ORAL SCI HOMEOST MED

Open Access
REVIEW

SPECIAL ISSUE: Celebrating the 80th Anniversary of Beijing Stomatological Hospital, Capital Medical University

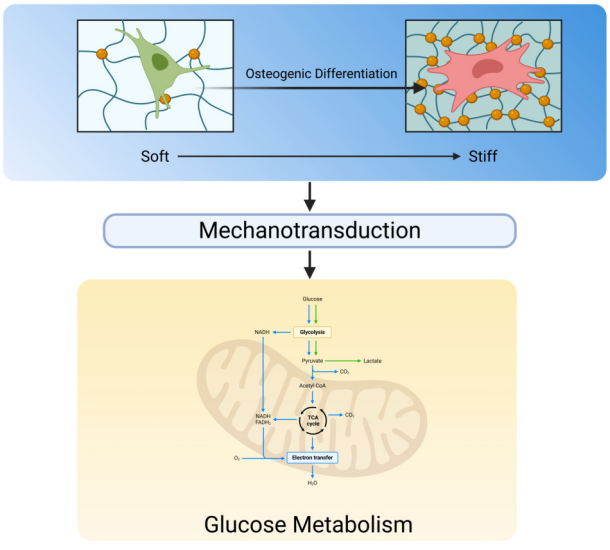
ECM stiffness-mediated regulation of glucose metabolism in MSCs osteogenic differentiation: mechanisms, intersections, and implications

Hao Yan[1], Jian Zhou[2,3,4] ✉, and Tingting Wu[1] ✉

[1] College & Hospital of Stomatology, Anhui Medical University, Key Lab. of Oral Diseases Research of Anhui Province, Hefei, 230032, China
[2] Salivary Gland Disease Center and Beijing Key Laboratory of Tooth Regeneration and Function Reconstruction, Beijing Laboratory of Oral Health and Beijing Stomatological Hospital, Capital Medical University, Beijing, 100071, China
[3] Department of VIP Dental Service, School of Stomatology, Capital Medical University, Beijing, 100050, China
[4] Laboratory for Oral and General Health Integration and Translation, Beijing Tiantan Hospital, Capital Medical University, Beijing, 100070, China

Cite This: *Oral Sci Homeost Med*, **2025**, 1, 9610025 Read Online

ABSTRACT: The mechanical characteristics of the extracellular matrix (ECM) are essential for cellular behaviors, particularly for mesenchymal stem cells (MSCs) that regulate osteogenic differentiation during bone formation. While mechanical cues and metabolic reprogramming have been studied separately, there is insufficient understanding of their interrelationship, representing an important gap in our overall knowledge about stem cell fate and bone tissue engineering. This review highlights recent research regarding the regulation of glucose metabolism by ECM stiffness during osteogenic differentiation, integrating data from mechanotransduction with metabolic pathways. It presents findings from both *in vitro* and *in vivo* studies that start to define additional important mechano-sensitive pathways that convert ECM stiffness into greater glycolysis and enhanced mitochondrial function providing the energy and biosynthetic resources needed for differentiation. In this review, we consolidate ongoing fragmented research into new frameworks that integrate pathways more comprehensively and demonstrate its importance to generating stiffness optimized biomaterials that have regenerative potential.



KEYWORDS: extracellular matrix stiffness, mesenchymal stem cells, glucose metabolism, osteogenic differentiation

1 Introduction

Bone formation represents one of the most energy-intensive biological processes, requiring coordinated mechanical and metabolic signals to direct mesenchymal stem cells (MSCs) differentiation toward the osteogenic lineage[1]. The extracellular matrix (ECM) provides a three-dimensional microenvironment for cells to inhabit. The ECM provides structural support to the cells, as well as an information delivery system through multidimensional signals[2]. Among the various physical properties of ECM, matrix stiffness has been established as an important physical signal to control the behavior of cells[3]. Extensive research has established that substrate stiffness directs lineage commitment in a predictable manner: soft matrices (0.1-1 kPa) promote neurogenic differentiation, intermediate stiffness (8-17 kPa) favors myogenic differentiation, while stiff substrates (25-40 kPa) drive osteogenic differentiation[4]. This stiffness-dependent differentiation reflects the mechanical properties of native tissues, with pre-mineralized bone exhibiting stiffness values around 30 kPa[5].

Cells sense and respond to biochemical signals and physical signals transmitted by the ECM through specific membrane receptors that bind to ligands in the ECM[6]. The mechanotransduction process, whereby cells sense and convert mechanical stimuli into biochemical signals, involves complex molecular machinery[7]. These mechanical signals ultimately converge on transcriptional programs that reshape cellular

Received: May 27, 2025; **Revised:** July 20, 2025
Accepted: July 31, 2025

✉Address correspondence to Jian Zhou, zhoujian@ccmu.edu.cn; Tingting Wu, wutingting_focus@163.com

metabolism to meet the demands of osteogenic differentiation[8].

Understanding glucose metabolism in MSCs is especially meaningful and relevant because it is essential for all aspects of their functions: survival, proliferation, differentiation, and therapeutic actions. Metabolism has significant roles in providing energy and precursors for synthesis, as well as for regulating signaling pathways. Given the latest advances in metabolomics technologies, studies show that differentiation processes clearly involve significant metabolic re-programming[9].

The interplay between mechanical properties and metabolic regulation is crucial not only for fundamental bone biology but also for developing regenerative strategies for complex tissue interfaces, including the periodontal soft-hard tissue interface where similar mechanometabolic principles may apply[10]. Cells must integrate information about their mechanical environment with nutrient availability and energy status to make appropriate differentiation decisions. This integration occurs through multiple signaling pathways that link mechanotransduction to metabolic regulation, creating a sophisticated regulatory network that ensures proper bone formation in response to mechanical demands.

This review provides a comprehensive examination of how ECM stiffness controls glucose metabolism during osteogenic differentiation. We explore the molecular mechanisms of mechanotransduction, examine key signaling pathways that link mechanical signals to metabolic regulation, and discuss the clinical implications of these findings. By integrating recent advances across multiple disciplines, we aim to provide a unified understanding of mechano-metabolic coupling in bone while highlighting future directions for research and therapeutic development.

2 Mechanotransduction pathways linking ECM stiffness to cellular responses

This chapter delves into the complex molecular pathways through which ECM stiffness influences the osteogenic differentiation of MSCs. We explore how cells sense mechanical properties of their environment. Various mechanotransduction pathways are involved in directing MSCs behavior (Fig. 1).

2.1 Integrin-mediated mechanosensing and focal adhesion dynamics

Integrins are cell surface transmembrane receptors that regulate cell-ECM adhesion. They connect ECM with the intracellular cytoskeleton and have a fundamental role in mechanotransduction. Integrins mainly communicate with ECM elements like fibronectin and collagen[11,12]. Integrin α5/β1 emerges as the predominant mechanosensor, with experimental evidence demonstrating a 2.1-fold increase in surface expression on substrates of 62-68 kPa compared to softer 13-16 kPa matrices[12]. This stiffness-dependent upregulation correlates directly with osteogenic commitment, as blocking antibodies against integrin α5 suppress osteogenic markers Collagen Type I Alpha 1 (COL1A1), Runt-Related Transcription Factor 2 (RUNX2), and Bone Gamma-Carboxyglutamate Protein (BGLAP) by 60-70%[12]. The integrin α2 subunit provides complementary mechanosensing, with siRNA knockdown reducing mineralization through disruption of downstream signaling[13].

The integrins cluster then recruit a number of focal adhesion proteins including talin, vinculin, and paxillin, forming larger protein complexes as focal adhesions. Focal adhesions (FAs) transduce ECM stiffness into intracellular signals by dynamically adjusting their size, composition, and lifetime[14]. On soft substrates (1-10 kPa), nascent adhesions containing primarily integrin β1, talin, and kindlin form transient connections. As substrate stiffness increases to osteogenic ranges (25-70 kPa), these adhesions mature into focal adhesion complexes incorporating over 150 proteins including vinculin, paxillin, focal adhesion kinase (FAK), and α-actinin[15]. In vitro experiment demonstrates that pre-osteoblasts forming 40% longer focal adhesions on 150 kPa versus 15 kPa substrates[16]. FAK is recruited to the cytoplasmic tails of integrin β subunits, then autophosphorylation. FAK's autophosphorylation results in binding and activation of Proto-oncogene Tyrosine-Protein Kinase Src (Src), facilitating the formation of the FAK/Src complex, which promotes the activity of many molecules[17].

