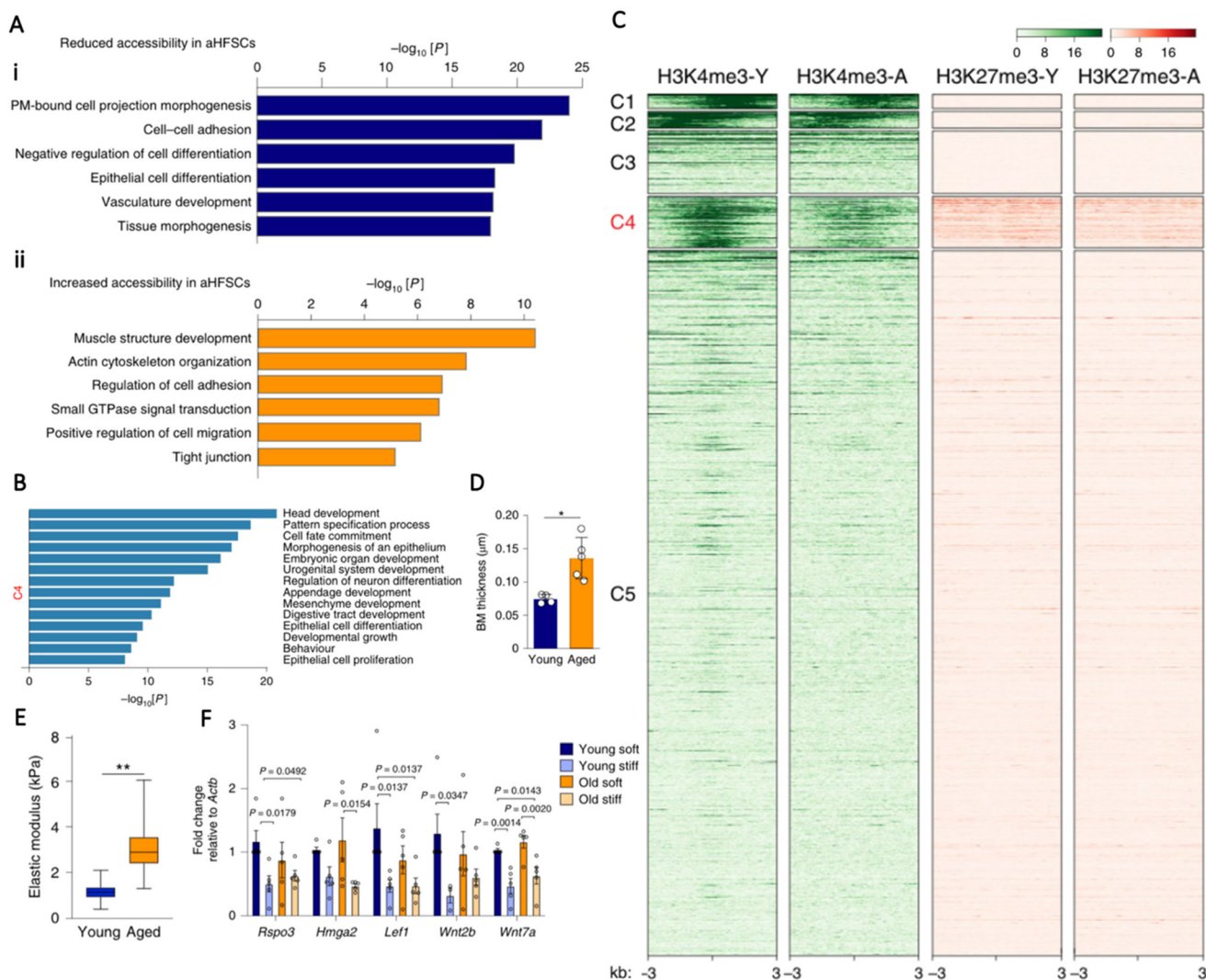


Fig. 2 (A) Gene ontology (GO) term analyses of peaks that have increased accessibility in young (i) and aged (ii) HFSCs demonstrate that developmental and cell differentiation processes have decreased accessibility, whereas genes that are involved in actin cytoskeleton organisation and the regulation of cell adhesion have increased accessibility in aged HFSCs. (B) GO term analyses of the genes identified in cluster 4 (C4) are related to developmental processes, cell fate, and differentiation. (C) *k*-means clustering of ATAC-seq peaks (not shown) with diminished accessibility in aged HFSCs. Peak cluster 4 (C4) shows differential epigenetic modification with both H3K4me3 and H3K27me3 in young HFSCs, and decreased H3K4me3 at these sites in aged HFSCs. Colour scales represent reads per genomic content. (D) Aged HFSCs exhibit increased basement membrane (BM) thickness compared to young HFSCs. (E) Aged HFSCs exhibit increased stiffness compared to young HFSCs. (F) RT-qPCR analysis of bivalent genes related to HFSC activation and self-renewal in young and aged HFSCs cultured in soft and stiff hydrogels. Note the downregulated expression in stiff hydrogels. $n = 5$ cultures per group. Statistical analysis was performed using ANOVA and Fisher's Least Significant Difference test. P values are indicated on the graph. Data are mean \pm s.e.m.⁷⁸ Reproduced from ref. 78 with permission from Springer Nature, copyright 2021.



thickening and stiffening of the BM (Fig. 2D and E), so they hypothesised that matrix stiffness, as a single parameter, was the dominating regulator of HFSC potency. Indeed, culturing both young and old HFSCs on a soft matrix ($E = 1\text{--}3$ kPa) produced similar activation of bivalent regions, whereas culturing young HFSCs on a stiff hydrogel matrix ($E = 5\text{--}6$ kPa) that recapitulated the aged BM was adequate to induce impaired stem cell self-renewal and deactivation of bivalent regions (Fig. 2F).⁷⁸ This finding emphasises the advantage in circumventing intrinsic manipulation of cells by exploiting the power of relatively simple external cues to modulate cell behaviour in a non-invasive manner.

