Current Research Status on the Influence of Mechanical Microenvironment on Mesenchymal Stem Cell Differentiation (Postprint)

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Abstract

Mesenchymal stem cells (MSCs) possess strong self-renewal capacity and multilineage differentiation potential, making them popular seed cells in recent research. The growth microenvironment of MSCs can influence and regulate their proliferation and differentiation, with mechanical stimulation being one of the factors affecting MSC differentiation. The effects of extracellular matrix stiffness, mechanical stress (shear stress, compressive stress, tensile stress), microgravity, and other mechanical cues on MSC differentiation are current research hotspots. This article reviews the influences of extracellular matrix stiffness, mechanical stress, and mechanical stress applied to three-dimensional scaffold culture on MSC differentiation.

Full Text

Effects of Mechanical Microenvironment on Mesenchymal Stem Cell Differentiation

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Abstract

Mesenchymal stem cells (MSCs) possess strong self-renewal capacity and multilineage differentiation potential, making them a popular seed cell type in contemporary research. The microenvironment in which MSCs reside can regulate their growth and differentiation, with mechanical stimulation being one of the key influencing factors. Current research hotspots include the effects of extracellular

matrix stiffness, mechanical stresses (shear stress, hydrostatic pressure, tensile stress), and microgravity on MSC differentiation. This review summarizes the influences of extracellular matrix stiffness, mechanical stress, and mechanical forces acting on three-dimensional scaffold cultures on MSC differentiation.

Keywords: Mechanical stimulation; Mesenchymal stem cells; Cell differentiation
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MSCs are stem cells derived from mesenchymal tissue with multi-lineage differentiation potential and self-renewal capacity. They can currently be isolated from various tissues including umbilical cord, umbilical cord blood, placenta, bone marrow, and adipose tissue. Due to their wide availability, ease of acquisition [1], strong proliferative capacity, and low tumorigenicity [2], MSCs have become a major focus of research. MSCs can be induced to differentiate into bone, cartilage, adipose tissue, and liver tissue in vitro [3, 4], making them promising seed cells for tissue engineering and cell therapy applications. However, their differentiation mechanisms remain unclear. MSC differentiation is associated with environmental factors such as cytokines, mechanical stimulation, and extracellular matrix [5-7].

This review summarizes the effects of extracellular matrix stiffness, mechanical stress, and three-dimensional scaffold culture on MSC differentiation.

1 Effects of Extracellular Matrix Stiffness on MSC Differentiation

Studies have shown that extracellular matrix (ECM) stiffness plays an important role in regulating cell morphology, proliferation, and differentiation [8]. ECM stiffness is a crucial regulatory factor for MSC differentiation.

The osteogenic and adipogenic differentiation capacities of MSCs vary with substrate stiffness. Xu et al. [9] cultured umbilical cord mesenchymal stem cells on polyacrylamide gel scaffolds with different stiffness values (Young' s moduli of 13-16, 35-38, 48-53, and 62-68 kPa). They found that MSC adhesion and proliferation differed across substrates, with proliferative activity decreasing as matrix stiffness increased—cells cultured on softer substrates showed superior proliferation compared to those on stiffer matrices. They also discovered that matrix stiffness influenced MSC lineage commitment: adipogenic differentiation occurred at 13-16 kPa, myogenic differentiation at 35-38 and 48-53 kPa, and osteogenic differentiation at 62-68 kPa.

Additionally, Chen et al. [10] cultured MSCs on three-dimensional scaffolds

of varying stiffness and induced osteogenic differentiation, then examined expression of two osteoblast-specific proteins: osteopontin (OPN) and osteocalcin (OCN). The results showed that MSCs on stiffer scaffolds expressed significantly higher levels of OPN and OCN compared to other groups, indicating that matrix stiffness promotes osteogenic differentiation.

Hwang et al. [11] proposed that ECM stiffness regulates MSC differentiation by activating intracellular signaling mediators. They induced osteogenic differentiation of MSCs using stiff hydrogel matrices and identified the transcriptional co-activator with PDZ-binding motif (TAZ) as an effector protein playing an important role in cell differentiation. TAZ activation positively correlated with activation of extracellular signal-regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) pathways. When ERK and JNK were inhibited, TAZ activation was also suppressed, leading them to conclude that ERK and JNK are important mediators through which matrix stiffness influences osteogenic differentiation, with TAZ serving as the effector protein. Shih et al. [12] found that cells likely perceive mechanical stimuli through integrins or focal adhesion kinase (FAK). They induced osteogenic differentiation of MSCs using polyacrylamide gel matrices and observed that as gel stiffness increased, osteogenic differentiation capacity enhanced concurrently with upregulated integrin and FAK activity, suggesting that ECM stiffness may mediate osteogenic differentiation through integrins.