Force transmission through focal adhesions activates multiple signaling cascades. When MSCs are plated on substrates of osteogenic stiffness (~60 kPa), integrin α5/β1 engagement induces FAK phosphorylation, which in turn promotes PI3K/Akt (Phosphoinositide 3-Kinase/Protein Kinase B) activation under these mechanical conditions. Blocking integrin α5 suppresses diminishes downstream Akt phosphorylation[12]. In addition, stiffness activates MAPK (Mitogen-Activated Protein Kinase) pathways, specifically ERK1/2 (Extracellular Signal-Regulated Kinase 1/2), and JNK (c-Jun N-terminal Kinase), during osteogenic differentiation. Hwang et al. provided evidences showing that human MSCs cultured on 4.47 kPa hydrogels exhibited significantly higher phosphorylated ERK and JNK levels compared to cells on softer 1.37 kPa substrates[18]. Sun et al. demonstrated that MSCs on stiff polyacrylamide hydrogels (62-68 kPa) showed robust ERK phosphorylation compared to soft substrates (13-16 kPa)[12].

2.2 Cytoskeletal force transmission

The cellular cytoskeleton experiences significant reorganization in response to substrate stiffness, with each of its components serving distinct and interconnected roles in mechanotransduction[19]. The cortical actin cytoskeleton develops tension in order to balance external forces imposed by the mechanical environment and internal forces being transmitted via the cytoskeleton. On polyacrylamide (PA) gels with stiffness greater than about 30 kPa, the actin stress fiber density and thickness increased with substrate stiffness, resulting in highly organized bundles at the cell periphery[20]. These stress fibers connect focal adhesion clusters to the nuclear envelope, provide rigorous links to provide continuous transmission of forces through the length of the entire cell[21].

Non-muscle myosin II pulls on the polymerized actin filaments and generates intracellular tension that then transmits through the focal adhesions to the ECM[22]. This acto-myosin contractile force is critical in allowing MSCs to generate the traction needed to “sense” ECM stiffness[23]. studies have demonstrated that myosin-driven contractility is critically needed for osteogenic differentiation. In stiff matrix environments, increased F-actin bundling and stress fiber formation facilitates generation of greater intracellular tension while reduced barriers to contraction arise in softer matrices allowing for cytoskeletal disorganization[24].

The Ras homolog family member A/Rho-associated protein kinase (Rho/ROCK) pathway serves as a central hub connecting mechanosensing to cytoskeletal dynamics[25]. ECM stiffness transmitted through the cytoskeleton activate RhoA GTPase, which stimulates ROCK. The Rho/ROCK signaling pathway enhances

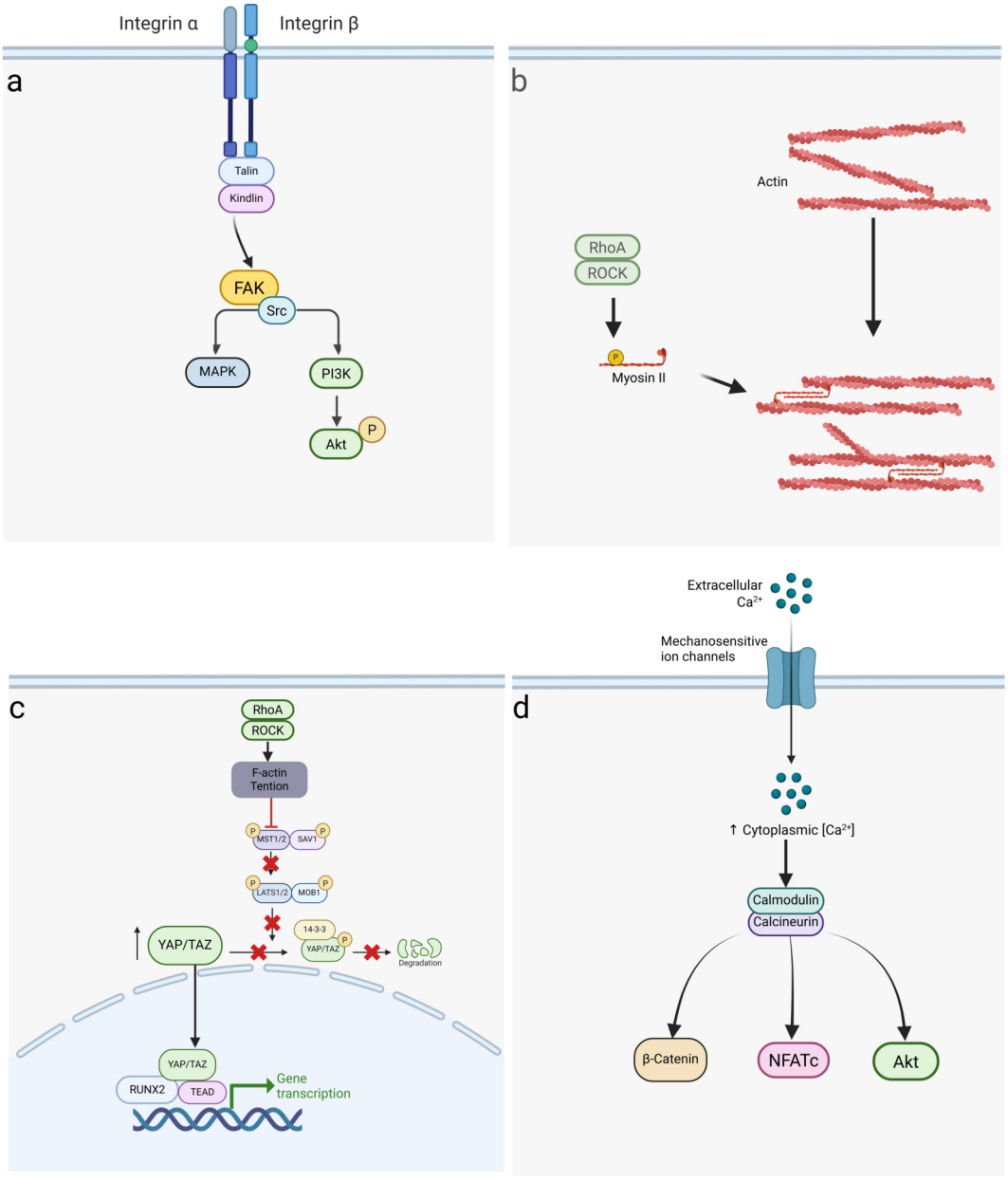


Figure 1 Mechanotransduction pathways linking ECM stiffness to cellular responses in MSCs osteogenicdifferentiation. (a) Integrin-mediated mechanosensing and focal adhesion dynamics: Integrins α and β formheterodimers that bind to ECM ligands and recruit focal adhesion proteins including talin, vinculin, andpaxillin. FAK autophosphorylation leads to Src binding and activation of downstream MAPK and PI3K/Akt signaling pathways. (b) Cytoskeletal force transmission: ECM stiffness regulates actin stress fiber formationand organization. RhoA/ROCK signaling enhances actomyosin contractility, promoting stress fiber assembly and cellular tension generation. (c) YAP/TAZ mechanotransduction: Cytoskeletal tension inhibits Hippopathway kinases (MST1/2, LATS1/2), leading to YAP/TAZ dephosphorylation and nuclear translocation. Nuclear YAP/TAZ complexes with TEAD transcription factors to regulate gene transcription. (d) Mechanosensitive ion channels: ECM stiffness activates mechanosensitive channels such as Piezo1 and TRPV4, leading to calcium influx. Increased cytoplasmic calcium activates downstream signaling pathways including calmodulin/calcineurin, NFAT, and Akt, which coordinate osteogenic differentiation programs.

actomyosin contractility via phosphorylation of the myosin light chains, increasing myosin II activation and enabling new actin stress fiber assembly. This is necessary to establish cytoskeletal tension and maintain cell shape[26]. The RhoA/ROCK pathway increases actomyosin contractility and spread cell morphology while increasing intracellular tension. A flattened and spread morphology of cells, especially osteoprogenitors, has been shown to promote osteogenic differentiation significantly. Wang et al. have shown that RhoA regulated cytoskeletal tension, which is important for directing differentiation behavior[27]. ROCK inhibition with Y-27632 reduces ERK1/2 phosphorylation by 60-70% and blocks osteogenic differentiation, while constitutively active RhoA rescues osteogenic marker expression even on soft substrates[18].

