Mechanical Properties of Materials for Stem Cell Differentiation

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Recent findings about cell fate change induced by physical stimuli have expedited the discovery of underlying regulatory mechanisms that determine stem cell differentiation. Progress with regards to micro-/nanofabrication technology have led to the development of advanced materials that can mimic biophysical features of in vivo related circumstances of the human body. Since the cellular microenvironment directly defines cellular structure and function, diverse material properties including stiffness, topology, and surface chemistry are investigated to regulate multiple signaling cascades involved in stem cell differentiation for the development of innovative tools that can be widely utilized in various fields ranging from basic research to medical applications. This progress report addresses essential biophysical regulation and alteration of material properties applied to control stem cell differentiation. It also presents novel strategies to regulate stem cell differentiation based on relationships between recently discovered mechanotransduction pathways and cell differentiation signaling. A new perspective on stem cell physiology will further provide a framework of biomedical applications such as regenerative medicine and stem cell therapy.

1. Introduction

Stem cells are nonspecialized cells that differentiate into any type of cells and their differentiation is regulated by various environmental signals. Stem cell differentiation in both embryos and adults is regulated by a systematic and complex process. It is essential for the development and maintenance of homeostasis. Due to the high differentiation potential to transform into specialized cells of desired tissues and their self-renewal ability, stem cells are considered as attractive cell sources for clinical applications. Stem cell differentiation is maintained and induced by various regulators of tightly balanced signaling pathways that depend on physiological properties of the microenvironment for response to differentiation demands.

Traditionally, understanding differentiation pathway has been achieved by studying biochemical signal pathways controlled by various growth factors and cytokines. However, since various physical factors including tissue stiffness and topology can also determine the differentiation pathway of stem cells, mechanobiological pathway for controlling differentiation has been emphasized. Moreover, newly identified mechanobiological pathways have encouraged efforts to interpret stem cell differentiation in terms of cell–material interaction and provided clues to accurately design microenvironment of stem cells to control the direction of differentiation.

Cells continuously recognize topographical and mechanical properties of the surrounding microenvironment and modulate their functional phenotypes through appropriate physiological responses to maintain homeostasis. Cell–cell and cell–extracellular matrix (ECM) interactions determine physical connections between the outside and the inside of individual cells to regulate various cellular functions, including adhesion, migration, proliferation, and cell differentiation. Integrin, a transmembrane protein, is actively involved in outside-in and inside-out signaling mediated by polymerization and contraction of the cytoskeleton known to control cellular mechanotransduction pathways. Therefore, a changed physical microenvironment can be detected by integrin–ECM interaction which has been traditionally considered as a primary target for controlling cell behavior through the material properties.

Recent studies have shown that nuclear mechanosensation is a key process in response to physical stimuli. Nuclear membrane is tightly connected to integrin-based focal adhesion through cytoskeletal fibers that can transmit external force or cytoskeletal tension to the nuclear membrane, causing structural deformation of the nucleus. Applied force not only changes nuclear shape, but also determines the conformation of many proteins located in nuclear membrane associated with various biochemical signals. Since transcriptional regulatory mechanisms, such as histone modification and transcription factor activity, are controlled by force-mediated nuclear deformation, signal pathways for nuclear mechanosensation have been focused to interpret cellular adaptation mechanism including stem cell differentiation.

Various methods have been used to determine cell functions by changing cell adhesion through the corresponding changes in external substrates using micropatterned cell confinement, micro-/nanosized topographic substrates, and substrate stiffness. Micropatterning of ECM proteins is a well-established method to

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control cell adhesion, where cells can adhere only to the ECM-protein-coated surfaces.[8] Cells placed in a confined space can adapt their shape and continue to remodel their cytoskeleton. For a micro-/nanosized topographic substrate, the surface roughness can be regulated by a post, a groove, or a porous structure.[9] The stiffness of a hydrogel, such as polyacrylamide gel or collagen can be controlled to study the fate of cells through changes in cell adhesion, migration, and differentiation according to mechanical properties of the extracellular matrix.[10] In addition, it is possible to artificially reproduce biophysical phenomena in vivo to induce cellular responses. Stretching or compression forces can be applied to cells (Figure 1).[11]

In this progress report, we focus on bioengineered materials that can induce stem cell differentiation into functionalized cells, including their mechanism of differentiation. Such studies using biofunctional materials for stem cell differentiation are crucial for treating organ-specific diseases.

2. Molecular Pathway of Stem Cell Mechano-sensation

Cellular recognition of surrounding environmental condition based on biophysical interactions is termed cellular mecha-

nosensation that regulates stem cell differentiation through various pathways. In this section, we will focus on the mechanical behavior and cognitive strategies of stem cells associated with signal transduction process.

2.1. Cell Mechanics in Stem Cell Mechano-sensation: From Focal Adhesions to Nucleus

Various characteristics of materials determine the cell structure and physiology and regulate stem cell differentiation. During fate determination of the stem cells, cellular mechanical communication, especially physical contact initiated by integrin and focal adhesion, has been emphasized. Integrin-based physical contact between cells and extracellular substrate is the beginning of mechanical interaction at the cellular level. Integrin connects inside and outside of the cell membrane, and it allows for cells to have bidirectional molecular signaling. Tethering integrin and ECM ligands can mature focal adhesion and induce actin polymerization.[12] Integrins can bind to specific ECM ligands and form adhesive molecular clusters named focal adhesions. They include integrins and related proteins such as vinculin, talin, kindlin, paxillin, and focal adhesion kinase (FAK) (Figure 2A).

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Figure 1. Stem cell fate changes induced by biophysical features of microenvironment. Material properties determined by substrate stiffness, surface topology, spatial distribution, and concentration of interacting ECM, and direct application of mechanical stress can act as biophysical signals that control cell physiology. Mechanical cues induce differentiation of stem cell into specific lineages through a complex signal transduction process.
Integrin signaling is highly associated with the generation of physical forces called actomyosin contractility through the actin filament and myosin, thus imparting motility and contractility to the cell. In fact, integrins, known to bind to the ECM, are physically linked to an internal adhesion complex and cytoskeletal fiber. This physical linkage transmits the intracellular contractility to the extracellular region, allowing cells to continuously communicate with the external environment. Therefore, cell shape and polarization are determined by spatial distribution, concentration, and affinity of the ECM components that cells have contact with. A higher density of integrin ligands is closely related to the organization of larger focal adhesions and matured actin stress fibers, leading to the formation of biased membrane protrusions and a wider cell spreading.

