When Epigenetics Meets Bioengineering – A Material Characteristics and Surface Topography Perspective

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Abstract

The field of tissue engineering and regenerative medicine (TE/RM) involves regeneration of tissues and organs using implantable biomaterials. The term epigenetics refers to changes in gene expression that are not encoded in the DNA sequence, leading to remodeling of the chromatin and activation or inactivation of gene expression. Recently, studies have demonstrated that these modifications are influenced not only by biological cues but also by mechanical and topographical signals. This review highlights the current knowledge on emerging approaches in TE/RM with a focus on the effect of materials and topography on the epigenetic expression pattern in cells with potential impacts on modulating regenerative biology.

Keywords: epigenetics; tissue engineering; surface topography; material energy, titanium, regenerative medicine.

Introduction

The field of tissue engineering and regenerative medicine (TE/RM) involves the process by which tissues and organs are regenerated by implantable biomaterials using *in vivo* or *in vitro* models.[1] The term epigenetics refer to changes in gene expression that are independent of mutations or changes in the genetic coding. Epigenetic changes leads to remodeling of the chromatin resulting in activation or inactivation of genes. Histones are grouped in complexes called nucleosomes that are responsible for packing the DNA into the nucleus. Post-translation modifications of histones can be mediated by lysine acetylation, serine phosphorylation, lysine methylation, arginine methylation and lysine ubiquitination. Acetylation of histones is associated with an activation of transcription of genes and is regulated by the histone acetyltransferases (HATs) responsible for adding acetyl groups to histones and histone deacetylases (HDACs) that remove acetyl groups. In turn, DNA methylation involves the addition of methyl groups to specific cytosine bases in the DNA sequence, altering the configuration of the DNA and hence the transcription.[2] Importantly, epigenetic mechanisms are reversible and have been considered potential treatment models for improving personalized drug therapy. This review highlights the present knowledge on current approaches in TE/RM with the focus on the influence of surface energy, mechanics and topography of materials on the epigenetic pattern in cells and implications in affecting tissue engineering and regenerative medicine (Table 1).

Surface energy

Surface energy is a critical component of cell response to a biomaterial and is dependent on both surface chemistry and topography. Multiple studies have shown that cell adhesion is mediated by surface hydrophobicity, with surfaces that have low hydrophobicity being more favorable to integrate with bodily fluids and protein adhesion. Protein adhesion to a surface changes the surface energy and influence cell response. For example, the rate of osteoblast cell spreading and proliferation on fibronectin-coated glass slides (i.e., a high energy surface) was found to increase with enhancing surface hydrophilicity and was linearly dependent on surface energy.[3] Glass is a high energy surface with a high affinity for water, making it hydrophilic and promoting the spread of water in order to lower the surface energy. Likewise, changes in surface topography through the incorporation of roughness results in highest surface energies if the existing surface is hydrophilic. Rough titanium implants have

improved osseointegration and mechanical stability due to a higher surface energy compared to a polished surface as a result of higher wettability which increases surface contact area.[4] Surface energy changes affect cell-specific responses such as cell growth, with high surface energy materials promoting cellular differentiation, mineral deposition, and osteogenic maturation.[5,6]

In tissue engineering, vital factors to ensure positive outcomes include adherence of cells to a scaffold and cell migration. Matrix characteristics influence the initial cell spreading, the 3D cell migration and the structure of cellular networks.[1] The influence of substrate characteristics on form and size of cells and nuclei has been known for several years as well as being linked to chromatin alterations.[7] A central factor inducing epigenetic changes in cells is the stiffness and structure of the surface matrix onto which cells adhere (Fig. 1). Cells adhering to a rigid substrate display a transcriptionally active chromatin (euchromatin), while soft substrates result in a chromatin shift to a transcriptionally inactive state (heterochromatin).[8] Using cell culture models, studies have shown that, not only do cells acquire specific matrix stiffness to maintain proper morphology and organization, but the matrix stiffness can also influence the differentiation of cells, e.g. stem cells grown on substrate stiffness that mimics bone develop into an osteoblastic lineage.[9] In line with these findings human umbilical vein endothelial (HUVECs) cells were shown to acquire a 3D matrix with the stiffness of greater than 15 kPa to maintain their proper morphology and organization.[10] These findings show not only that mechanical signals are important in forming the chromatin structure and hence, cell responses and gene expression, but also presents a link between ECM and cytoskeleton that controls both cell shape and chromatin structure.[11] Therefore, adjusting the matrix properties of putative target cells and tissues may improve regeneration as well as induce the development of specific cell lineages (Fig. 2). A recent study using a 3D cell culture model mimicking soft tissue showed that a stiffness gradient affected both the migration and organization of different cell types.[12]