Sun et al. [13] further investigated how ECM stiffness affects intracellular signal transduction. They induced osteogenic differentiation of MSCs using polyacrylamide hydrogels with Young' s moduli of 13-16 kPa and 62-68 kPa. The stiffer matrix (62-68 kPa) was more conducive to osteogenic differentiation, showing increased expression of osteogenesis-related markers type I collagen (COL I), osteocalcin, and runt-related transcription factor 2 (RUNX2). They also detected increased expression of integrin 5/ 1 and downstream signaling molecules including FAK, p-ERK, phosphorylated protein kinase B (p-Akt), glycogen synthase kinase 3 (GSK-3), p-GSK-3 , and -catenin. After blocking integrin 5 with an antibody inhibitor, expression of COL I, osteocalcin, and RUNX2 decreased while Akt, p-Akt, GSK-3 , and p-GSK-3 expression increased. Additionally, inhibiting Akt reduced p-Akt and p-GSK-3 expression. They concluded that at 62-68 kPa ECM stiffness, p-GSK-3 expression is regulated by p-Akt, and integrin 5 mediates osteogenic differentiation of MSCs.

These studies demonstrate that ECM stiffness is an important regulator of MSC differentiation, and understanding its regulatory mechanisms will be crucial for future research.

2 Effects of Mechanical Stress on MSC Differentiation

Under physiological conditions, cells are inevitably subjected to mechanical stresses. Fluid flow generates shear stress, hydrostatic pressure, and tensile stress on cell surfaces. Studies have shown that mechanical factors in the stem

cell microenvironment regulate physiological activities including MSC growth, proliferation, and differentiation, altering cell size, shape, and arrangement [14]. For example, under cyclic pressure, MSC proliferative capacity increases with strain amplitude and cycle number, and MSCs can differentiate into smooth muscle cells even without growth factor supplementation [15].

2.1 Effects of Fluid Shear Stress on MSC Differentiation

Fluid shear stress, generated by tissue fluid movement within various tissues, results from tissue compression, stretching, or fluid flow causing interstitial fluid movement around cells. In vivo, multiple cell types are exposed to shear forces from flowing biological fluids such as blood and lymph.

Studies have demonstrated that fluid shear stress regulates osteogenic differentiation of MSCs. Yourek et al. [16] applied a fluid perfusion device to induce osteogenic differentiation and found that exposure to fluid shear stress significantly increased alkaline phosphatase (ALP) activity in culture medium, with markedly higher mRNA expression of bone morphogenetic protein-2 (BMP2) and OPN compared to static induction conditions, indicating that fluid shear stress promotes osteogenic differentiation. Zhao et al. [17] utilized a perfusion bioreactor system with flow rates of 0.1 ml/min and 1.5 ml/min to induce osteogenic differentiation of MSCs under otherwise identical conditions. Compared to the 0.1 ml/min flow rate, the 1.5 ml/min rate significantly increased intracellular ALP activity and calcium deposition levels, demonstrating that fluid shear stress promotes osteogenic differentiation. Even minimal medium flow significantly enhances extracellular matrix secretion, MSC growth, and osteogenic differentiation. Moreover, cells grown in three-dimensional structures are more susceptible to mechanical stimulation than those in two-dimensional cultures, as minimal flow effects can alter the three-dimensional structure and morphogenesis of the cellular microenvironment [18].

Shear stress regulates multiple mechanotransduction pathways, including calcium ion channels and activation of AKT, MAPK, and FAK [19]. Liu et al. [20] proposed that the effects of fluid shear stress on MSCs depend on its application mode. They induced MSC differentiation using intermittent and continuous fluid shear stress and found that intermittent shear stress resulted in significantly higher osteogenic gene expression and ALP activity than continuous shear stress. They attributed this to intermittent shear stress upregulating ERK1/2 and FAK activity, making it more effective for inducing osteogenic differentiation. Kim et al. [21] demonstrated that fluid shear stress can activate cellular signals for proliferation, differentiation, and migration. Using an osmotic pump with a microfluidic chip, they showed that fluid shear stress activates TAZ and its target genes to induce osteogenic differentiation. These studies collectively indicate that fluid shear stress promotes MSC differentiation.