On the other hand, actomyosin contractility determines the mechanical feedback, which is the main principle of cellular physical communication. Multiple integrins present on cell membranes are organically linked through actin stress fibers and organized actin stress fibers are mechanically connected to focal adhesion complex. Filamentous actin (F-actin) polymerized by globular actin (G-actin) is bundled and cross-linked to form stress fiber in assistance with actin–myosin network. Molecular activity of myosin generates a contractile force in actin fibers, and highly regulated force generation by multiple myosins is used as the basis of cellular mechanical communication. Cytoskeleton is anchored to nuclear membrane through LINC molecular complex located in nuclear membrane. Nesprin that is directly bound to the cytoskeleton, is linked to nuclear lamina through KASH domain located at the outer nuclear membrane and SUN protein at the inner nuclear membrane. The physical stimulus delivered by connected cytoskeleton is transmitted through the LINC complex into the nucleus, regulating various genetic activities.
the integrins,[16] leading to structural deformation of attached ECM to different cell adhesion levels depending on the substrate stiffness and deformability.[17] In fact, ECM deformation or release of integrin tethered by cell contraction can lead to a disruption of focal adhesion clusters and actin fibers, resulting in attenuated downstream signal transduction. By contrast, if ECM supports a force exerted by the cell, it will induce a stronger integrin signaling and maintain positive feedback. This mechanical feedback is considered a basic strategy for cellular mechanosensivation.[18]

Thus, damage and malfunctions of proteins involved in the formation of intracellular physical linkage can disturb stem cell differentiation regulated by mechanosensivation. In bone-marrow-derived mesenchymal stem cells (BMMSCs), soft substrates can markedly increase the internalization of $\beta_1$ integrins, while inhibition of integrin internalization can block neural lineage differentiation of the BM MSCs on a soft substrate.[19] Multipotent cells derived from cardiac tissues show an enhanced endothelial differentiation when the matrix stiffness closely matches that of the myocardium. In this process, pi190rhoGAP, a regulator of the RhoA pathway plays a decisive role in the differentiation regulated by $\beta_1$ integrin.[20] The stiffness-dependent osteogenic differentiation of the mesenchymal stem cells (MSCs) is regulated by RhoA/Rho-associated protein kinase signaling and FAK activity. Each inhibitor can disrupt the mechanical regulation of bone differentiation.[21] Another study has shown that integrin $\alpha_5$ acts as a major regulator of osteogenic differentiation of MSCs because antibody blocking integrin $\alpha_5$ reduces expression of osteoblast markers.[22]

Cell nucleus is an organelle that contains genetic information in eukaryotic cells and it plays an important role in cell mechanics.[23] The interior of the nucleus is physically isolated from the cytoplasm by the nuclear envelope that is divided into an inner nuclear membrane and an outer nuclear membrane. The linker of the nucleoskeleton and cytoskeleton (LINC) complex is known to consist of proteins such as lamin, nesprin, Sad1 and UNC-84 (SUN) proteins, Klarsicht, ANC-1, Syn homology (KASH) proteins, and emerin that are physically connected to each other. These connections have been proposed as key pathways to transmit mechanical forces from the cytoskeleton to the inside of the nucleus (Figure 2C).[23,24] Nuclear lamina, a mesh network composed of A- and B-type lamins and accessory proteins, resides inside the inner nuclear membrane and plays an important role in the structural stability of the nucleus. Expression of lamin proteins is regulated by LMNA genes encoding A- and C-type lamins and LMNB1 and LMNB2 genes encoding B1 and B2 lamins, respectively. Lamin A is present in most cells. However, the protein content of lamin A differs depending on the cell type during the developmental stage.[23,25]

The LINC complex allows lamins that supports the nuclear membrane in the nucleoplasm to have a direct physical interaction with cytoplasmic stresses.[26] Interestingly, mechanical interaction between the nucleus and cytoskeleton determines various aspects of cell physiology. This phenomenon, called nuclear mechanosensing, is a result of mechanical stress transmitted into the nucleus, which induces various molecular signals inside the nucleus along with structural changes in the nucleus. Mechanical stresses can increase the tension of nuclear membrane, nuclear import of transcription factors, and chromosome condensation.[27,28] Therefore, an abnormal function of proteins belonging to LINC resulting from genetic deletion or mutation can potentially lead to the loss of mechanical linkages, resulting in abnormal cell phenotypes.[29]

One unique feature of the nuclear lamin is that the ratio of lamin A to lamin B is determined by the mechanical state of the cell. The combination of lamin A/C and lamin B determines physical properties of the nucleus. Lamin A regulates nuclear viscoelasticity, while lamin B controls nuclear elasticity.[20,31] Changes in the external environment, such as the substrate stiffness, can regulate the expression of lamin A/C.[32] Previous studies have shown that differences in lamin expression are strongly correlated with tissue-stiffness-specific stem cell differentiation. The difference in the expression of lamin A is related to tissue stiffness. Lamin A expression is well-known to be increased in hard tissues such as bone or muscle, whereas the expression of lamin A is suppressed in soft tissues such as brain and fat. The expression of stiffness-specific lamin A also serves as an indicator of stem cell differentiation. High levels of lamin A/C are strongly correlated with increased MSC differentiation for osteoblasts. By contrast, they are associated with reduced adipogenic differentiation.[30] Damage to nuclear lamin, a key protein in the LINC complex, can disrupt the mechanotransduction of cells, leading to unexpected differentiation of stem cells. Mutations in LMNA, a gene that regulates lamin A/C expression, can result in abnormal regulation of signaling pathways and osteogenic protein expression in MSC differentiation.[33] Partial knockdown of lamins also promotes bone differentiation of MSCs in soft substrates.[30] These results clearly show a relationship between nuclear lamin A/C and cell differentiation, suggesting the importance of nuclear mechanics in stem cell differentiation.

### 2.2. Mechanoregulation of Key Transcription Factors for Osteogenesis and Adipogenesis

Multistep regulatory mechanisms for stimulus recognition and inhibition play an important role in the early and late stages of stem cell differentiation. In many cases, transcription factors downstream of signal cascade directly regulate the expression of various genes by interacting with DNA in the nucleus. However, previous studies have shown that the activity of several specific transcription factors commonly determine the differentiation pathways.[14] By activating transcription factors, differentiation into specific pathways can be induced by upregulating the expression of genes responsible for the induction and progression of specific cell type differentiation. Therefore, inducing stem-cell-specific transcription factors for stem cell differentiation has become a key strategy for in vivo stem cell differentiation and medical applications.

Physical factors such as substrate stiffness and extracellular forces can regulate the phenotype of stem cells possibly accompanied by the expression or functional activation of key transcription factors for the transcription of differentiation-specific proteins. Many studies have suggested that each signaling pathway involved in adipogenesis and osteogenesis of stem cells can inhibit other signals, that is, the adipogenic pathway can interfere with osteogenic signaling and vice versa. This biased differentiation is determined by interactions of multiple
transcription factors. Recent studies have shown that intracellular mechanics determined by mechanical cues can regulate the activity of transcription factors (Figure 3). Therefore, this section examines how physical stimuli regulate key transcription factors in the differentiation pathways.