2.3 YAP/TAZ as master mechanotransducers

Although YAP/TAZ (Yes-Associated Protein/Transcriptional Co-Activator with PDZ-Binding Motif) are classical effectors of the Hippo kinase cascade, Studies showed that stiffness-driven nuclear translocation persists even when Large Tumor Suppressor Kinase 1/2 (LATS1/2) activity is unaltered, indicating a Hippo-independent

mechanism tethered to cytoskeletal tension[28]. Increased cytoskeletal tension mediated by ROCK mechanically inhibits the activity of MST1/2 (Mammalian Sterile 20-like Kinase 1/2) and LATS1/2. This leads to decreased phosphorylation of YAP/TAZ. Dephosphorylated YAP is free to translocate. ECM stiffness deforms the nucleus by flattening it and stretching nuclear pores, thus promotes their nuclear translocation and subsequent activation of gene expression[29]. RhoA/ROCK also triggers ERK. ERK decreased LATS1/2 activity, reduced YAP phosphorylation, enhancing YAP/TAZ nuclear localization[30]. Researchers first reported that human MSCs cultured on stiff matrices (40 kPa) exhibit robust nuclear YAP/TAZ localization and upregulation of osteogenic markers, whereas those on soft gels (0.5 kPa) display cytoplasmic retention and neurogenic fate. Genetic knockdown of YAP/TAZ abrogated stiffness-induced differentiation, while expression of constitutively active YAP bypassed physical constraints, demonstrating functional requirement for these co-activators in mechanotransduction.

2.4 Mechanosensitive ion channels orchestrate rapid calcium signaling

Mechanosensitive ion channels provide rapid calcium-mediated responses to substrate stiffness. On bone-mimetic rigid substrates, enhanced focal adhesion assembly and actomyosin contractility amplify membrane tension and local deformation. Actomyosin contractility transmits forces to the plasma membrane, increasing bilayer tension and local curvature, which gates mechanosensitive channels, such as Piezo1 and TRPV4. Piezo1 opening via strain-induced conformational changes to permit Ca^{2+} influx[31]. Piezo1 channels demonstrate maximal currents of 152.3 ± 21.6pA in MC3T3-E1 osteoblasts under mechanical stimulation, with knockout cells showing significantly reduced responses[32]. In addition, TRPV4 senses matrix viscoelasticity in three-dimensional microenvironments and engages in a reciprocal feedback loop with cell volume expansion[33].

Mechanosensitive ion channels elicit ion calcium flux that is decoded downstream by calcium sensors, such as calmodulin and calcineurin, which initiates downstream signaling pathways, such as nuclear factor of activated T cells (NFAT), MAPK, and Akt pathways[34,35]. In one example, calcium/calcineurin-NFAT signaling pathway enhances Wnt/β-catenin signaling partially by providing a mechanism by retaining β-catenin in the nucleus. calcium influx into the cytosol has also been shown to have a coupling with PI3K/Akt and MAPK to create a feed-forward loop to support stabilization of RUNX2. Furthermore, the function of calcineurin has been shown to support RhoA/ROCK signaling to control cytoskeletal contractility to subsequently support later activation of mechanosensitive calcium channels and maturation of focal adhesions[31].

3 Glucose metabolism in osteogenic differentiation

The transition from a stem/progenitor state to a differentiated lineage often necessitates significant metabolic reprogramming, shifts in the utilization and flux through key metabolic pathways[36]. This metabolic transformation is orchestrated by an intricate network of transcriptional regulators, signaling pathways, and metabolite driven mechanisms that coordinate energy production with biosynthetic demands for bone matrix formation.

3.1 Glycolysis and oxidative phosphorylation

The metabolic reprogramming of MSCs during osteogenic differentiation represents one of the most dynamic and controversial areas in stem cell biology. Undifferentiated MSCs are usually found in a hypoxic environment. Nevertheless, they mainly rely on glycolysis for energy, even in aerobic environment, to support their demand for fast proliferation and self-renewal. Additionally, undifferentiated MSCs exhibit lower mitochondrial respiratory activity, producing relatively low amounts of Reactive Oxygen Species (ROS) to protect cells from oxidative harm[36].

As cells commit to the osteogenic lineage, they exhibit a biphasic metabolic pattern that reflects the changing energy demands of differentiation. Upon osteogenic induction, cells progressively enhance mitochondrial biogenesis and Oxidative Phosphorylation (OXPHOS) activity[37]. During this process, energy metabolism shifts from glycolysis to an increased dependence on mitochondrial respiration[38]. In the early commitment and proliferation phases days 1-7, cells maintain moderate glycolysis metabolic activity while dramatically enhance their mitochondrial capacity[39]. The first critical metabolic switch occurs between days 7-9, marked by initial Hexokinase 2 (HK2) and Pyruvate Kinase M2 (PKM2) downregulation. Cells then transition to a phase where they preferentially use aerobic glycolysis despite maintaining their enhanced mitochondrial capacity[39]. Days 14-16 represent the second major transition point, where glycolytic enzyme expression reaches its nadir while oxidative phosphorylation achieves peak capacity. By days 21-28, the mature metabolic phenotype stabilizes with sustained low glycolytic enzyme levels but preserved glycolytic capacity for emergency energy needs[39]. Oxygen consumption rates increase significantly, with differentiated osteoblasts showing 2-fold higher mitochondrial ATP synthase flux compared to undifferentiated MSCs[40]. This metabolic shift is crucially important to help accommodate the increased energy demands associated with matrix mineralization and bone matrix protein synthesis by the osteoblasts[41,42]. The use of acute mitochondrial complex inhibitors such as rotenone, have been shown to inhibit osteoblast differentiation[43]. As MSCs commit to osteoblast differentiation, mitochondrial fusion is also important for retaining metabolic bioenergetic capacity[44].

3.2 Glucose transporter (GLUT) dynamics

The increased glucose demands of differentiating osteoblasts require coordinated upregulation of glucose transporter systems[45]. GLUT1 serves as the primary transporter in early proliferating precursors, with expression levels critical for differentiation capacity[36]. GLUT1, abundant in undifferentiated MSCs, undergoes rapid downregulation during osteoblast differentiation[46]. GLUT1 expression decreases by up to 80% within 12 days of differentiation, establishing a critical metabolic checkpoint[47]. This downregulation is mediated by tumor suppressor p53, which directly binds to the SLC2A1 (GLUT1) gene promoter[48]. GLUT3 maintains stable expression throughout differentiation, providing high-affinity glucose transport capacity. Its high-affinity transport capacity ensures basal glucose uptake even in low-glucose conditions. GLUT4, traditionally associated with insulin-responsive tissues, shows remarkable upregulation during osteoblast differentiation, with expression increasing up to 5-fold[46].

3.3 The pentose phosphate pathway (PPP)

The PPP, divided into oxidative and non-oxidative branches, serves

dual functions in osteogenic differentiation. The oxidative branch produces Nicotinamide Adenine Dinucleotide Phosphate (NADPH), maintaining cellular redox balance and protecting osteoblasts from oxidative stress damage; the non-oxidative branch produces ribose-5-phosphate, providing key precursors for nucleic acid synthesis[49]. NADPH production through the PPP is essential for maintaining cellular redox balance, especially during the high metabolic demand[50]. Notably, the relationship between PPP and osteogenic differentiation warrants further investigation. Some studies suggest that proliferating MSCs exhibit elevated PPP activity to generate NADPH and ribose‐5‐phosphate for nucleotide synthesis and reductive biosynthesis[50]. Stable isotope tracing with 13C-labeled glucose in bone marrow stromal cells (BMSCs) and osteoblasts revealed high labeling in PPP intermediates but low in late glycolysis or TCA cycle metabolites. This indicates glucose is mainly routed to PPP for biosynthesis, while glutamine supports mitochondrial metabolism. This pattern persists during 15 days of osteogenic differentiation, highlighting PPP's role in sustaining proliferation and matrix production[49]. Treatment of MSCs with inhibiting the key PPP enzyme G6PD (glucose-6-phosphate dehydrogenase) during osteogenic induction reduced ALP staining and activity by >50% and lowered intracellular NADPH levels, directly impairing early osteogenic markers[49].

3.4 Regulatory networks orchestrate glucose metabolism

3.4.1 mTOR

The mammalian target of rapamycin (mTOR) signaling pathway responds to multiple growth-factor, nutrient and energy signals, integrating those signals to adapt cellular metabolism to distinct stages of cellular commitment. Through the mTORC1 (Mammalian Target of Rapamycin Complex 1) and mTORC2 (Mammalian Target of Rapamycin Complex 2) complexes it regulates osteoblast proliferation, osteoblast differentiation, and osteoblast matrix production, albeit with distinct context with respect to osteoblast lineage commitment stages[51].

During the early stage of osteoblast differentiation, mTORC1 activates and subsequently phosphorylates HIF-1α (Hypoxia-Inducible Factor 1 Alpha) factors to initiate aerobic glycolysis. Alongside the stimulation of the enzymatic process, mTORC1 drives the glycolytic flux to one pathway to synthesize NADPH to support redox balance, and ribose-5 phosphate to support nucleotide biosynthesis through the PPP pathway[52]. As osteoblast differentiation progresses, mTORC1 impacts expression of PGC-1α (Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1 Alpha), promoting translation of factors needed for mitochondrial biogenesis and electron-train complexes. Increased mitochondria and assembly of the electron transport chain complexes promotes ATP synthesis via aerobic processes advancing efficient energy for supporting collagen cross linking and matrix mineralization[53].