The TRPV4 channel is a member of the transient receptor potential (TRP) family, a non-selective calcium channel activated by various physical and chemical

stimuli including heat, mechanical forces, and phorbol ester derivatives, participating in normal cell function maintenance. Hu et al. [22] found that fluid shear stress promotes early osteogenic differentiation of MSCs, with increased expression of early osteogenic markers ALP and osterix but no significant changes in late osteogenic marker OCN. When TRPV4 was blocked, osteogenic differentiation was inhibited, suggesting that TRPV4 plays an important role in early osteogenic differentiation of MSCs.

2.2 Effects of Hydrostatic Pressure on MSC Differentiation

Hydrostatic pressure refers to the force exerted perpendicular to contact surfaces within a relatively static fluid due to gravity or other external forces. Hydrostatic pressure can promote better differentiation of MSCs. Studies have found that pressure stimulation enhances chondrogenic differentiation of mesenchymal stem cells. Yi et al. [23] used a self-developed hydraulic loading device to apply continuous and cyclic fluid pressure to cultured bone marrow MSCs, dividing cells into groups receiving continuous pressures of 45 kPa or 90 kPa, and cyclic pressures of 0-45 kPa or 0-90 kPa. Pressure stimulation significantly promoted expression of chondrogenic genes, with 90 kPa continuous pressure showing the most significant chondrogenic effect. Chen et al. [24] applied hydrostatic pressure during TGF- 1 and insulin-like growth factor-1-induced differentiation of MSCs into nucleus pulposus-like cells. Compared to the drug-only group, the hydrostatic pressure group showed better MSC viability and enhanced capacity for proliferation and functional extracellular matrix production in vivo. Immunohistochemical staining revealed positive type II collagen staining with obvious nucleus pulposus-like cell phenotypes, while the drug-only group showed only minimal type II collagen expression, demonstrating that hydrostatic pressure promotes MSC differentiation into nucleus pulposus-like cells. Additionally, hydrostatic pressure promotes neural differentiation of MSCs. Javanmard et al. [25] applied different pressures to MSCs during neural differentiation using a custom hydrostatic pressure model. At 25 mmHg pressure, total neurite length increased significantly compared to the non-pressurized control group, but further pressure increases to 50 mmHg and 100 mmHg did not continue this growth and instead reduced neurite length compared to controls, indicating that low or appropriate hydrostatic pressure is beneficial for neural differentiation.

Hydrostatic pressure affects the MSC growth microenvironment. Ye et al. [26] encapsulated MSCs in alginate beads and applied dynamic or static pressure. Dynamic pressure was more conducive to glycosaminoglycan (GAG) accumulation, a major component of cartilage extracellular matrix that is synthesized de novo and serves as evidence of chondrogenic differentiation [27]. They proposed two reasons: first, the static pressure group showed significantly decreased pH due to increased CO partial pressure, acidifying the culture medium and inhibiting chondrogenic differentiation, while dynamic pressure maintained stable CO partial pressure. Second, the important chondrogenic factor TGF- 1 was continuously activated under dynamic pressure but only transiently activated under

static pressure, suggesting dynamic pressure is more favorable for chondrogenesis. They concluded that mechanical stimulation is more effective for inducing cartilage formation when it maintains microenvironmental homeostasis [26].

In Liu et al.' s study [28] on hydrostatic pressure mechanisms in osteogenic differentiation, they applied static pressure (23 kPa) or dynamic pressure (10-36 kPa at 0.25 Hz frequency) to MSCs and examined osteogenic transcription factor expression and ERK1/2 and p38 MAPK phosphorylation on days 0, 3, and 7. The results indicated that ERK signaling regulated early osteogenic differentiation and played a positive role in mechanotransduction, while p38 MAPK was not involved. Liu et al. [29] reported that hydrostatic pressure effects on Wnt10b and Wnt4 are ERK-dependent. Hydrostatic pressure promoted Wnt10b and Wnt4 expression, but with ERK inhibition, Wnt10b expression decreased while Wnt4 expression increased. They also found that p-ERK peaked at 1-3 days under dynamic pressure, whereas static pressure showed slower effects, further demonstrating that dynamic pressure is more beneficial for early osteogenic differentiation.

These studies demonstrate that hydrostatic pressure promotes MSC differentiation, with dynamic pressure potentially being more significant.

2.3 Effects of Tensile Stress on MSC Differentiation

Tensile stress refers to pulling forces applied along a specific direction, such as muscle contraction generated by cellular traction. In enclosed organs or culture systems, cells inevitably experience tensile forces due to hydrostatic pressure.