Differentiation of stem cells into osteoblasts, termed osteogenesis, is an essential cellular mechanism underlying bone growth, bone regeneration, and bone turnover process governed by osteoblasts and osteoclasts. Osteogenic differentiation is regulated by several key soluble factors and signal transduction pathways. Among proteins known to be involved in osteogenic pathways, transcription factors such as Runx2, Smad, and β-catenin are key regulators. Runx2 transcription factor is an essential regulator of bone formation in stem cells. It directs stem cells including MSCs to differentiate into osteoblasts and inhibits other differentiation pathways, especially differentiation into adipocytes. The expression of Runx2 is regulated simultaneously by various signaling pathways, including the bone morphogenic protein (BMP) and Wnt signaling pathways.

BMPs are the major growth factors associated with skeletal tissue. They are well-known for their ability to induce the formation of bone and cartilage. BMP2 interacts with BMP receptor to activate the transcription factors Smad1, 5, and 9 that can interact with Smad4 for osteogenesis. Activated Smad moves into the nucleus and initiates the transcription of proteins for bone differentiation, where Runx2 expression is also promoted by Smad. Thus, the deletion of the BMP ligand inhibits bone formation. However, the importance of Smad in bone differentiation has been emphasized in that the production of Runx2 and its interaction with Runx2 are essential for bone-differentiation-related transcription.

Wnt signaling is initiated by binding of Wnt proteins to Frizzled family receptors. In the absence of Wnt signaling, β-catenin is continuously degraded without its function as

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**Figure 3.** Stem cell mechanotransduction in regulation of adipogenesis and osteogenesis. Adipogenesis and osteogenesis of stem cells are determined by the activity of key transcription factors regulated by various mechanical signals. The mechanical properties of natural and artificial materials in contact with stem cells trigger integrin signaling and cytoskeleton reorganization associated with generation of contractile forces, which are converted into biochemical signals regulating in transcription factors involved in stem cell differentiation. Adipogenesis and osteogenesis are precisely regulated by key transcription factors, which can interfere with each other by promoting differentiation pathways of same lineage or inhibiting differentiation pathways of other lineages. Osteogenesis is increased by Runx2, Smads, β-catenin, and YAP, whereas adipogenesis is induced by C/EBPα, C/EBPβ, and PPAR-γ. Mechanical stress applied directly to the cell can regulate intracellular Ca²⁺ concentration, which serves as a major secondary messenger that regulates various signaling pathways. Mechanical stress of cell nucleus, which is highly associated with nuclear translocation of YAP, is determined by cytoskeletal tension generated by the cells or by external stress transferred into the nucleus.
a transcription factor. Activation of the Wnt pathway causes accumulation of β-catenin in the cytoplasm and translocation into the nucleus. In the nucleus, β-catenin activates transcription of downstream genes by binding to T-cell factor/lymphoid enhancer factor.β-catenin is known to play an important role in stem cell differentiation into osteoblasts, and the absence of β-catenin blocks osteogenic differentiation.α-

As discussed above, rigid matrix stiffness, increased cell spreading, and enhanced actomyosin contractility can promote stem cell osteogenesis. Biophysiologically enhanced osteogenic differentiation is mostly accompanied by increases of Runx2, Smad, and β-catenin activities, where mechanosensing of the contacted material plays a significant role in this differentiation. As substrate stiffness increases, Runx2 is also more expressed, which is closely related to the increase of integrin expression.β-catenin is known to play an important role in stem cell differentiation. β-catenin and Smad levels, which are closely associated with an increase in cell volume, which is regulated by transcription with an increase in Smad signaling.γ-

β-catenin is also sensitive to substrate stiffness. During the differentiation of adipose-derived stromal cells, β-catenin can increase nuclear translocation when stiff substrates are used, thus promoting osteogenesis with increases of Runx2 expression.γ-

A recent study has shown that osmotic pressure or ECM stretching can induce intracellular calcium influx along with an increase in cell volume, which is regulated by transient receptor potential cation channel subfamily V member 4 (TRPV4), one of the mechanosensitive ion channels present in cell membrane. TRPV4-mediated calcium influx controls Runx2 nuclear translocation and osteogenesis of MSCs, suggesting that mechanosensitive ion channels are main components that regulate osteogenesis under mechanical stimulation.γ-

The application of nanovibrations can also enhance β-catenin and Smad levels, which are closely associated with an increase of calcium influx through TRPV4, consequently leading to enhanced bone differentiation.γ-

Ingestion of excess nutrients above energy consumption promotes proliferation and enhancement of adipocytes. An increase in the number of adipocytes is caused by signal transduction factors that can induce differentiation of MSCs into adipocytes.γ-

Although numerous transcription factors are involved in adipogenesis, peroxisome proliferator-activated receptor-γ (PPAR-γ), C/EBPα, and C/EBPβ (CCAAT/enhancer-binding proteins) are the most important transcription factors.γ-

PPAR-γ is one of transcription factors that regulate the expression of genes responsible for adipogenesis differentiation.γ-

PPAR-γ is upregulated during adipogenesis differentiation of MSCs. Inhibition of this transcription factor inhibits adipogenesis.γ-

One interesting characteristic of PPAR-γ is its competitive inhibitory relationship with β-catenin. PPAR-γ activation leads to degradation of β-catenin, and vice versa, β-catenin activation leads to the degradation of PPAR-γ.γ-

C/EBPβ is also a transcription factor that is essential in the process of adipogenesis. Expression and nuclear translocation of C/EBPβ can initiate the transcription of PPAR-γ, another adipogenic transcription factor, and other proteins essential for adipogenesis.γ-

 Destruction of the C/EBPβ gene can reduce fat mass caused by developmental disorders of adipose tissue.γ-

Moreover, C/EBPβ is involved in inhibition of Wnt/β-catenin signal transduction, which inhibits fat production.γ-

This suggests that C/EBPβ, along with PPAR-γ, can act as a competitive inhibitor of stem cell osteogenesis to enhance adipogenesis.γ-

PPAR-γ and C/EBPα of stem cells are also regulated by physical cues. For instance, reduced substrate stiffness increased the expression of PPAR-γ and C/EBPα in adipose-derived stromal cells as well as in MSCs.γ-

Inhibition of cell spreading by micropatterns also increases adipogenesis, in association with nuclear translocation of PPAR-γ, which is closely related with an increase in the cell height and a decrease in the cell spreading area.γ-

This suggests that intracellular tension determined by the cell morphology regulates the activity of PPAR-γ in mechanotransduction of the stem cell to regulate adipogenic transcription. Restriction of cell area through the tripeptide Arg-Gly-Asp (RGD) patterning also can determine nuclear translocation of PPAR-γ, supporting the logic that the activity of PPAR-γ increases in inverse proportion to cell spreading.γ-

However, if the ligand density is too small, differentiation of stem cells is suppressed regardless of cell size, which suggests that mechanical regulation of stem cell differentiation occurs only when there is a certain level of internal tension to determine the differentiation pathway according to the mechanical state.γ-

Another mechanical signaling pathway that regulates the transcription in stem cell differentiation is hippo pathway. The hippo pathway is regulated by various signals, including intra- and extracellular physical signals.γ-