Inflammation

Bionanomaterials' properties have recently been shown to elicit epigenetic changes, including DNA and/or RNA methylation, hydroxymethylation, histone modification, and changes in miRNA expression, linked to oxidative stress due to ROS overproduction, MAPK signalling cascade dysregulation, and inflammation.²⁰ Indeed, nanoparticle composition, size and shape, as well as biomaterial surface charge and stiffness can control both immunostimulation and immunosuppression.⁷⁹ For example, Sima *et al.*⁸⁰ studied the mRNA and miRNA expression of the full genome of human embryonic lung fibroblasts (HEL 12469) as well as DNA methylation after exposure to positive or negative carbon dots. Generally, negative dots showed more differences in expression than positive dots, with only a few of them overlapping in both groups. After a pathway analysis, it was shown that positive dots influenced pathways related to dysregulation of immune response processes, tumorigenesis, and cell cycle regulation, whereas the negative dots induced changes in cell proliferation, apoptosis, oxidative stress, and cycle regulation. Similar trends were shown when studying miRNAs. Interestingly, within the same group, the number of dysregulated mRNAs increased when increasing dose and exposure time.⁸⁰ Taken together, these observations demonstrate that nanoparticle charge can induce epigenetic changes. Interestingly, characteristics of the host organism also play a critical role in epigenetic response to bionanomaterials. Ma *et al.*⁸¹ treated two groups of mice, comprised of young and adult populations, with TiO₂ nanoparticles *via* nasal inhalation. Global methylation and hydroxymethylation in lungs were measured. As a result of the treatment, pulmonary inflammation and fibrosis were induced more severely in young mice due to a decrease in global methylation and hydroxymethylation only in this group. The altered methylation affected the *TNF- α* and *Thy-1* promoters. Overall, the young mice presented 197 genes that were upregulated compared to the adult group.⁸¹ Deciphering nanoparticle-tissue interactions and underlying mechanisms will allow for a more comprehensive and personalised use of materials in biomedicine and avoid possible toxicity and safety concerns.

Biomaterials have also been explored as useful tools to probe the biology underlying fibrosis, which is a consequence of dysregulated wound healing. Walker *et al.*⁸² used photo-softening PEG hydrogels to investigate aberrant fibroblast activation, which is

known to contribute to fibrotic tissue development. They showed that activated fibroblasts, also known as myofibroblasts, cultured on stiff hydrogels (storage modulus, $G' = 4.5$ kPa) for 1–3 days were capable of 'deactivating' upon hydrogel softening ($G' = 1.7$ kPa), whereas cells that were cultured for 1–5 days were only partially deactivated. Interestingly, a period for more than 7 days rendered the cells unable to deactivate, even after the pathological 'stiff' stimulus was withdrawn. They subsequently identified that the persistently activated myofibroblasts possessed globally condensed chromatin, compared with transiently activated fibroblasts, which was correlated with increased HDAC activity.

Conclusions and future directions

Advances in chemistry, physics, and biology, integrated with medicine, have enlightened the path to a better understanding of biomaterials and their interactions with cells and tissues. The biomaterials field has focused on stiffness as the predominant material property that affects cell behaviour. Whilst stiffness has been proven to be a potent stimulus for epigenetic remodelling particularly in cancer, other material parameters whose epigenetic effects in cells remain under-characterised include topography and stress relaxation. Highly uniform topography (2D micro/nanogrooves) has been shown to alter cell shape and thus promote epigenetic marks that support cell reprogramming, which will be useful for optimised iPSC/cardiomyocyte/neuron generation. Further elucidation of the effects that 3D hydrogel- or scaffold-based culture has on cell shape and subsequent epigenetic consequences warrants further investigation, especially in organ modelling/tissue engineering applications. Furthermore, viscoelastic hydrogels that mimic the natural viscoelasticity of biological tissues have been shown to enhance several cell outputs, as opposed to purely elastic hydrogels. To our knowledge, there have been no studies as of yet that have explored the effects of viscoelasticity/stress relaxation on epigenetic remodelling to guide cell fate or phenotype. In the future, these parameters should be considered equally as significant as stiffness to generate highly representative tissue/organ models. Equally interesting is the understanding of how bio- and nanomaterials alter gene expression, and whether they have the potential to overcome the limited drugability of some targets, like tumour suppressor genes.

Despite the significant leaps taken, there is inevitably a wealth of bionanomaterial-cell interactions that remain uncharacterised. The increasing availability of highly informative molecular techniques will enable a better understanding of this relatively young interface, leading to bioactive materials capable of modulating cell epigenetics in a therapeutic manner.

Author contributions

A. D. Y. R. and J. A. D. M. wrote the original draft manuscript. A. D. Y. R., J. A. D. M. and N. O. reviewed and



edited the manuscript. N. O. conceptualised the ideas and acquired the funding.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

N. O. acknowledges an Imperial College Research Fellowship.

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