Harvesting cells for tissue engineering requires trypsinization or scraping of the cells from the cell culture plate, methods that may influence cell functions. A recent study showed that, compared to cells grown on a rigid, cell culture plate, cells grown on thermoresponsive hydrogels with different stiffness induced a sustainable mechanical memory as well as a stable chromatin thereby maintaining the cells functions and phenotype induced by the material. The stiffness of the hydrogel influenced the DNA methylation pattern and the

thermal harvesting method was found to stabilize the chromatin with the global methylation pattern and histone configuration intact. Furthermore, using an in vivo murine skin wound model it was shown that the cells obtained using the thermoresponsive hydrogels improved wound healing, thus indicating a new tool within the field of mechanobiology (Fan et al. 2017).

Surface topography

In addition to surface stiffness, nanotopographical structures, geometry and surface energy of material interfaces influence cell function and differentiation. Both micro and nano-scaled topography of a substrate can induce epigenetic changes in cells. Culturing cells on microscaled substrates improved cellular reprogramming of cells into induced pluripotent stem cells (iPSCs) for application in gene therapy. A substrate with microgroves of 10 μm width and spacing enhanced reprogramming of cells into iPSCs. A similar result was also obtained by using aligned nanofibers.[13] Interestingly, it has been reported that cells were more affected by changes in surface topography at the nanometer scale as compared to micro- or macro scales [14]. It was suggested that scaffolds with nanostructured topography may, therefore, be a tool to improve periodontal tissue engineering.[14]

A key mechanism involved in cellular responses to matrix topography and structure is the alteration in the level of histone acetylation. MSCs grown on micropatterned, flexible substrates showed a decrease in HDAC activity and an increase in histone acetylation indicating an increase in gene expression in these cells. Furthermore, mechanical compression and stretching of the substrate alters the nuclear shape and the histone acetylation pattern based on the directionality of the strain.[15] Matrix elasticity was suggested to be able to influence cells in direct contact with the biomaterial.[16] MSCs cultured on microgrooved poly(dimethyl siloxane) (PDMS) membranes (10 μm width, 3 μm height, 10 μm space between grooves) display an elongated nuclear shape as well as a decrease in HDAC activity with corresponding increase in histone acetylation and gene transcription compared to cells cultured on unpatterned surfaces.[15] Mammary epithelial cells grown on a flat plastic substrate formed stress fibers and assembled as a monolayer on the surface, while cells cultured in a 3D laminin rich extracellular matrix (ECM) model and grown on 2D patterned substrate displayed a rounded morphology similar to their native *in vivo* phenotype. A correlation between cellular shape and histone acetylation has been reported.[11] Adipose-tissue-derived

mesenchymal stromal/stem cells (AMSCs) grown on 3D porous titanium discs in vitro presented a different expression of epigenetic regulators of histone methylation, as well as expression of genes related to bone extracellular proteins, compared to cells grown in a 2D tissue culture polystyrene dish.[17] A similar effect on cancer stem cells has been shown, with high levels of histone H3 observed in cancer cells growing in monolayer, but when cultured in low-adhesion conditions (holospheres), the levels of acetylated H3 decreased.[18]