In contrast, mTORC2 is thought to be an energy metabolism fine-tuner likely integrating pathways involving AKT that control factors associated with aerobic glycolysis, and throughout osteoblast maturation, mTORC2 will promote the transition from aerobic glycolysis to OXPHOS through by impacting PGC-1α and the pathway to mitochondrial biogenesis[54]. The genetic deletion of mTORC2 components impairs osteoblast differentiation while also promoting adipogenesis[55]. This highlights its essential role in osteogenic lineage specification.

3.4.2 HIF1-α

HIF1-α is another transcription factor that regulates glycolysis. HIF1α upregulates glycolytic enzymes pyruvate dehydrogenase kinase 1 (PDK1), which inactivates Pyruvate Dehydrogenase (PDH) and thereby blocks the use of pyruvate into acetyl-CoA for entry into the tricarboxylic acid (TCA) cycle. Additionally, lactate has been documented to stabilize HIF1-α, thereby inducing osteogenic differentiation[56]. Once stabilized, HIF1-α transcriptionally induces glycolytic enzymes Lactate Dehydrogenase A (LDHA) and the monocarboxylate transporter (MCT4) thereby increasing both glycolytic flux and lactate export. This feed forward loop maintain a sustained high lactate state while reinforcing osteogenic gene programs in differentiating osteoblasts[57]. Notably, HK2 also plays a role in regulating HIF1-α stability and thereby induces glycolysis related gene expression to supportive feed forward loop to promoting the state of osteogenic differentiation[58].

3.4.3 RUNX2

RUNX2 is best known as the “master switch” of osteoblast gene expression, but emerging evidence shows it also directly reprograms metabolism, during differentiation. Upon osteogenic induction, RUNX2 directly upregulates the glucose transporter GLUT1 and rate-limiting glycolytic enzymes HK2, boosting glucose uptake and glycolytic flux in osteoprogenitors[36]. High levels of intracellular glucose stabilize Runx2 protein by inhibiting AMP-activated protein kinase (AMPK). AMPK is activated during energy deficiency and promotes proteasomal degradation of RUNX2; high glucose levels inhibit this process, preventing RUNX2 degradation and allowing it to accumulate[59]. RUNX2 also cooperates with PGC-1α by upregulating factors NRF1(Nuclear Respiratory Factor 1) and TFAM(Transcription Factor A, Mitochondrial) that promote mitochondrial DNA replication and electron transport chain assembly, increasing mitochondrial mass and activity shift ATP production from glycolysis to OXPHOS[60].

3.4.4 AMPK

AMPK is a crucial energy sensor that maintains cellular energy homeostasis. Activation of AMPK occurs when ATP levels drop, then activate catabolic pathways that generate ATP and inhibiting energy-consuming processes[61]. During differentiation, AMPK is activated by a low intracellular energy status. AMPK promotes glucose uptake by enhancing the GLUT4 to the plasma membrane[62], AMPK can also directly promote glycolytic flux through phosphorylating and activating 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFKFB), increasing expression of hexokinase II and modulating pyruvate kinase M2 (PKM2) activity[63]. Osteogenic differentiation of mesenchymal stem cells was associated with early (day 1) activation of AMPK and its target Raptor, coinciding with the inhibition of mTOR and its substrate p70S6 kinase. This was followed by the late activation of Akt/mTOR at days 3-7 of differentiation[64].

4 Molecular mechanisms of ECM stiffness-mediated glucose metabolic regulation

Extensive research demonstrates that metabolic reprogramming is not merely a concurrent phenomenon of osteogenic differentiation but a necessary driver of the differentiation process. This perspective positions cellular metabolism not as a passive response

to differentiation cues but as an active determinant of cell fate decisions in the osteoblast lineage. The stiffness of the ECM profoundly impacts glycolytic activity during osteogenic differentiation through multiple related mechanotransduction Mechanisms (Fig. 2).

4.1 Cytoskeletal tension links ECM stiffness to metabolism

The finding that mechanical forces directly regulate cellular metabolism has uncovered a complex mechanism of mechanometabolic coupling[36]. Cytoskeletal tension directly modulates glycolytic enzyme activity through remarkable spatial organization[65]. On stiff substrates, stress fiber formation sequesters the E3 ubiquitin ligase TRIM21 (Tripartite Motif Containing 21), preventing it from targeting phosphofructokinase (PFK) for degradation. Conversely, soft substrates trigger stress fiber disassembly, releasing TRIM21 and reducing glycolytic capacity through PFK degradation[66]. ECM stiffness also controls the subcellular localization of metabolic machinery. Stiff substrates promote perinuclear clustering of glycolytic enzymes, creating metabolic microdomains that efficiently channel ATP to energy-demanding processes[67]. This spatial organization optimizes metabolic efficiency during the energy-intensive process of matrix synthesis. The actin cytoskeleton serves as a mechanical rheostat