Among key components of the hippo pathway, yes-associated protein (YAP) and transcriptional coactivator with postsynaptic density 95, PSD-85; Discs large, Dlg; Zonula occludens-1, ZO-1-binding motif (TAZ) play an important role in regulating cellular mechanotransduction. Control of the hippo pathway via YAP/TAZ is regulated by phosphorylation and nuclear translocation of YAP/TAZ.γ-

The nuclear translocation of YAP/TAZ is also regulated by intracellular dynamics, unlike other signaling pathways that rely on receptor–ligand responses. Multiple biophysical factors such as cell density, ECM stiffness, and modification of cell structure can regulate the localization and activity of YAP/TAZ.γ-

Translocation of YAP/TAZ in response to mechanical stimulation is directly determined by structural and functional regulation of cytoskeletal components such as actin and myosin that regulate cellular tension.γ-

Recent studies have shown that biophysical forces acting on the nucleus can immediately alter nuclear translocation of YAP because mechanical stress applied to the nucleus can increase the nuclear membrane tension and expand nuclear pores, thereby promoting spontaneous nuclear inflow of YAP.γ-

YAP/TAZ also plays an important role in stem cell differentiation. In the differentiation of MSCs, TAZ acts as a coactivator of Runx2 to promote bone formation, while it inhibits PPAR-γ-dependent
gene transcription and attenuates adipogenic differentiation.\textsuperscript{[66]} Similarly, knockdown of YAP inhibits osteogenesis and promotes adipogenesis, whereas overexpression of YAP promotes osteogenesis and inhibits adipogenesis.\textsuperscript{[67]} These results clearly support that YAP/TAZ activity aids osteogenesis and inhibits the adipogenesis pathway of MSCs.

These unique properties of YAP/TAZ that determine nuclear translocation by reflecting cellular mechanics confirm that YAP/TAZ is a key regulator of stiffness-specific stem cell differentiation. Interaction between the ECM and skeletal stem cells mediated by \(\beta1\) integrin regulates cytoskeletal reorganization, cell shape, and nuclear localization of YAP/TAZ. It also increases levels of YAP, thus promoting bone differentiation.\textsuperscript{[68]} Downregulation of YAP/TAZ via small interfering RNA treatment can decrease osteogenesis in MSCs, which increased in stiff substrates, while adipogenesis that is suppressed by stiff substrates is increased by YAP/TAZ knockout.\textsuperscript{[63]} These findings show the importance of nuclear translocation of YAP/TAZ as a mechanical responder in the decision-making process of stem cell differentiation under physical stresses, suggesting the potential of YAP/TAZ as an appropriate biomolecular indicator to predict the direction of cell differentiation.

3. Mechanobiological Strategies to Control Stem Cell Differentiation

Cell–material interactions modulate diverse intracellular structural connections spanning from focal adhesions to cell nucleus that are key steps in the decision of biophysical environmental recognition as well as differentiation pathways of stem cell, as described previously. To achieve optimal differentiation efficiency, many efforts have been made to specifically mimic tissue-specific niches in vivo by adjusting multiple material properties. In this section, we will describe the physiological regulation of stem cell’s phenotype, mainly focusing on the alteration of material properties that applies mechanical stimuli to modulate cellular mechanics.

3.1. Alteration of Matrix Rigidity

Materials stiffness represents the extent to resist the deformation of a material when a force is applied. Materials with a high stiffness can withstand strong forces without permanent deformation, while materials with low stiffness easily deform. The Young’s modulus, determined by the ratio of stress to strain, is used to represent the material stiffness, and harder materials exhibit a higher Young’s modulus. Individual organs display a distinct stiffness values that are one of the most important biological features in the context of mechanobiology because they are determined by the composition and density of the ECM proteins as well as presentation of cross-linking proteins that make up the tissues. In turn, these proteins are regulated and maintained by the cells that reside in each tissue.\textsuperscript{[69]} The interaction between the stem cells and the matrix stiffness is critical to regulate the early stage differentiation pathways. These cells are physically bound to these biological substrates through adhesive molecules such as integrins, and they recognize substrate stiffness through cytoskeletal contraction.\textsuperscript{[70]} The physical interaction between the cell and the substrate is converted into biochemical signals and determines cell differentiation.\textsuperscript{[71]}

The physical interactions between cells and their external microenvironment have been studied by utilizing a variety of natural and synthetic materials that can mimic the mechanical properties of individual organs in the body. For instance, not only natural polymers, e.g., alginate, gelatin, and collagen, but synthetic polymers, e.g., polycrylamide, polydimethylsiloxane, and polyethylene glycol (PEG) have been adopted. While physical properties of these polymeric materials can be conveniently determined by controlling concentration of cross-linkers, recently, smart hydrogels that can alter their mechanical characteristics in response to various physical stimuli (e.g., light, temperature) and biochemical stimuli (e.g., pH, ionic strength) have been developed.\textsuperscript{[72]} Since these materials can effectively simulate varying stiffness of soft tissues such as brain, fat, and bone marrow as well as hard tissues such as muscle and bones, they have been extensively used to understand the mechanical responsibility of cells in vitro.\textsuperscript{[73]}

Polycrylamide (PA) hydrogels are the most representative synthetic hydrogels applied to control stem cell differentiation. Early efforts studying the mechanical sensitivity of stem cells have led to the development of diverse stiffness of PA hydrogels that can specifically mimic tissue stiffness since their stiffness can be simply controlled by adjusting ratio between acrylamide and cross-linker monomer, \(N,N’\)-methylene-bis-acrylamide.\textsuperscript{[74]} The substrate elasticity of PA hydrogel clearly regulates the differentiation of MSCs, with soft substrates like brain tissue (0.1–1 kPa) resulting in an increased expression of neuronal-related marker proteins. Muscle-like substrates (8–17 kPa) also show increased expression of myogenic marker proteins. A rigid stiffness (25–40 kPa) enhances osteogenic differentiation. Difference in stem cell differentiation is determined by the stiffness of PA hydrogel, independent of porosity or protein tethering.\textsuperscript{[75]} Recently, stiffness-dependent regulation of stem cell differentiation has been investigated using stiffness gradient PA hydrogels, where local stiffness of hydrogels is gradually changing due to the differential diffusion of cross-linkers during the synthesis of PA hydrogels.\textsuperscript{[76]} In this experimental setup, differentiation of human adipose-derived stem cells (ASCs) is promoted differently depending on the location of the cells, leading to maximum osteogenesis at stiff region (≈36 kPa), smooth muscle cell (SMC) at middle stiffness (≈12 kPa), and adipogenesis at soft region (<3 kPa), consistent with the results introduced above. Furthermore, induced differentiation of cells through transforming growth factor \(\beta\) known to induce differentiation of MSCs into chondrocytes or smooth muscle cells is also affected by substrate stiffness. A rigid matrix promotes SMC differentiation, while a soft matrix promotes chondrogenic and adipogenic differentiation.\textsuperscript{[77]} This demonstrates that a combination of physical factors such as substrate stiffness, and biochemical factors such as cytokines can determine the differentiation pathway of stem cells. 3D hydrogels are often used as carriers to introduce cells into the body. Engineering stiffness of the hydrogels is important to form the optimum niche for cell differentiation. When MSCs are implanted into the body with structurally engineered void-forming hydrogels based on alginate, stiffness of the hydrogel
determines the efficiency of bone regeneration,[78] where the elasticity of the hydrogel could be optimized at ≈60 kPa for the maximum bone regeneration. This suggests that matrix stiffness is the essential physical variable that can lead to more effective results in the development of materials for medical applications.