In addition to histone acetylation, the methylation pattern of histones is also affected by surface structures. In a recent study human adipose stem cells (hASCs) were grown on titanium dioxide (TiO_2) nanotubes with sand-blasted, large grit, acid-etched (SLA) or smooth surfaces. TiO_2 with 70 nm grooves influenced the histone methylation pattern to promote an osteogenic differentiation of hASC.[19] Besides exposure to titanium particles changes in DNA methylation pattern can be observed upon changes on the nano-topographical architecture.[20] Embryonic stem cells (ESCs) grown on nano-topographical polycarbonate substrates changed the DNA methylation level in parts correlated to MSCs and early osteogenic progenitor cells phenotypes. In contrast no difference was found between surface topography regarding global methylation compared to that of normal hESCs.[20] Cellular and nuclear morphology along with histone modifications (acetylation) can also be observed upon cell culturing on micro- and nanotopography surfaces. Interestingly, seeding cells over high grooves (10 μm width and 3 μm height) increases global histone acetylation of fibroblasts when compared to cell culture over flat surfaces. Of note, increased histone acetylation correlates with reduced HDAC activity. Besides enhanced histone acetylation, fibroblasts seeded over microgroove structure undergo a mesenchymal-to-epithelial transition with the result in transcription of epithelial-associated genes and the development of more polarized cells.[13] The process of mesenchymal-to-epithelial transition is often observed during embryogenesis suggesting the potential ability of modified surfaces to favor cellular reprogramming as previously described.[11]

Biomaterial Influences

At present, there exist a limited number of studies that have investigated how different biomaterials alter cellular epigenetic patterns, with most studies focusing on titanium and titanium dioxide (TiO_2), and a few on silica, glass, and graphene. The most prominent readout

between different materials and epigenetic modification has been the DNA damage pathway and the phosphorylation of Histone H2A.X (γH2AX), which is an early marker of DNA damage. γH2AX play a crucial role in the DNA damage response of cells. Upon double strand breaks, γH2AX is one of the first responders to a DNA insult. Interestingly, however, the efficacy of γH2AX in response to an injury can be epigenetically controlled by histone acetylation. Enhanced histone H3 acetylation at lysine 56 (H3K56ac) enhances the DNA damage response in stem cells.[21] Therefore, γH2AX/H3K56ac interaction is suggested to play a major role in the control of hypersensibility of cells to DNA damage repair. Along these lines, exposure of cells to TiO_2 particles may directly influence histone acetylation leading to enhanced DNA double strand breaks.

TiO_2-induced γH2AX is observed at a low concentration of 10 μg/ml, compared to terbium-doped-gadolinium oxide (Tb-Gd2O3) that required a concentration of 1000 μg/ml to induce damage to the DNA or poly(lactic-co-glycolic acid) (PLGA) nanoparticles that did not induce any DNA damage.[22] Interestingly, TiO_2 particles induce γH2AX independent of reactive oxygen species (ROS) produced by inflammatory cells in an innate immune response. This indicates that TiO_2 itself can cause DNA damage in cells that come in contact with the particles.[23] Particles of nano-size induced γH2AX damage DNA more efficiently than larger TiO_2 particles.[23] The structure of the titanium can further induce changes in the epigenetic pattern. Analysis of TiO_2 nanotubes showed that an increase in TiO_2 diameter resulted in a corresponding increase in the binding of histone IIA, with an 8 fold higher binding to the 100 nM nanotubes compared to titanium foil.[24] Also, stem cells cultured on titanium nanotubes showed enhanced adhesion and collagen secretion, improving periodontal tissue regeneration and formation of collagen bundles. Furthermore, using a titanium/cell sheet/HA construct not only promotes periodontal regeneration but also induces blood vessels forming among the collagen fibers and a cementum-like tissue. Several sizes of titanium tubes were tested with 10 μm and 5 μm showing the optimal results with a structure most closely resembling the natural periodontal ligament structure.[25]