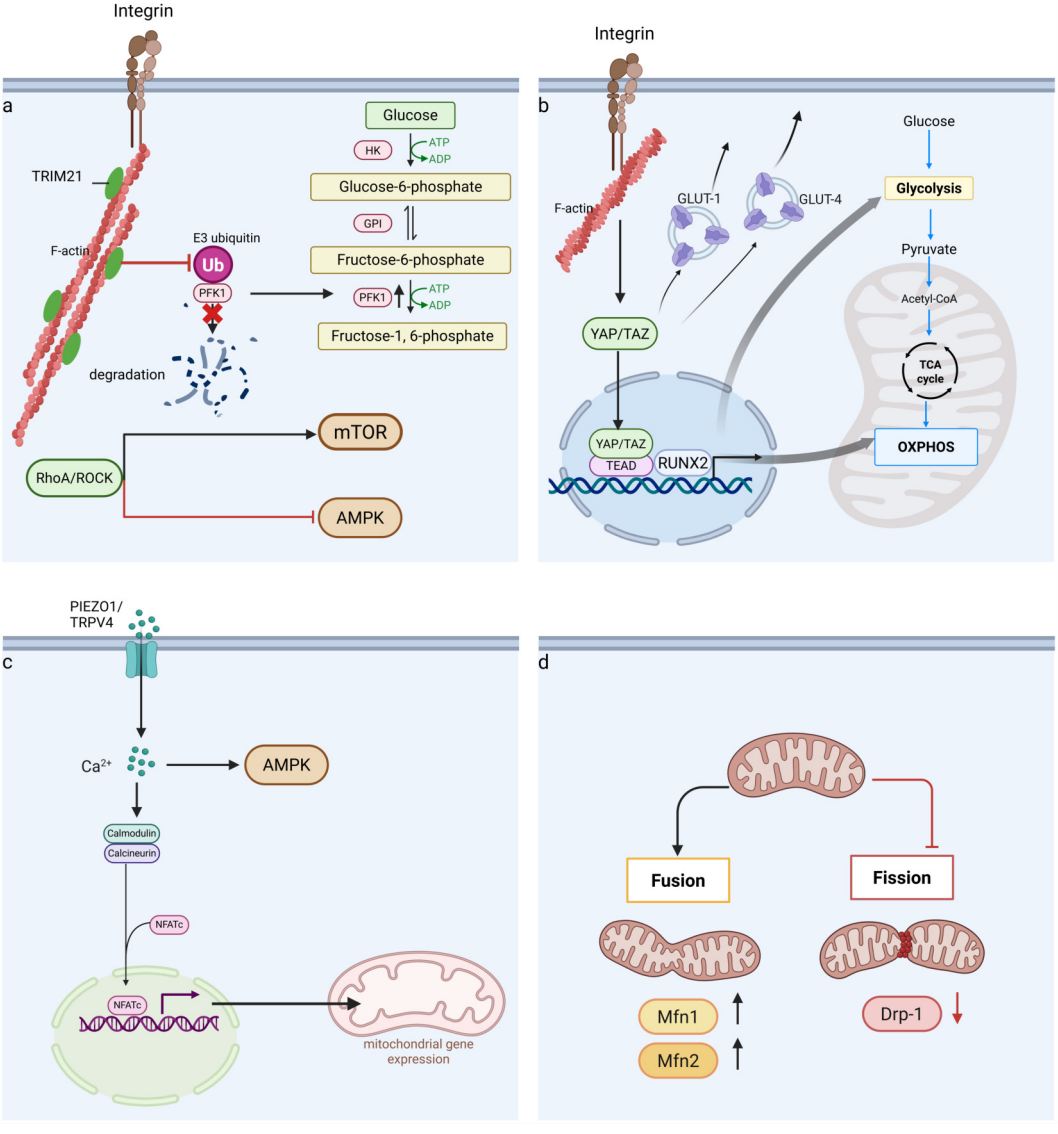


Figure 2 Molecular mechanisms of ECM stiffness-mediated glucose metabolic regulation during osteogenicdifferentiation. (a) Cytoskeletal tension-mediated metabolic regulation: ECM stiffness promotes stress fiberformation, which sequesters E3 ubiquitin ligase TRIM21, preventing phosphofructokinase (PFK) degradationand maintaining glycolytic capacity. Stress fibers also facilitate perinuclear clustering of glycolytic enzymes, creating metabolic microdomains. RhoA/ROCK signaling activates mTOR and inhibits AMPK, promoting glycolytic flux. (b) YAP/TAZ-mediated metabolic reprogramming: Nuclear YAP/TAZ complexes with TEADtranscription factors to directly upregulate glycolytic enzymes and glucose transporters (GLUT1, GLUT4). YAP/TAZ also promotes mitochondrial biogenesis through PGC-1α activation, facilitating the metabolic transition from glycolysis to oxidative phosphorylation (OXPHOS). (c) Calcium signaling pathway: Mechanosensitive ion channels (Piezo1/TRPV4) mediate calcium influx, which activates AMPK through calcium/calmodulin-dependent protein kinase (CaMKK). Calcium signaling also activates NFAT, which regulates both nuclear and mitochondrial gene expression to fine-tune the balance between glycolysis andoxidative phosphorylation. (d) Mitochondrial dynamics: ECM stiffness promotes mitochondrial fusion through increased mitofusin1/2 (Mfn1/Mfn2) expression and decreased DRP1 activity, leading to elongated, interconnected mitochondrial networks that enhance respiratory efficiency and ATP production during osteogenic differentiation.

controlling metabolic enzyme activity. F-actin polymerization state correlates directly with glycolytic flux capacity, while depolymerization reduces metabolic activity[68]. The mechanosensitive metabolic regulation involves both direct enzyme-cytoskeleton interactions and indirect effects through mechanotransduction signaling. The dynamic nature of cytoskeletal remodeling enables rapid metabolic adaptation to changing mechanical environments.

Cytoskeletal tension induced RhoA/ROCK signaling pathway activate numerous mechanistic alterations in glycolysis. RhoA/ROCK signaling can activate mTORC1, which inhibits prolyl hydroxylases and stabilizes HIF-1α under normoxic conditions[69]. In addition to the latter physiological anticipations, there exists cross-talk between the RhoA/ROCK signaling pathway and AMPK pathways. On stiff substrates, RhoA/ROCK signaling inhibits AMPK activity and relieves AMPK-mediated constraint on glycolysis; thus decreasing PFK phosphorylation and degradation thereby enabling higher glycolytic flux[70]. A study has shown that the inhibition of ROCK reduces cytoskeletal tension, thus leading to AMPK activation[71].

4.2 YAP/TAZ-mediated metabolic reprogramming

The ECM stiffness modulates the transcription of glycolytic pathway components via the activation of YAP/TAZ in cells, thereby propelling the cells to a higher ATP state[72,73]. YAP/TAZ binds to TEAD (TEA Domain Transcription Factor) transcription factors to assemble a complex that assists with the direct regulation of expression for metabolic genes. The complex facilitates elevated levels of expression for the glycolytic metabolic enzymes HK2, PFK1 and LDHA[74]. YAP/TAZ also enhances GLUT1 and GLUT4 translocation to the plasma membrane[75]. The complex also co-activates RUNX2 indirectly as a key regulatory prompt to surge glycolysis. Beyond glycolysis, the YAP/TAZ signaling pathway regulatory influences regarding mitochondrial biogenesis via activated transcription of PGC-1α[65].

Recently, the concept of mechanical memory represents a paradigm shift in understanding cellular mechanotransduction. MSCs retain information from past mechanical environments through YAP/TAZ nuclear retention and stable epigenetic modifications[76]. Assay for Transposase-Accessible Chromatin using Sequencing (ATAC-seq) studies reveal ~2,000 mechanosensitive enhancers with differential activity on soft versus stiff substrates. These "mechanoenhancers" show enrichment for TEAD and Activator Protein 1 (AP-1) binding motifs, connecting YAP/TAZ and stress-activated pathways to chromatin accessibility Stiff matrices induce extensive chromatin remodeling characterized by increased H3K27ac (Histone H3 Lysine 27 Acetylation) at osteogenic enhancers and H3K4me3 (Histone H3 Lysine 4 Trimethylation) at promoters[77]. In another study, mechanical priming for >3 days creates irreversible changes, with memory persisting 3-10 days after stimulus removal. The memory involves H3K9me3 remodeling, DNA hypomethylation at osteogenic promoters, and 3D chromatin architectural changes including TAD (Topologically Associating Domain) reorganization on stiff matrices[78].

4.3 Calcium signaling pathway

Multiple studies showed that the activity of calcium pathways was important in maintaining high glucose metabolism especially during osteogenic differentiation. The stiffness of the three-dimensional (3D) matrix modulates the energy metabolism that is associated with osteogenic differentiation via the mechanosensitive ion channel Piezo1 and the activation of the AMPK signaling axis. AMPK can also activate by upstream kinases, predominantly liver kinase B1 (LKB1) and calcium/calmodulin-dependent protein kinase (CaMKK), as CaMKK-mediated activation is more pronounced during acute changes in cellular energy states, such as those induced by calcium signaling. Specifically, a higher matrix stiffness significantly increases expression of Piezo1 and intracellular calcium levels, which in turn activates the AMPK-ULK1 signaling axis regulating autophagy and increase ATP utilization, thus promoting differentiation of osteoblasts toward an osteogenic lineage[79]. Extracellular calcium influx through Piezo calcium channels activates NFAT that regulates genes in the nucleus and binds to the D-loop (Displacement Loop) of mitochondrial DNA to regulate nuclear and mitochondrial gene expression that fine-tune the balance between oxidative phosphorylation and glycolysis[80].

4.4 Mitochondrial dynamics

Stiffness greatly influences mitochondrial morphology and dynamics, particularly fission and fusion. Stiffness increases mitochondrial fusion by increasing mitofusin1/2 expression. Cells show enhanced mitochondrial biogenesis with increased mass and number and coordinated increases in antioxidant enzymes to manage elevated reactive oxygen species production[81]. Decreasing DRP1 (Dynamin-Related Protein 1) activity leading to elongated, interconnected networks that maintain maximal respiratory efficacy[82]. Oxygen consumption rate (OCR), ATP production, and expression of the respiratory chain complexes increase significantly on stiff matrices during mid to late stages of osteogenic differentiation. Low stiffness leads to short, round mitochondria with an imbalance of fusion/fission leading to decrease in OXPHOS efficiency[83]. Bioenergetic analysis reveals that stiff substrates promote 2-4 fold increases in maximal respiratory capacity, improved mitochondrial coupling efficiency, enhanced spare respiratory capacity providing stress resilience, and optimized proton leak for efficient ATP synthesis[84].

4.5 PI3K/Akt pathway

Gene chip analyses revealed the pathway was highly correlated with ECM stiffness, and AKT phosphorylation was evidently affected in 5th-7th days after ECM stiffness stimulation in human bone marrow mesenchymal stem cells[85]. hMSCs on stiff ECM (62-68 kPa) showed upregulated integrin α5, FAK, p-ERK, p-Akt, and β-catenin, with enhanced osteogenic markers[12]. In another hand, the PI3K/Akt pathway is well-established as a regulator of glucose metabolism during osteogenic differentiation[86,87]. However, the direct mechanistic link showing ECM stiffness specifically regulates glucose metabolism via the PI3K/Akt pathway is not definitively established in the current literature. Causal relationship requires further investigation.