Rather than simply maintaining a constant stiffness, materials that change their stiffness in response to specific stimuli such as induced light, pH, and temperature have also been continuously developed because these materials can induce extracellular physical-cue-mediated cell adaptation and functional variation. Photosensitive hydrogels, e.g., gelatin methacrylate (GeLMa), for instance, can be applied for artificial control of stiffness by inducing light. They are polymerized via a photoinitiator such as Irgacure 2959 or VA-086. The stiffness of the GeLMa hydrogel whose polymerization is promoted by blue light is determined by solution concentration (15%: 41.78 ± 1.10 kPa, 10%: 33.69 ± 1.13 kPa, 75%: 25.59 ± 2.09 kPa).[79] MSCs encapsulated in GeLMa gel differentiate into endothelial-like cells in soft gel, whereas osteogenesis is dominant in intermediate stiffness. Recently, stiffness-dependent regulation of ASC differentiation has been investigated using GeLMa hydrogels with stiffness gradients.[80] The stiffness of the gradient gel is determined by controlling UV dose through a photomask. With lower stiffness (3.5 kPa), ASCs differentiate into adipogenic cells, while stiffer gel (12 kPa) induces myogenic differentiation. Besides GeLMa, photoinitiators can be applied to synthesize light-dependent stiffness changing substrates such as PA hydrogel,[81] PEG hydrogel,[82] and gelatin/hyaluronic acid hydrogel.[83]

pH-sensitive hydrogel is composed of polymer backbones with acidic or basic functional groups that undergo protonation/deprotonation process depending on the pH level of the external microenvironment. Therefore, pH-dependent swelling and deswelling of these hydrogels can directly modulate cell adhesion.[84] In addition, pH-sensitive polymers are often used as dual-sensitive polymers that are sensitive to both pH and temperature by polymerizing together with thermosensitive polymers such as poly(N-isopropylacrylamide).[85] Unique properties of the dual-sensitive biodegradable hydrogels are beneficial to development of regenerative biomaterials for cardiac cell therapy after heart damage.[86] This hydrogel solidifies at pH 6.5 (shear modulus: 1418.7 ± 39.8 Pa), the pH of infarcted heart. However, they do not solidify at pH 7.4 (381.3 ± 26.1 Pa), the pH of blood. In addition, cardiosphere-derived cells encapsulated in this hydrogel that have an optimal matrix modulus for cardiac cell differentiation can differentiate into cardiac cells with the expression of cardiac markers. Dual-sensitive microgels have also been developed using acrylic acid and N-isopropylacrylamide.[87] Since acrylic acid switches hydrophobicity depending on pH level (e.g., hydrophilic at increased pH value), the microgels can expand as the pH changes from 4 to 7. On the other hand, N-isopropylacrylamide is a temperature-sensitive polymer that shrinks at high temperatures. Thus, the microgels that swell at pH 7 and 37 °C can enhance physical contact between cells and these materials, which consequently increases the osteogenic differentiation of MSCs.

Like synthetic polymeric hydrogels, real tissues are viscoelastic materials that exhibit both elastic and viscous characteristics when undergoing deformation. Viscoelastic property has a greater effect on soft tissues such as brain tissues that display large deformation in response to mechanical input. Elasticity of the tissue is changed according to the change in viscosity over time, and thus viscoelastic property is critical to determine cellular mechanosensation.[88] For instance, 3D hydrogels synthesized by alginate of different molecular weights are utilized to control the degree of stress relaxation, a property of viscoelastic materials where the stress decreases over time under constant stress.[89] In the lower molecular weight alginate hydrogels, stress relaxation exhibits faster, which improves cell adhesion, proliferation, as well as osteogenic differentiation of the encapsulated MSCs. These results demonstrate that viscoelasticity, along with material stiffness, is also a critical physical factor that regulate the cell–substrate mechanical interaction.

### 3.2. Controlling Integrin–Substrate Surface Interaction

As described above, cell mechanics can be controlled by modifying surface characteristics in various ways. Cell mechanics can be altered by using other 2D materials such as graphene, gold nanoparticles, or changing composition of integrin ligands (e.g., RGD peptide) on the substrate that affects the integrin–ligand interaction between the cell and the substrate.

Graphene is a carbon monolayer featuring honeycomb structure and displays superior mechanical stability and physicochemical characteristics. Graphene surfaces have hydroxyl, carbonyl, carboxyl, and carboxylate groups that can increase the interaction between proteins through electrostatic, covalent, and hydrogen bonds. These characteristics enable the graphene to be widely used as a cell stimulator that induces osteogenic and neurogenic differentiation of stem cells.[90,91] Graphene oxide (GO) is chemically modified graphene form that has epoxide and hydroxyl groups in the defects of the carbon lattice and carboxyl groups on the outer edges. These functional groups result in a high solubility of GO. When MSCs are differentiated in osteogenic medium and adipogenic medium on graphene and GO surfaces, MSCs anchored on graphene surfaces are more mineralized than those placed on GO surfaces.[92] On the other hand, in adipogenic medium, MSCs grown on GO exhibit more adipogenesis than those grown on graphene. These results demonstrate that different affinities of cells for graphene and GO surfaces regulates cell differentiation.

Coating graphene and graphene-based materials on the surface with modified topology can lead to more efficient stem cell differentiation. A combination of graphene and electrospun nanofibers can promote differentiation of neural stem cells (NSCs) into oligodendrocytes (Figure 4D).[93] In this structure, the differentiation enhances as the concentration of graphene in the scaffolds increases. In addition, graphene scaffolds used with polydimethylsiloxanes (PDMSs) of different stiffnesses can regulate the differentiation of impregnated MSCs.[94] MSC-impregnated graphene scaffolds exhibit enhanced bone-related markers RunX2 and osteopontin in vivo. Moreover, osteogenesis and integrin–FAK-related proteins were more expressed on graphene-coated PDMS rather than on bare PDMS in vitro, suggesting that graphene induces osteogenesis of the MSCs.
by enhancing integrin-mediated cell adhesion and related signaling.