Other materials used in TE/RM that influence epigenetic patterns are silica, bioglass, and graphene. The use of silica substrate induces cell alignment and nuclear elongation related to induction of histone acetylation.[26] Silicon wafers with microgrooves (10 μm wide, 3 μm deep) induce histone acetylation and was also shown to make the cells more responsive to treatment

with HDAC inhibitors (HDACi).[26] Gene expression in osteoblasts exposed to hydroxylapatite (HAp) nanoparticles, silica nanoparticles, calcium oxide and phosphate, showed a unique expression in the cells exposed to nano-HAp. A decrease in alkaline phosphatase expression and an increase in DNA methylation of the ALP promoter region were reported.[27] Nano bioglass ceramic particles reduced the levels of HDAC enzyme increasing osteoblast differentiation through induction of the microRNA miR-30c.[28] Graphene, a substrate made of 2D-structures of carbon atoms enhanced cell growth and differentiation.[29] By comparing reprogramming of mouse fibroblasts grown on graphene with cells grown on glass, it was shown that graphene induces an increase in histone methylation at the transcription start site for genes associated with inducing mesenchymal-to-epithelial changes in cells.[29] Graphene also improved alkaline phosphatase staining in iPSCs, presenting a potential tool in bone tissue regeneration.

Scaffold design and combining scaffolds with epi-drugs for gene therapy

Regenerative medicine includes the use of biochemical molecules to induce changes in cells to improve the outcome of tissue regeneration by targeting specific biological mechanisms. The combined use of scaffolds with small molecules like novel epigenetic drugs (epi-drugs), may improve differentiation of cells and tissue engineering by enhancing and regulating epigenetic mechanisms.[30] Silica has been approved by the U.S. Food and Drug Administration (FDA) and used as a delivery model for DNA methylation inhibitor 5-aza. Scaffolds present a good delivery system in regenerative medicine, due to their ability to immobilize a drug (e.g., small molecules, nucleases, or viruses), thereby locally distribute the molecule to the specific site of tissue damage.[31] Not only do scaffolds present an attractive method for efficient local delivery, but the structure of the scaffold itself can also induce epigenetic changes that influence cell behavior, gene expression and hence, tissue regeneration. Small molecules, such as HDAC inhibitors have been delivered extracellularly, and embedded in 3D-scaffolds, with microparticles and genetically modified cells.[31] A local delivery approach could also reduce the potential side effects of the HDACi.[32] Histones have non-chromatin related function that facilitates permeability of membranes thereby mediating transport of substances. Histones immobilized on microspheres facilitates adhesion, proliferation and network formation.[33] This presents an additional tool for using epigenetic molecules to improve surface modifications of scaffold design and tissue regeneration. [34]

Several small epigenetic molecules are available and approved by the FDA that are currently being used in clinical trials for the treatment of various cancers.[35] HDACi are small compounds able to inhibit HDACS thereby inducing transcription of genes. HDACi, such as TSA, Valproate and MS-275, have been investigated for the potential use in regulating bone formation and it has been suggested that HDACi are suitable agents for both local and systemic treatment of bone loss.[36] Interestingly, a positive effect of HDACi on both bone and inflammation in animal models of rheumatoid arthritis (RA), provide a treatment option for simultaneously targeting both the inflammation, bone and tissue destruction thereby presenting a new treatment option in bone tissue engineering.[32]

By treating human bone marrow stromal cells with either an HDACi (TSA) or with a DNA methylation inhibitor (5-aza-dC) stimulated cells to differentiate into osteogenic and chondrogenic, populations respectively [37]. This indicates a use for epigenetic regulatory compounds to induce different cells lineage from one line of stem cells. Coating of surfaces with keratin and 5-Azacytidine induced differentiation of hMSCs into a cardiomyocyte lineage.[38]

Valproic acid (VPA) is considered safe for use in clinical settings and promotes regeneration of nerves *in vitro* using a silicon tube connecting two nerve ends. VPA applied in the silicon tube created a microenvironment resulting in nerve bundles properly oriented and shaped.[39] Collagen sponges and macroporous biphasic calcium phosphate scaffolds mixed with HDAC inhibitors induced woven bone formation and newly formed bone at the contact with the scaffold. [40]