5 Clinical implications transform bone regeneration strategies

5.1 Biomaterial design principles leverage mechanometabolic coupling

Understanding ECM stiffness-mediated metabolic regulation has

revolutionized biomaterial design for bone tissue engineering. The established optimal stiffness range of 25-40 kPa guides material selection and processing to achieve mechanical properties that maximize osteogenic differentiation while supporting cellular metabolism[88]. Modern scaffolds incorporate this knowledge through multiple strategies: using calcium phosphate ceramics like β-tricalcium phosphate with 70±5% porosity to balance mechanical support with nutrient transport[89]. Tunable hydrogels demonstrate that bone-mimicking stiffness combined with adhesive ligands optimizes metabolic energy allocation for osteogenic differentiation, as measured by increased calcium deposition and protein expression[90]. Material chemistry must support appropriate mechanotransduction while permitting metabolic adaptation. For instance, silicon-enhanced biomaterials have been shown to facilitate functional mitochondrial transfer, significantly improving vascularization (up to 1.5-fold) and innervation in bone defects, while optimizing metabolic responses to ECM stiffness[91]. Hydrogels based on polyethylene glycol, alginate, or hyaluronic acid can be tuned to desired stiffness while incorporating Arginine-Glycine-Aspartic Acid (RGD) peptides for integrin engagement[92]. Emerging personalized strategies include patient-specific scaffolds created through 3D printing to match individual anatomy and mechanical requirements, biomarker-guided therapy using mechanotransduction markers to optimize treatment selection, and adaptive systems with materials responding to patient-specific mechanical environments[93]. Future developments point toward smart biomaterials that sense local mechanical and metabolic conditions to adjust properties accordingly, integration with wearable devices for real-time monitoring of bone mechanical loading and metabolic responses.

5.2 Clinical implications

Dysregulation of ECM stiffness is linked to pathological conditions. Study noted that ECM stiffness dysregulation contributes to bone-related disorders, suggesting that understanding stiffness could help in diagnosing and treating such conditions. In the aging process and glucocorticoid-induced osteoporosis, bone loss is caused not only by unbalanced bone resorption but also by impairment of MSCs' commitment towards the osteogenic lineage[94]. Age-related changes decrease mechanosensitivity in elderly patients, requiring adjusted therapeutic strategies. Disease states like osteoporosis and diabetes alter baseline bone metabolism and mechanical responses. For example, hyperglycemia associated with diabetes produces advanced glycation end-products (AGEs) in the ECM, which will alter mechanical properties, interfering with the nature of normal mechanosensing. This alteration of mechanical properties is an interaction that is both mechanically and metabolically mediated[95]. Understanding ECM stiffness-metabolism interactions offers therapeutic targets for enhancing osteogenic commitment and metabolic interventions to improve bone quality.

6 Conclusion

This comprehensive review illustrates the complex relationship between ECM stiffness and glucose metabolism in the process of mesenchymal stem cells osteogenic differentiation. Our review finds that the two regulatory mechanisms function as a complete system rather than parallel processes. The interaction of mechanotransduction pathways and metabolic regulation presents a major advancement in unpacking the complexity of bone formation mechanisms.

Cytoskeletal tension, YAP/TAZ, and calcium signaling pathways represent important overlapping molecular intersections that not only respond to matrix mechanics but also orchestrate metabolic reprogramming. Evidence indicates that coordination between mechanical sensing and metabolic shifts is essential for proper osteogenic progression. This interaction is suggested to be designed to be self-regulating by design, where the ECM stiffness contributes to cell metabolism, which then contributes to the cells capacity to generate and remodel the ECM.

The value of this integrated perspective is not only theoretical. It also gives mechanistic insight into how metabolic disorders like diabetes, which are able to degrade bone quality. It gives a biomimetic rationale for developing next-generation bone tissue engineering scaffolds that capture the mechanical and metabolic signals necessary for osteogenesis.

While these insights are well-founded, there are limitations that must also be recognized. A large number of the currently available evidence was derived from *in vitro* examples using artificial substrates that cannot fully simulate the complex, dynamic microenvironment in which native bone develops. In addition to this some technological limitations have still meant that greatly significant aspects of studying the differentiation process; including mechanical forces, metabolic fluxes, and matrix remodeling cannot be considered simultaneously which has been a limitation on studying differentiation *in vivo*.

Acknowledgements

This study was supported by National Natural Science Foundation of China 25 (81600845), Anhui Province Scientific Research Preparation Program Project 26 (2022AH050734), and "Feng Yuan" Program (2022xkfyhz03, 2023xkfytszd02). The schemes were created with BioRender.

Data available statement

N/A

Author contribution

H.Y. wrote the manuscript, J.Z. and T.W. revised the manuscript. The authors read and approved the final manuscript.

Ethics approval and consent

N/A

Consent for publication

All authors agree to publish.

Conflicts of interest

The authors declare that they have no competing interests.

References

[1] Greenhill, C. Role of bone in glucose metabolism. *Nat. Rev. Endocrinol.* **2018**, *14*, 191.

[2] Karamanos, N. K.; Theocharis, A. D.; Piperigkou, Z.; et al. A guide to the composition and functions of the extracellular matrix. *Febs J.* **2021**, *288*, 6850–6912.

[3] Zhang, Y. Y.; Xing, Y. H.; Li, J.; et al. Osteogenesis-related behavior of MC3T3-E1 cells on substrates with tunable stiffness. *Biomed Res. Int.* **2018**, *2018*, 4025083.

[4] Engler, A. J.; Sen, S.; Sweeney, H. L.; et al. Matrix elasticity directs stem cell lineage specification. *Cell* **2006**, *126*, 677–689.

[5] Guimarães, C. F.; Gasperini, L.; Marques, A. P.; et al. The stiffness of living tissues and its implications for tissue engineering. *Nat. Rev. Mater.* **2020**, *5*, 351–370.

[6] Seetharaman, S.; Etienne-Manneville, S. Integrin diversity brings specificity in mechanotransduction. *Biol. Cell* **2018**, *110*, 49–64.

[7] Stewart, S.; Darwood, A.; Masouros, S.; et al. Mechanotransduction in osteogenesis. *Bone Joint Res.* **2020**, *9*, 1–14.

[8] Romani, P.; Valcarcel-Jimenez, L.; Frezza, C.; et al. Crosstalk between mechanotransduction and metabolism. *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 22–38.

[9] Li, H. Y.; Dai, H. W.; Li, J. Immunomodulatory properties of mesenchymal stromal/stem cells: The link with metabolism. *J. Adv. Res.* **2023**, *45*, 15–29.

[10] Dang, G. P.; Wei, Y.; Wan, Q. Q.; et al. Regulatory mechanisms and regeneration strategies of the soft–hard tissue interface in the human periodontium. *BMEMat* **2024**, *2*, e12069.

[11] Guo, R.; Lu, S.; Merkel, A. R.; et al. Substrate modulus regulates osteogenic differentiation of rat mesenchymal stem cells through integrin β1 and BMP receptor type IA. *J. Mater. Chem. B* **2016**, *4*, 3584–3593.

[12] Sun, M. Y.; Chi, G. F.; Xu, J. J.; et al. Extracellular matrix stiffness controls osteogenic differentiation of mesenchymal stem cells mediated by integrin α5. *Stem Cell Res. Ther.* **2018**, *9*, 52.

[13] Shih, Y. V.; Tseng, K. F.; Lai, H. Y.; et al. Matrix stiffness regulation of integrin-mediated mechanotransduction during osteogenic differentiation of human mesenchymal stem cells. *J. Bone Miner. Res.* **2011**, *26*, 730–738.

[14] Atherton, P.; Stutchbury, B.; Jethwa, D.; et al. Mechanosensitive components of integrin adhesions: Role of vinculin. *Exp. Cell Res.* **2016**, *343*, 21–27.

[15] Stutchbury, B.; Atherton, P.; Tsang, R.; et al. Distinct focal adhesion protein modules control different aspects of mechanotransduction. *J. Cell Sci.* **2017**, *130*, 1612–1624.

[16] Gao, S. H.; Chen, B.; Gao, M.; et al. Substrate stiffness of bone microenvironment controls functions of pre-osteoblasts and fibroblasts *in vitro*. *Biomimetics (Basel)* **2023**, *8*, 344.

[17] Tan, X. M.; Yan, Y. H.; Song, B.; et al. Focal adhesion kinase: From biological functions to therapeutic strategies. *Exp. Hematol. Oncol.* **2023**, *12*, 83.

[18] Hwang, J. H.; Byun, M. R.; Kim, A. R.; et al. Extracellular matrix stiffness regulates osteogenic differentiation through MAPK activation. *PLoS One* **2015**, *10*, e0135519.

[19] Khan, A. U.; Qu, R. M.; Fan, T. Y.; et al. A glance on the role of actin in osteogenic and adipogenic differentiation of mesenchymal stem cells. *Stem Cell Res. Ther.* **2020**, *11*, 283.

[20] Katsuta, H.; Okuda, S.; Nagayama, K.; et al. Actin crosslinking by α-actinin averts viscous dissipation of myosin force transmission in stress fibers. *iScience* **2023**, *26*, 106090.

[21] Chen, J. C.; Jacobs, C. R. Mechanically induced osteogenic lineage commitment of stem cells. *Stem Cell Res. Ther.* **2013**, *4*, 107.