In Haasper et al.' s study [30] on MSC osteogenic differentiation, a custom motor-driven device applied tensile stress at 1 Hz frequency with 3% or 8% strain. Mechanical tensile stress enhanced FosB expression, which belongs to the transcription activator protein-1 (AP-1) family that regulates osteoblast differentiation and bone formation. Enhanced FosB expression significantly promotes MSC osteogenic differentiation, leading them to conclude that mechanical tensile stress promotes osteogenic differentiation. Nam et al. [31] found that when applying tensile stress at 1 Hz frequency with 4% strain, cell proliferation rate was highest, while 0.5 Hz showed minimal effect. At 1 Hz frequency with 8% strain, collagen formation and tenocyte gene expression peaked, with no further increase in cell proliferation or tenogenic differentiation at higher tensile forces, suggesting that appropriate mechanical stimulation is necessary for MSC proliferation and differentiation.

Mechanical tensile stress may be equally effective for MSC differentiation into mesodermal and ectodermal lineages. Studies have shown that tensile stress promotes neural differentiation of MSCs [32]. Leong et al. applied uniaxial cyclic tensile loading at different frequencies and amplitudes to MSCs. At 0.5 Hz frequency with 0.5% strain, MSCs showed significant filopodia growth and upregulated neural gene expression, demonstrating that low-amplitude, low-frequency cyclic tensile loading induces neural differentiation through GTPase regulation,

even without neural induction factors.

Wu et al. [33] investigated the effect of tensile stress duration on MSC osteogenic differentiation and its underlying mechanisms. They applied 10% tensile stress for 1-7 days and examined osteoblast gene mRNA levels and core-binding factor 1 (CBF 1) expression. Osteoblast genes and CBF 1 expression increased with tensile stress duration. Knockdown of CBF 1 reduced osteoblast gene expression, and ERK1/2 inhibition also suppressed CBF 1 and osteoblast gene expression, suggesting that prolonged mechanical tensile stress promotes osteogenic differentiation through the ERK1/2-activated CBF 1 signaling pathway.

Some studies suggest that intermittent tensile stress regulates MSC osteogenic differentiation through the p38 MAPK pathway [34]. Xiao et al. found that intermittent tensile stress increased expression of osteogenic markers ALP, COL I, and OCN, along with increased p-p38 MAPK and osterix expression. Blocking the p38 MAPK pathway reduced osteogenic marker expression, indicating that mechanical tensile stress promotes osteogenic differentiation and that the p38 MAPK-osterix pathway plays an important role in controlling bone formation-related gene expression.

These studies demonstrate that mechanical tensile stress promotes MSC differentiation, and its rational application is beneficial for MSC differentiation.

2.4 Effects of Microgravity on MSC Differentiation

Microgravity, also known as zero gravity, is caused by residual atmospheric factors in space rather than Earth' s gravity. A microgravity environment is one where the system' s apparent weight is much less than its actual weight under gravitational effects. Current methods for generating microgravity include drop towers, aircraft, rockets, and spacecraft.

Microgravity also affects MSC differentiation. Chen et al. [35] used a microgravity model to observe its effects on MSC differentiation and found that simulated microgravity inhibited osteogenic differentiation while suppressing the osteogenic regulator TAZ, suggesting that microgravity may inhibit osteogenic differentiation by suppressing TAZ activation. Additionally, Zhang et al. [36] found that even under osteogenic induction conditions, microgravity inhibited osteogenic differentiation while promoting adipogenic differentiation. Analysis of osteogenic signaling pathways revealed decreased expression and activity of RUNX2, BMP2, and SMAD proteins, along with significantly reduced FAK and ERK1/2 activity. They proposed that microgravity inhibits osteogenic differentiation by downregulating RUNX2 expression through dual effects on BMP2/SMAD and integrin/FAK/ERK pathways. They also found that microgravity increased MAPK and AKT activity, which promotes adipogenic differentiation.

Xue et al. [37] discovered that microgravity duration affects MSC differentiation. Short-term microgravity (72 hours) promoted endothelial, neuronal, and

adipogenic differentiation, while prolonged stimulation (10 days) promoted osteogenic differentiation. RhoA activity decreased during short-term microgravity but increased with long-term stimulation, suggesting that sustained microgravity regulates MSC differentiation through the RhoA pathway.

These studies demonstrate that microgravity can regulate MSC differentiation, though its effects on osteogenic differentiation remain inconsistent across literature, and the mechanisms require further investigation.