Biological interface determines cell adhesiveness based on ECM density, spatial distribution, and motility. Integrin ligands such as fibronectin, vitronectin, and collagen are present in an extracellular matrix. The integrin ligand in various ECM proteins such as RGD peptide has also been utilized to better understand physical interactions between cells and biomaterials (Figure 5). ECM density is an essential factor that directly determines the amount of integrin-bound cells to the surface of the biomaterial (Figure 5B). Gradient of collagen concentration can promote bone differentiation of MSCs. Increasing collagen concentration can increase Runx2 of MSCs, demonstrating that higher integrin adhesion can promote osteogenesis. Cell differentiation has also been studied through changes in ligand density in the hydrogel. For instance, methacrylated gelatin with RGD motif and polyethylene glycol diacrylate (PEGDA) are used to simultaneously control the hydrogel stiffness and integrin ligand density. When osteoblastic cells (hFOB1.19 cells) are cultured on PEGDA/GelMA gels with high density of RGD, expression levels of osteogenic markers are also increased. These results reconfirm that osteogenesis is increased with an increasing integrin ligand density.

Spatial distribution of integrin ligands can be artificially controlled by altering the spacing between ligands and arrangement of the ligand sites (Figure 5C). Ligand distribution determines the formation of integrin clusters and focal adhesion, which could potentially control cell traction forces. To alter the ligand distribution, materials such as Au and graphene have often been used. RGD-conjugated gold nanorods (AuNRs) with different aspect ratios can be used to investigate the effect of spatial distribution of integrin ligands on cell adhesion and differentiation. RGD–AuNRs coated on the surface have the same surface perimeters with different aspect ratios of 1, 2, 4, and 7. MSCs form mature focal adhesions, enhance expression of integrin-mediated proteins, as well as improve osteogenic differentiation of MSCs as the aspect ratios of AuNRs increase. Nanoscaled adhesion sites that are uniformly arranged on the surfaces by coating graphene on Au nanoelectrodes can change the differentiation of MSCs. When MSCs are incubated on
graphene–Au hybrid nanoelectrode arrays with different diameters of 200, 400, 600, and 800 nm, expression levels of alkaline phosphatase (ALP) and Runx2 (early osteogenic markers), and calcification markers are the highest at 400 nm group. On the surface of randomly distributed microsized and nanosized graphene oxides (MGO, NGO) with the same concentration of graphene oxide, the physicochemical characteristics between MGO and NGO surface are similar, but in terms of cell adhesion, more active filopodia is formed in MGO.[100] In the differentiation of human adipose-derived mesenchymal stem cells on MGO and NGO surfaces, higher levels of osteogenic marker proteins are expressed in MGO than in NGO. These studies confirm that it is essential to properly set biophysical factors, such as ligand spacing and size when forming mature osteoblasts.

In general, the ligand is coated on the surface through covalent bonds, allowing the ligand to be immobilized on the surface.[100] However, an actual cellular microenvironment has rapid, dynamic, and adaptive properties. To understand these properties, it is necessary to understand the mobility of the ligand. Supported lipid bilayers (SLBs) can easily change their

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**Figure 5.** Schematic view of three factors affecting integrin–ligand interactions: ligand density, ligand distribution, and ligand motility. A) Schematic of integrin–ligand interaction in stem cell differentiation. B) Ligand density controllable surface: gradient ethanol and propionaldehyde polymer on the surface, 3D hydrogel embedded different concentrations of ligand polymer. Enhanced expression of differentiation marker proteins in high density of ligands. C) Surface coated with materials for distribution of integrin–ligand interaction: surface coated with various aspect ratio Au rods, nanoelectrode array using Au–graphene to manipulate size and distribution of array. Cell differentiation depends on the physical features of coated surface. D) Surface modification for regulating ligand motility: surface-coated lipid with different motilities (DOPC: high, DPPC: low), controlling ligand dynamics (motility and oscillation) using magnetic field. High potent of differentiation is observed in slow motion of ligand rather than fast or static motion.
features, such as characteristics of their biological interfaces (ligand density, mobility) (Figure 5D). In the case of SLBs containing RGD ligands, the density and mobility of ligands can be altered to control the adhesion or differentiation of MSCs.\[102\] The lipid layer composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) and 1,2-dipalmityl-sn-glycero-3-phosphocholine (DPPC) lipids has different characteristics in ligand mobility. The DPPC lipid has a high melting temperature, and thus has an immobile ligand at physiological temperature. On the other hand, the DOPC lipid has a low melting temperature, so it can have a mobile ligand. Cell spreading increases more in DOPC SLB, which has high mobility than in DPPC SLB. When MSCs are cultured on the SLB, osteogenic differentiation as well as cell spreading is enhanced in DOPC than in DPPC. These results indicate that the motility of the ligand is important in the differentiation of stem cells, which was also confirmed using magnetic fields.

RGD-grafted magnetic nanoparticles can be tethered to the surface via PEG linker to control RGD motility under a magnetic field.\[103\] This surface allows a larger number of cells to adhere under the application of a magnetic field, and also enhances cell spreading. In addition, application of a magnetic field on magnetic nanoparticles increases expression of osteogenic markers ALP and Runx2. Furthermore, magnetic fields have been used to induce ligand oscillation to control integrin–ligand binding.\[104\] By adjusting the oscillation frequency and magnetic field, it is possible to delicately control magnetic nanoparticles combined with RGD peptide, where both cell adhesion and cell spreading increase at lower levels of RGD oscillation than at static or faster oscillation. Cell differentiation can also be promoted under the slow oscillation of RGD, the cells cultured in osteogenic medium for 7 days expressed 222% more Runx2 under slower RGD oscillation than in static conditions. These results indicate that the cellular functions such as cell adhesion and differentiation require a low motility of ligand rather than the static state.

3.3. ECM Micropatterning and Surface Topology

The extracellular matrix and neighboring cells act as physical boundaries and regulate morphological properties of attached cells such as spreading area, shape, and polarity.\[105\] ECM micropatterning allows limiting the cell shape by mimicking tissue-specific microenvironments in vitro. A key strategy for ECM micropatterning is to induce spatial arrangement of integrin binding ligands, such as collagen and fibronectin onto substrates. Precisely controlled presentation of limited binding sites for cellular attachment facilitates intracellular signal transduction that determines cytoskeletal assembly, turnover, and contraction, therefore determining cell morphology. Advances in microfabrication techniques have led to ECM patterning methodologies to control ECM molecules in various shapes at the micrometer scale. Microcontact printing, a type of soft lithography, can print ECM molecules on culture substrates such as glass or plastic using PDMS stamps obtained by etching microarrays,\[106\] where transferred ECM molecules act as restricted cell attachment region, thus reconstructing the cytoskeletal architecture depending on the printed ECM pattern.\[107\]

This approach has demonstrated that cell morphology can affect stem cell differentiation. Restricting cell spreading with a fibronectin pattern can effectively inhibit osteogenesis of MSCs without significantly inhibiting fat formation.\[108\] Similarly, the ECM patterns that induce cytoskeletal tension enhance osteogenesis of MSCs.\[109\] By contrast, adipogenesis is enhanced by patterns that inhibit cell spreading or attenuate cellular tension. This distinct differentiation is strongly correlated with the F-actin formation and actomyosin contractility, i.e., cell morphology that can induce higher actomyosin contractility and enhance bone differentiation of stem cells, but inhibit adipose formation, whereas low levels of myosin activity induce adipogenesis and weaken osteogenesis.