[22] Clark, K.; Langeslag, M.; Figdor, C. G.; et al. Myosin II and mechanotransduction: A balancing act. *Trends Cell Biol.* **2007**, *17*, 178–186.

[23] Asparuhova, M. B.; Gelman, L.; Chiquet, M. Role of the actin cytoskeleton in tuning cellular responses to external mechanical stress. *Scand. J. Med. Sci. Sports* **2009**, *19*, 490–499.

[24] Bernal, R.; Van Hemelryck, M.; Gurchenkov, B.; et al. Actin stress fibers response and adaptation under stretch. *Int. J. Mol. Sci.* **2022**, *23*, 5095.

[25] Shi, H. X.; Zhou, K. X.; Wang, M. F.; et al. Integrating physicomechanical and biological strategies for BTE: Biomaterials-induced osteogenic differentiation of MSCs. *Theranostics* **2023**, *13*, 3245–3275.

[26] Guan, G. Z.; Cannon, R. D.; Coates, D. E.; et al. Effect of the rho-kinase/ROCK signaling pathway on cytoskeleton components. *Genes* **2023**, *14*, 272.

[27] Wang, Y. K.; Yu, X.; Cohen, D. M.; et al. Bone morphogenetic protein-2-induced signaling and osteogenesis is regulated by cell shape, RhoA/ROCK, and cytoskeletal tension. *Stem Cells Dev.* **2012**, *21*, 1176–1186.

[28] Dupont, S.; Morsut, L.; Aragona, M.; et al. Role of YAP/TAZ in mechanotransduction. *Nature* **2011**, *474*, 179–183.

[29] Cai, X. M.; Wang, K. C.; Meng, Z. P. Mechanoregulation of YAP and TAZ in cellular homeostasis and disease progression. *Front. Cell Dev. Biol.* **2021**, *9*, 673599.

[30] Zinatizadeh, M. R.; Miri, S. R.; Zarandi, P. K.; et al. The hippo tumor suppressor pathway (YAP/TAZ/TEAD/MST/LATS) and EGFR-RAS-RAF-MEK in cancer metastasis. *Genes Dis.* **2021**, *8*, 48–60.

[31] Zhu, S. Y.; Chen, W.; Masson, A.; et al. Cell signaling and transcriptional regulation of osteoblast lineage commitment, differentiation, bone formation, and homeostasis. *Cell Discov.* **2024**, *10*, 71.

[32] Sun, W. J.; Chi, S. P.; Li, Y. H.; et al. The mechanosensitive Piezo1 channel is required for bone formation. *eLife* **2019**, *8*, e47454.

[33] Lee, H. P.; Stowers, R.; Chaudhuri, O. Volume expansion and TRPV4 activation regulate stem cell fate in three-dimensional microenvironments. *Nat. Commun.* **2019**, *10*, 529.

[34] You, J.; Reilly, G. C.; Zhen, X.; et al. Osteopontin gene regulation by oscillatory fluid flow *via* intracellular calcium mobilization and activation of mitogen-activated protein kinase in MC3T3-E1 osteoblasts. *J. Biol. Chem.* **2001**, *276*, 13365–13371.

[35] Du, Y. G.; Xu, B. W.; Li, Q.; et al. The role of mechanically sensitive ion channel Piezo1 in bone remodeling. *Front. Bioeng. Biotechnol.* **2024**, *12*, 1342149.

[36] Bertels, J. C.; He, G. X.; Long, F. X. Metabolic reprogramming in skeletal cell differentiation. *Bone Res.* **2024**, *12*, 57.

[37] Li, Q. Q.; Gao, Z. W.; Chen, Y.; et al. The role of mitochondria in osteogenic, adipogenic and chondrogenic differentiation of mesenchymal stem cells. *Protein Cell* **2017**, *8*, 439–445.

[38] Shares, B. H.; Busch, M.; White, N.; et al. Active mitochondria support osteogenic differentiation by stimulating β-catenin acetylation. *J. Biol. Chem.* **2018**, *293*, 16019–16027.

[39] Sigmarsdottir, T. B.; McGarrity, S.; de Lomana, A. L. G.; et al. Metabolic and transcriptional changes across osteogenic differentiation of mesenchymal stromal cells. *Bioengineering (Basel)* **2021**, *8*, 208.

[40] Sigmarsdottir, T. B.; McGarrity, S.; Yurkovich, J. T.; et al. Analyzing metabolic states of adipogenic and osteogenic differentiation in human mesenchymal stem cells *via* genome scale metabolic model reconstruction. *Front. Cell Dev. Biol.* **2021**, *9*, 642681.

[41] Jin, Z. X.; Kho, J.; Dawson, B.; et al. Nitric oxide modulates bone anabolism through regulation of osteoblast glycolysis and differentiation. *J. Clin. Invest.* **2021**, *131*, 138935.

[42] Wang, Z. Q.; Chen, H. G.; Tan, Q. Y.; et al. Inhibition of aberrant Hif1α activation delays intervertebral disc degeneration in adult mice. *Bone Res.* **2022**, *10*, 2.

[43] Yoon, H.; Park, S. G.; Kim, H. J.; et al. Nicotinamide enhances osteoblast differentiation through activation of the mitochondrial antioxidant defense system. *Exp. Mol. Med.* **2023**, *55*, 1531–1543.

[44] Forni, M. F.; Peloggia, J.; Trudeau, K.; et al. Murine mesenchymal stem cell commitment to differentiation is regulated by mitochondrial dynamics. *Stem Cells* **2016**, *34*, 743–755.

[45] Karner, C. M.; Long, F. X. Nutrient uptake and metabolism in osteoblasts. *Curr. Opin. Endocr. Metab. Res.* **2023**, *30*, 100447.