To address a critical first step of initiating and controlling stem cell differentiation, a vital component in TE/RM, the use of the histone H3K4 demethylase LSD1 on hASCs-scaffolds containing an inhibiting molecule for LSD1 was investigated. Compared to anorganic bovine bone-collagen scaffold the prototype scaffold resulted in an increase in H3K4me in osteogenic associated genes along with higher osteogenic differentiation.[41] Furthermore, when adding an HDAC inhibitor to a soft substrate, it preserved the euchromatin structure, compensating for the unfavorable effect of the soft substrate.[8] An additional use for HDACi is as a tool to improve ex vivo genome engineering by restoring gene expression of gene delivery by lentiviral vectors. [42]

Mechanical stimulation and microspheres

Interestingly, not only does the material and structure of a scaffold that cells grow on/come in contact with induce epigenetic changes, recently mechanical stimulation was found to be an additional factor. Mechanical stimulation with fluid flow experiments reduced DNA methylation and increased gene expression, thus indicating that the mechanical micro-environment can induce epigenetic changes that not only can control the present cells but also pass this change on to the daughter cells. [43] Induction of cells into iPSC may also be regulated by biomechanics and biophysical signals inducing changes in the nucleus and the chromatin, suggesting the use of surface topography as a tool for improving TE/RM and replacing the use of small-molecules .[13]

Future considerations

In summary, this review presents novel insights on the delivery of epigenetic modifications that are affected by biomaterial surfaces and scaffolds affecting TE/RM. At present, there is limited knowledge about the epigenetic effects of substrate biomaterials and topography on cellular activities. Greater investigation in these domains is important for the better understanding of stem cell differentiation and for the improvement of bone and soft tissue regeneration. Using the surface topography to induce a specific epigenetic pattern is an advantage since it is highly local and specific to cells in direct contact with biomaterials. Such approaches could enhance the function of local Epi-drug delivery. Finding the critical epigenetic mechanisms involved in stem cell differentiation may be imperative in the control of stem cell differentiation for clinical translation in TE/RM.

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Figure legends

Figure 1. A central factor inducing epigenetic changes in cells are the stiffness and structure of the biomaterial or tissue surface matrix. Cells that adhere to high energy surfaces have a more open and transcriptionally active chromatin structure (i.e., euchromatin) while low energy surfaces induce a shift into a heterochromatin structure (i.e., transcriptionally inactive and more dense chromatin).

Figure 2. A schematic drawing and summary on the structural and functional changes in cells grown on surfaces with different surface structure, stiffness and energy.

Table 1. Overview of the current evidence on the effect of material topography and energy as well as different materials on chromatin configuration and epigenetic mechanisms in cells.

	Reference	Evidence
Topography	Li *et al.* 2011	Increase histone acetylation and decreased HDAC activity in MSCs grown on micropatterned substrates.
	Lv *et al.* 2015	hASCs grown on TiO_2 nano grooves showed increase in H3K4 tri-methylation and inhibition of the demethylase RBP2.
	Kingham *et al.* 2013	ESCs grown on nanotopographical substrates changed DNA methylation in parts of chromatin related to MSCs and early osteogenic progenitor cells.
Material energy	Rabineau *et al.* 2015	Stiff surface induced a transcriptionally active chromatin structure in kidney epithelial cells. Decreasing the substrate softness shifted the chromatin into a transcriptionally inactive structure.
	Li *et al.* 2011	Increase histone acetylation and decreased HDAC activity in MSCs grown on elastic PDMS substrate.
Materials		
Titanium	Setyawati *et al.* 2013	Induced DNA damage in fibroblasts.
	Toyooka *et al.* 2012	TiO_2 Induced DNA damage by phosphorylation of histone H2AX in adenocarcinoma epithelial cell line.
	Lv *et al.* 2015	TiO_2 enhanced methylation of histone H3K4 in hASCs.
Silica	Morez *et al.* 2015	Silicon microgrooves induced histone acetylation.
PLGA	Setyawati *et al.* 2013	Did not induced DNA damage in fibroblasts.
Bioglass ceramic	Moorthi *et al.* 2013	Reduced the level of HDACs in human osteoblastic cells.
Graphene	Yoo *et al.* 2014	Mouse fibroblasts grown on graphene showed increased histone methylation in genes associated with mesenchymal-to-epithelial changes.