Although confining cell spreading area to control cell shape is possible with microlevel ECM patterning as described above, technological advances have enabled nanoscale ECM patterning that can directly control the size or the formation of focal adhesion. For example, nanoscale ECM islands could spatially control the formation of adhesion clusters that determine the size and intracellular position of the structured focal adhesions.\[109\] Moreover, ECM islands that are smaller than specific size inhibits the formation of integrin clusters, which demonstrates that threshold tension exerted by cytoskeletal structure is required for clustering of adhesion molecules, e.g., talin and vinculin.\[110\] Forcing the distance between attached integrins also determines the formation of integrin cluster. By regularly placing integrin–ligand-bound gold nanoparticles on 2D surface, spatial distribution of integrin attachment sites can be controlled. When the distance between gold nanoparticles increases, maturation of focal adhesions and pulling force generated by integrin cluster are decreased.\[111\] Similarly, spatial distribution of gold particles can be further fine-tuned to evaluate the threshold of distance between integrins for focal adhesion formation. These results demonstrate that biophysical interactions between integrins are in the range of about 30 nm,\[112\] which further suggest that a sufficient level of integrin density is required to form a focal adhesion cluster through interaction between activated integrins. Therefore, nanoscale ECM patterning could be used in stem cell differentiation as a tool to control clustering of adhesion molecules.

Surface characteristics of materials such as roughness and isotropy can modulate cell adhesion, orientation, alignment, and movement, which in turn alters downstream cell physiology including cell survival and terminal differentiation. To control cell behaviors as desired, various micro-/nanostructures such as nanofiber, micro-/nanogrooves, and micro-/nanoposts are fabricated by using micro-/nanofabrication techniques including electrospinning, electron beam lithography, and photolithography (Figure 4).

For micro-/nanoscale fiber scaffolds made using electrospinning, the fiber alignment determines cell spreading and elongation and has a significant effect on cell differentiation. For example, on well-aligned fiber scaffolds, cell elongation is dominant, while randomly arranged fibers inhibit cell elongation,\[113,114\] which suggests that controlling cell morphology through the fiber scaffolds could regulate cell differentiation pathway. Aligned nanofiber scaffolds can promote MSCs to differentiate into tendon-like cells at four weeks postimplantation. Random nanofiber scaffolds, by contrast, cause MSCs to
differentiate into cartilage, i.e., chondrogenesis (Figure 4A). Aligned nanofibers can also promote differentiation of renal stem cells into kidney podocyte precursors independent of biochemical factors. These electrospun fibers can also be combined with nanoparticles or 2D materials to induce mechanobiological effects on the stem cell differentiation. For instance, gold nanoparticles can be decorated on nanofiber scaffolds to improve the surface roughness, which increase the neuronal differentiation of PC12 cells.

Unlike the other methods described above, post arrays can be used to assess and control the traction force. ECM-functionalized micro-/nanoposts provide cells with a limited area of attachment. The pulling force of the cells generated around the focal adhesion causes bending of the posts, and by changing the height of the posts, the resistance of the posts to traction force can be controlled. Differentiation of MSC is regulated by the height of the microsized posts. In short post, which is not easily deformed by cell contraction, osteogenic differentiation of human MSC is favored, while in high posts, adipogenic differentiation increases. In addition, embryonic stem cells (ESCs) on nanosized gradient posts (120–360 nm in diameter) can enhance the formation of colonies as the size of post increases (Figure 4B). During early differentiation, ESCs highly express early cardiac lineage marker Mesp1 on the 200/280 nm sized posts with large colonies, while other functionalized marker proteins are suppressed. ESCs on gradient nanoposts reorganize the cytoskeleton via p-cofilin transduction, which induces differentiation of ESCs into mature cardiomyocytes.

Similar to fiber alignment, the grooved surface can direct cell orientation along the groove patterns. Width of a groove and distances between grooves are major controlling factors to determine cell morphology. Cells placed on wide groove surface show better alignment compared to narrow surface, but expression of osteogenic markers is not increased, indicating that osteogenic differentiation is not related to cell alignment. On the other hand, adipogenic differentiation is induced as nanosized width and spacing by facilitating alignment of actin stress fibers.

Porous surfaces can also regulate cell differentiation by adjusting pore size. Osteogenesis and myogenesis can be induced by culturing MSCs on a honeycomb-shaped porous surface according to the size of the pore and frame width. Osteogenic differentiation occurs with small pore sizes (pore size: 1.6 µm), while myogenic differentiation occurs with large pores (pore size: 4.7 µm), small median spacing, and significant cell elongation. On the other hand, osteogenic differentiation of bone-marrow-derived stem cells depends on the pore size (10, 80, 200 nm) and surface roughness on the porous surface. These results reveal that osteogenic differentiation occurs more effectively in a pore structure of several hundred nanometers than on a surface having a pore structure of a few nanometers, suggesting that too small porous structures cannot enhance osteogenesis. In addition, the 200 nm nanopore structures induce pancreatic differentiation with the upregulation of pancreatic and duodenal homeobox 1, which are involved in downregulation of TAZ (Figure 4C). When comparing porous surface and linear groove patterns, it has been found that myoblasts differentiate into musculoskeletal tissue on a linear groove surface, whereas preosteoblasts promote the regeneration of bone tissue on hexagonal porous scaffolds. These results indicate that micro-/nanosized porous structures can enhance osteogenesis, while enlarged porous structures and linear surfaces can induce differentiation of stem cells into muscle-like cells. The representative works introduced in this chapter is summarized in Table 1.

4. Concluding Remarks

Extracellular physical cues are critical modulators of cellular function. Thus, the role of interactions between cells and the environment in biological activities needs to be understood in addition to biochemical factors that have been traditionally investigated. Cell organelles such as focal adhesion, cytoskeletons, and LINC complex, are organically linked to each other. They are essential for cell movement and force generation, acting as sensors for physical external stimuli and simultaneously transmitting physical signals out of the cell.

Mechanical properties of the surrounding environment determine the differentiation pathway of stem cells. Intracellular mechanical linkages can convert physical stimulus to produce biochemical signals for various pathways that interfere with the differentiation pathway signal. Transcriptional activities of the transcription factors are determined by a convergence of mechanical and biochemical signals through complex interactions between signaling molecules in multiple pathways. These physiological properties allow these transcription factors to be used as indicators to predict differentiation. However, it is not yet clear how the majority of regulatory molecules in differentiation pathways are modulated by mechanical stimuli. In addition to material-property-based regulation of stem cell differentiation, maintenance of stemness is also important for cellular mechanoadaptation and functional variation, which is further highlighted in terms of mechanical memory effects in response to substrate stiffness. Immunomodulation is also emphasized to prevent the functional degradation of materials and to suppress side effects of implanted materials by innate immunity. Biomaterials designed to reflect these cellular cognitive and adaptive mechanisms are expected to be complementary to existing stem cell therapy and become a novel approach to more precisely regulate cell functions.