[46] Arponen, M.; Jalava, N.; Widjaja, N.; et al. Glucose transporters

GLUT1, GLUT3, and GLUT4 have different effects on osteoblast proliferation and metabolism. *Front. Physiol.* **2022**, *13*, 1035516.
[47] Donat, A.; Knapstein, P. R.; Jiang, S.; et al. Glucose metabolism in osteoblasts in healthy and pathophysiological conditions. *Int. J. Mol. Sci.* **2021**, *22*, 4120.
[48] Ohnishi, T.; Kusuyama, J.; Bandow, K.; et al. Glut1 expression is increased by p53 reduction to switch metabolism to glycolysis during osteoblast differentiation. *Biochem. J.* **2020**, *477*, 1795–1811.
[49] Catheline, S. E.; Smith, C. O.; McArthur, M.; et al. Energy metabolism in osteoprogenitors and osteoblasts: Role of the pentose phosphate pathway. *J. Biol. Chem.* **2025**, *301*, 108016.
[50] Loopmans, S.; Rohlenova, K.; van Brussel, T.; et al. The pentose phosphate pathway controls oxidative protein folding and prevents ferroptosis in chondrocytes. *Nat. Metab.* **2025**, *7*, 182–195.
[51] Chen, J. Q.; Long, F. X. mTOR signaling in skeletal development and disease. *Bone Res*. **2018**, *6*, 1.
[52] Szwed, A.; Kim, E.; Jacinto, E. Regulation and metabolic functions of mTORC1 and mTORC2. *Physiol. Rev.* **2021**, *101*, 1371–1426.
[53] Morita, M.; Gravel, S. P.; Hulea, L.; et al. mTOR coordinates protein synthesis, mitochondrial activity and proliferation. *Cell Cycle* **2015**, *14*, 473–480.
[54] Fu, W. X.; Hall, M. N. Regulation of mTORC2 signaling. *Genes* **2020**, *11*, 1045.
[55] Lai, P. L.; Song, Q. C.; Yang, C.; et al. Loss of Rictor with aging in osteoblasts promotes age-related bone loss. *Cell Death Dis.* **2016**, *7*, e2408.
[56] Minami, E.; Sasa, K.; Yamada, A.; et al. Lactate-induced histone lactylation by p300 promotes osteoblast differentiation. *PLoS One* **2023**, *18*, e0293676.
[57] Zhang, J. Y.; Yao, M. X.; Xia, S. T.; et al. Systematic and comprehensive insights into HIF-1 stabilization under normoxic conditions: Implications for cellular adaptation and therapeutic strategies in cancer. *Cell. Mol. Biol. Lett.* **2025**, *30*, 2.
[58] Palomäki, S.; Pietilä, M.; Laitinen, S.; et al. HIF-1α is upregulated in human mesenchymal stem cells. *Stem Cells* **2013**, *31*, 1902–1909.
[59] Wei, J. W.; Shimazu, J.; Makinistoglu, M. P.; et al. Glucose uptake and Runx2 synergize to orchestrate osteoblast differentiation and bone formation. *Cell* **2015**, *161*, 1576–1591.
[60] Matthijssens, F.; Sharma, N. D.; Nysus, M.; et al. RUNX2 regulates leukemic cell metabolism and chemotaxis in high-risk T cell acute lymphoblastic leukemia. *J. Clin. Invest.* **2021**, *131*, 141566.
[61] Yan, Y.; Zhou, X. E.; Xu, H. E.; et al. Structure and physiological regulation of AMPK. *Int. J. Mol. Sci.* **2018**, *19*, 3534.
[62] Wadley, G. D.; Lee-Young, R. S.; Canny, B. J.; et al. Effect of exercise intensity and hypoxia on skeletal muscle AMPK signaling and substrate metabolism in humans. *Am. J. Physiol. Endocrinol. Metab.* **2006**, *290*, E694–E702.
[63] Wu, S. B.; Wei, Y. H. AMPK-mediated increase of glycolysis as an adaptive response to oxidative stress in human cells: Implication of the cell survival in mitochondrial diseases. *Biochim. Biophys. Acta* **2012**, *1822*, 233–247.
[64] Jaafar, R.; Tran, S.; Shah, A. N.; et al. mTORC1 to AMPK switching underlies β-cell metabolic plasticity during maturation and diabetes. J. Clin. *Invest.* **2019**, *129*, 4124–4137.
[65] Na, J.; Yang, Z.; Shi, Q.; et al. Extracellular matrix stiffness as an energy metabolism regulator drives osteogenic differentiation in mesenchymal stem cells. *Bioact Mater* **2024**, *35*, 549–563.
[66] Park, J. S.; Burckhardt, C. J.; Lazcano, R.; et al. Mechanical regulation of glycolysis *via* cytoskeleton architecture. *Nature* **2020**, *578*, 621–626.
[67] Daga, P.; Thurakkal, B.; Rawal, S.; et al. Matrix stiffening promotes perinuclear clustering of mitochondria. *Mol. Biol. Cell* **2024**, *35*, ar91.
[68] Lee, S.; Kumar, S. Actomyosin stress fiber mechanosensing in 2D and 3D. *F1000Research* **2016**, *5*, 2261.
[69] Papavassiliou, K. A.; Sofianidi, A. A.; Spiliopoulos, F. G.; et al. YAP/TAZ signaling in the pathobiology of pulmonary fibrosis. *Cells* **2024**, *13*, 1519.
[70] Noom, A.; Sawitzki, B.; Knaus, P.; et al. A two-way street–cellular metabolism and myofibroblast contraction. *NPJ Regen. Med.* **2024**, *9*, 15.
[71] Evers, T. M. J.; Holt, L. J.; Alberti, S.; et al. Reciprocal regulation of cellular mechanics and metabolism. *Nat. Metab.* **2021**, *3*, 456–468.
[72] Trujillo, S.; Vega, S. L.; Song, K. H.; et al. Engineered full-length fibronectin-hyaluronic acid hydrogels for stem cell engineering. *Adv. Healthc. Mater.* **2020**, *9*, e2000989.
[73] Zhao, X. B.; Chen, Y. P.; Tan, M.; et al. Extracellular matrix stiffness regulates DNA methylation by PKCα-dependent nuclear transport of DNMT3L. *Adv. Healthc. Mater.* **2021**, *10*, e2100821.
[74] Ge, H. M.; Tian, M. X.; Pei, Q.; et al. Extracellular matrix stiffness: New areas affecting cell metabolism. *Front. Oncol.* **2021**, *11*, 631991.
[75] Cox, A. G.; Tsomides, A.; Yimlamai, D.; et al. Yap regulates glucose utilization and sustains nucleotide synthesis to enable organ growth. *EMBO j.* **2018**, *37*, e100294.
[76] Yang, C.; Tibbitt, M. W.; Basta, L.; et al. Mechanical memory and dosing influence stem cell fate. *Nat. Mater.* **2014**, *13*, 645–652.
[77] Na, J.; Tai, C. Z.; Wang, Z. Y.; et al. Stiff extracellular matrix drives the differentiation of mesenchymal stem cells toward osteogenesis by the multiscale 3D genome reorganization. *Biomaterials* **2025**, *312*, 122715.
[78] Scott, A. K.; Casas, E.; Schneider, S. E.; et al. Mechanical memory stored through epigenetic remodeling reduces cell therapeutic potential. *Biophys. J.* **2023**, *122*, 1428–1444.
[79] Wu, Y. Q.; Xu, X. X.; Liu, F. Y.; et al. Three-dimensional matrix stiffness activates the Piezo1-AMPK-autophagy axis to regulate the cellular osteogenic differentiation. *ACS Biomater. Sci. Eng.* **2023**, *9*, 4735–4746.
[80] Lambertini, E.; Penolazzi, L.; Morganti, C.; et al. Osteogenic differentiation of human MSCs: Specific occupancy of the mitochondrial DNA by NFATc1 transcription factor. *Int. J. Biochem. Cell Biol.* **2015**, *64*, 212–219.
[81] Li, B. E.; Shi, Y.; Liu, M. Y.; et al. Attenuates of NAD(+) impair BMSC osteogenesis and fracture repair through OXPHOS. *Stem Cell Res. Ther.* **2022**, *13*, 77.
[82] Chen, K.; Wang, Y. L.; Deng, X. Y.; et al. Extracellular matrix stiffness regulates mitochondrial dynamics through PINCH-1- and kindlin-2-mediated signalling. *Curr. Res. Cell Biol.* **2021**, *2*, 100008.
[83] Ma, S. Y.; Ding, R.; Cao, J.; et al. Mitochondria transfer reverses the inhibitory effects of low stiffness on osteogenic differentiation of human mesenchymal stem cells. *Eur. J. Cell Biol.* **2023**, *102*, 151297.
[84] Shum, L. C.; White, N. S.; Mills, B. N.; et al. Energy metabolism in mesenchymal stem cells during osteogenic differentiation. *Stem Cells Dev.* **2016**, *25*, 114–122.
[85] Shen, Y.; Jing, D.; Zhao, Z. H. The effect of AKT in extracellular matrix stiffness induced osteogenic differentiation of hBMSCs. *Cell. Signal.* **2022**, *99*, 110404.
[86] Aierken, A.; Li, B. L.; Liu, P.; et al. Melatonin treatment improves human umbilical cord mesenchymal stem cell therapy in a mouse model of type II diabetes mellitus *via* the PI3K/AKT signaling pathway. *Stem Cell Res. Ther.* **2022**, *13*, 164.
[87] Ma, J. L.; Li, X.; Li, Q. Y.; et al. Niacin regulates glucose metabolism and osteogenic differentiation *via* the SIRT2-C/EBPβ-AREG signaling axis. *Biomed. Pharmacother.* **2024**, *180*, 117447.
[88] Zhang, C.; Xia, D. D.; Li, J. J.; et al. BMSCs and osteoblast-engineered ECM synergetically promotes osteogenesis and angiogenesis in an ectopic bone formation model. *Front. Bioeng. Biotechnol.* **2022**, *10*, 818191.
[89] Bose, S.; Roy, M.; Bandyopadhyay, A. Recent advances in bone tissue engineering scaffolds. *Trends Biotechnol.* **2012**, *30*, 546–554.
[90] Yu, X. H.; Tang, X. Y.; Gohil, S. V.; et al. Biomaterials for bone regenerative engineering. *Adv. Healthc. Mater.* **2015**, *4*, 1268–1285.

[91] Ma, Y. X.; Lei, C.; Ye, T.; et al. Silicon enhances functional mitochondrial transfer to improve neurovascularization in diabetic bone regeneration. *Adv. Sci. (Weinh)* **2025**, *12*, e2415459.
[92] Kozaniti, F. K.; Deligianni, D. D.; Georgiou, M. D.; et al. The role of substrate topography and stiffness on MSC cells functions: Key material properties for biomimetic bone tissue engineering. *Biomimetics (Basel)* **2021**, *7*, 7.
[93] Wang, M. X.; Xu, Y. P.; Cao, L. X.; et al. Mechanical and biological properties of 3D printed bone tissue engineering scaffolds. *Front. Bioeng. Biotechnol.* **2025**, *13*, 1545693.
[94] Valenti, M. T.; Dalle Carbonare, L.; Mottes, M. Osteogenic differentiation in healthy and pathological conditions. *Int. J. Mol. Sci.* **2016**, *18*, 41.
[95] Zhang, W. L.; Meng, H. Z.; Yang, M. W. Regulation of DMT1 on bone microstructure in type 2 diabetes. *Int. J. Med. Sci.* **2015**, *12*, 441–449.