HDAC=histone deacetylase, MSCs=mesenchymal stem cells, hASCs=human adipose stem cells, ESCs=embryonic stem cells, PDMS=poly (dimethyl siloxane).

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Graphene	Yoo *et al.* 2014	Mouse fibroblasts grown on graphene showed increased histone methylation in genes associated with mesenchymal-to-epithelial changes.

HDAC=histone deacetylase, MSCs=mesenchymal stem cells, hASCs=human adipose stem cells, ESCs=embryonic stem cells, PDMS=poly (dimethyl siloxane).

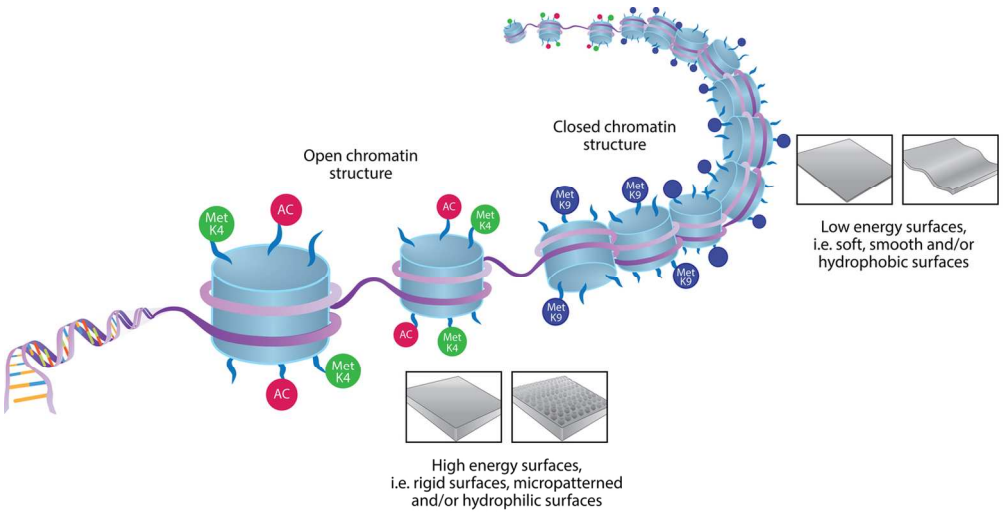

Figure 1

135x73mm (300 x 300 DPI)

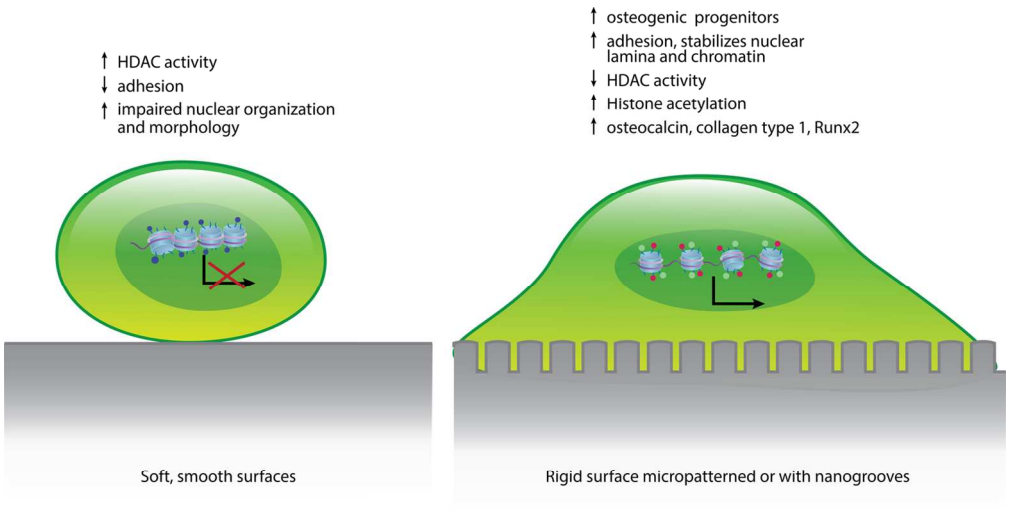

Figure 2

135x75mm (300 x 300 DPI)