Interactions between cells and substrates discussed in this review describe cellular cognitive processes in which cells actively recognize and adapt to physical properties of contacted substrates, such as tissue ECM and artificially designed physical environments. However, real body tissues continually create physical movements, and living cells are exposed in a dynamic physiological environment including tensile, compressive, and shear stresses. Therefore, mimicking these biophysical events is another way to regulate cellular physiology, including stem cell differentiation. Expansion and contraction of blood vessels, periodic heart motion, tensile strength, and compression of skeletal tissues can cause deformation of the ECM, which contains various types of cells. Stretching a substrate to which adherent cells are attached to mimics these mechanical events, which can be used to regulate the cell differentiation pathway. For example, rat adipose stem cells show decreased adipogenesis and increased osteogenesis under cyclic stretching. In addition, cyclic stretching applied to ESCs embedded in gelatin-based scaffolds...
can result in a higher proportion of cardiomyocytes with a more mature phenotype.[128] Cyclic stretching of the material can be applied in conjunction with the topographical modification of the material surface to create synergy. For instance, topographic micropatterns can induce cell alignment and increase SMC phenotype of MSCs when cyclic stretching is applied parallel to the alignment direction.[129] A compressive force on the tissue also promotes differentiation of stem cells contained in the tissue. A mechanical compression on adult *Drosophila* midgut increases enteroendocrine cell population, which is regulated by facilitated Ca$^{2+}$ signaling pathway. This suggests that recognition of mechanical signals by mechanosensitive ion channels such as piezo plays a decisive role in regulating cell distribution through stem cell differentiation.[130]

Table 1. Summary of strategies to induce stem cell differentiation by changing material properties or applying external mechanical stimuli.

| Method                        | Characteristic                        | Cell line          | Outcome                                                                                      | Ref. |
|-------------------------------|---------------------------------------|--------------------|---------------------------------------------------------------------------------------------|------|
| Stiffness                     | Polycrylamide hydrogel                | Human MSCs         | 0.1–1 kPa: Neuronal differentiation  
8–17 kPa: Myogenic differentiation  
25–40 kPa: Osteogenic differentiation                                                                 | [74] |
|                               | Polycrylamide hydrogel (gradient)     | Human MSCs         | −3 kPa: Chondrogenic and adipogenic differentiation  
3–15 kPa: Smooth muscle cell differentiation  
15–36 kPa: Osteogenic differentiation                                                                 | [76] |
|                               | Void-forming 3D hydrogel               | Human MSCs         | Optimized in vivo bone generation at 60 kPa                                                                 | [78] |
|                               | Gelatin methacrylate hydrogel (photosensitive) | MSCs               | 25.59 ± 2.09 kPa: Endothelial differentiation  
33.69 ± 1.13 kPa: Osteogenic differentiation                                                                 | [79] |
|                               | Gelatin methacrylate hydrogel (photosensitive, gradient) | ASCs               | 3 kPa: Adipogenic differentiation  
12 kPa: Myogenic differentiation                                                                 | [80] |
|                               | pH/thermosensitive hydrogel            | Cardiosphere-derived cells | pH 6.5: 1418.7 ± 39.8 kPa  
pH 7.4: 381.3 ± 26.1 kPa, Cardiac differentiation                                                                 | [86] |
| Controlling integrin–ligand interaction | Monolayer graphene film | Human neuroblastaoma (SH-SY5Y) | Stimulation neuronal differentiation                                                                 | [91] |
|                               | Graphene-decorated nanofiber scaffold  | Human NSCs         | Enhanced oligodendrocyte differentiation                                                                 | [93] |
|                               | Graphene-decorated PDMS               | Human NSCs         | Enhanced osteogenic differentiation                                                                 | [94] |
|                               | 3D PEGDA–GelMA hydrogel (PEGDA: controlling stiffness, GelMA: controlling ligand density) | hFOB1.9           | Increased osteogenic differentiation as GelMA concentration increased                                                                 | [96] |
|                               | RGD-bearing gold nanorods with different aspect ratios (from 1 to 7) | Human MSCs         | Increased osteogenic differentiation in high AR                                                                 | [98] |
|                               | Graphene–Au hybrid nanoelectrode array (controlling size and distance of graphene–Au array) | Human MSCs         | High osteogenic differentiation in 200 nm array                                                                 | [99] |
|                               | Supported lipid bilayers (DOPC: high motility, DPPC: low motility) | Human MSCs         | High osteogenic differentiation in DOPC                                                                 | [102] |
|                               | Remote control of ligand oscillation using magnetic field (high: 2 Hz, low: 0.1 Hz, static: 0 Hz) | Human MSCs         | High osteogenic differentiation in low oscillation                                                                 | [104] |
| ECM pattern                   | Circular patterns of 1000 µm²          | Human MSCs         | Decreased osteogenic differentiation on microislands                                                                 | [108] |
|                               | Various types of patterns             | Human MSCs         | Increased osteogenesis and decreased adipogenesis on patterns that induce increased myosin activity                                                                 | [109] |
| Topology                      | PLLA nanofiber scaffold (aligned, randomly oriented fiber structure) | C3H10T1/2          | Aligned: Tenogenic differentiation  
Random: Osteogenic differentiation                                                                 | [113] |
|                               | Gold-nanoparticle-decorated nanofiber scaffold | Rat pheochromocytoma (PC12) cells | Enhanced neurogenic differentiation                                                                 | [116] |
|                               | Micorsized post (0.97, 12.9 µm in heights) | Human MSCs         | 12.9 µm (soft rigidity): Adipogenic differentiation  
0.97 µm (stiff rigidity): Osteogenic differentiation                                                                 | [117] |
|                               | Nanosized gradient post (120–360 nm in diameter) | Fetal liver kinase 1-positive mesodermal precursor cells (Flk1+ MPCs) | High cardiomyocyte differentiation in 200–280 nm sized posts                                                                 | [118] |
|                               | Nanogroove pattern on polyurethane acrylate mold | 3T3-L1 preadipocytes | Enhanced adipogenic differentiation                                                                 | [120] |
|                               | Microsized honeycomb-shaped pore (1.6, 4.7 µm in diameter) | Bone-marrow-derived stem cells | 1.6 µm pore: Osteogenic differentiation  
4.7 µm pore: Myogenic differentiation                                                                 | [121] |
|                               | Nanosized pore (10, 80, 200 nm in diameter) | Bone-marrow-derived stem cells | =100 nm pore: Osteogenic differentiation                                                                 | [122] |
In summary, an understanding of cellular regulatory mechanisms in response to materials properties will lead to the development of precise controlling methods for selective stem cell differentiation. Integrating biophysical and biochemical considerations, complementing technical limitations of traditional regenerative medicine, and facilitating the development of smart materials will lead to an accurate and efficient stem cell therapy.

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Conflict of Interest

The authors declare no conflict of interest.

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