3 Effects of Mechanical Stimulation on MSC Differentiation Under Three-Dimensional (3D) Culture Conditions

Cells grow in 3D conditions in vivo, so from a physiological perspective, culturing MSCs on 3D scaffolds that more closely mimic the living environment may better facilitate their functional performance. Different modes of mechanical stimulation affect MSC differentiation on 3D scaffolds differently, with distinct mechanisms [Figure 1: see original paper]. Chen et al. [38] used a custom 3D oscillating flow device to apply oscillatory perfusion on scaffolds and studied MSC growth and osteogenic differentiation under 3D oscillatory perfusion. The results showed that mechanical stress improved viable cell distribution on 3D scaffolds, enabling more uniform MSC distribution. Additionally, fluid oscillation provided adequate nutrition and gas exchange while removing metabolic waste and dead cells from scaffold pores, creating a better survival environment for MSCs. Compared to static culture, oscillatory shear stress promoted collagen secretion, mineral deposition, and osteogenic differentiation.

Continuous medium flow benefits MSC proliferation, and appropriate fluid pressure aids osteogenic differentiation. Tang et al. [39] fabricated polyurethane-based polymer scaffolds and used a custom bioreactor for perfusion and fluid pressure stimulation to study MSC survival, proliferation, and osteogenic differentiation. At a perfusion rate of 10 ml/min and fluid pressure of 60 mmHg at 0.5 Hz, MSC viability on scaffolds was maintained. After 2 weeks, ALP activity and calcium deposition increased significantly, with no further improvement observed when fluid pressure was increased to 120 mmHg, indicating that appropriate fluid pressure is more beneficial for MSC differentiation.

Li et al. [27] used fibrous polyurethane scaffolds for 3D culture of MSCs and applied cyclic pressure and shear stress using a custom bioreactor. Within a certain range, increasing load frequency and amplitude increased GAG production and chondrogenic gene expression, demonstrating that chondrogenic differentiation of MSCs in 3D biomaterials is modulated by the frequency and amplitude of cyclic pressure and shear stress. These studies suggest that applying appropriate mechanical loading is necessary in MSC-based cartilage tissue engineering. Compared to cyclic static pressure, intermittent shear stress is more effective for stimulating MSCs. Becquart et al. [40] studied early mechanosensitive genes and found that MSCs exposed to intermittent shear stress showed activation of all sensitive genes, while only partial activation occurred under cyclic static

pressure. Moreover, only intermittent shear stress activated the ERK1/2 pathway, leading them to conclude that intermittent shear stress stimulation is more effective.

Studies have shown that cyclic tensile stress promotes fibroblastic differentiation of MSCs. Qiu et al. [41] attached MSCs to a 3D collagen fiber scaffold and applied cyclic tensile stress at 1 Hz frequency with 10% strain. Expression of transcription factors related to tendon fibroblast phenotype was significantly upregulated under cyclic tensile stress. Compared to static culture, MSCs under cyclic tensile stress secreted more extracellular matrix, including collagen I, collagen III, and tenascin (important markers of tendon/ligament fibroblastic differentiation). The collagen fiber scaffold' s high tensile strength allowed better application of cyclic tension, suggesting that combined use of cyclic tensile stress and collagen fiber scaffolds can better promote fibroblastic differentiation of MSCs.

These findings demonstrate that mechanical stimulation affects MSC growth and differentiation into bone [38], cartilage [26], fibroblasts [31], neurons [32], and other lineages. The multi-lineage differentiation capacity of MSCs gives them tremendous potential in regenerative medicine and tissue engineering. Research on MSC culture conditions, directed differentiation, and differentiation factors has become a hotspot. Identifying appropriate culture conditions is crucial for MSC differentiation and application studies. As mechanical stimulation is one of the important factors affecting MSCs, in-depth research is necessary. A thorough understanding of mechanical stimulation and its mechanisms will have scientific value for revealing fundamental stem cell properties and clinical significance for tissue engineering and regenerative medicine applications.

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Figure 1
Schematic diagram of mechanical stimulation effects on MSC differentiation in 3D scaffolds

ECM: extracellular matrix; FAK: Focal Adhesion Kinase; ERK1/2: extracellular signal-regulated kinase 1/2; Src: proto-oncogene encoding tyrosine protein kinase; Ras: Ras protein; Raf: Raf protein kinase; Talin: talin protein; Actin: actin protein.

Note: Figure translations are in progress. See original paper for figures.