

Therapeutic Implants: Mechanobiologic Enhancement of Osteogenic, Angiogenic, and Myogenic Responses in Human Mesenchymal Stem Cells on 3D-Printed Titanium Truss

Se-Hwan Lee, Ali Kiapour, Brendan D. Stoeckl, Ellen Y. Zhang, Matthew R. Begley, Jarod Oldham, Lewis Harrison, Jessee Hunt, Robert L. Mauck,* and Su Chin Heo*

This study investigates the effect of mechanotransduction on human mesenchymal stem cells (hMSCs) attached to structural elements of an implant, specifically a titanium truss element. It is found that surface features generated by 3D printing technology promote osteogenic activity, comparable to conventional treatments such as acid etching. Cell morphology and differentiation are evaluated using test coupons with either smooth or rough surface features. hMSCs on smooth titanium surfaces exhibit a flat, spread morphology consistent with fibrous tissue formation. In contrast, cells on 3D printed hierarchical surfaces cluster within surface asperities, resembling a trabecular bone-like orientation, and exhibited enhanced osteogenic gene expression. Building on this static surface-induced osteogenesis, we further investigated the effect of load-induced strain on hMSCs attached to the rough surface. Upon applying dynamic mechanical loading, RNA-sequencing analysis reveals that strain not only amplified osteogenic gene expression but also significantly upregulated angiogenic and myogenic pathways compared with static conditions. These findings demonstrate a synergistic effect between surface topography and mechanical strain in promoting osteogenesis. This study highlights the potential of utilizing mechanical strain through an implant's structural design to enhance osteogenic responses, offering valuable insights for the development of next-generation therapeutic implants.

1. Introduction

The number of Spinal fusion surgeries has steadily increased over the past two decades, primarily due to the growing size of the elderly population.^[1] One of the leading conditions requiring these surgeries is Degenerative disc disease, which is caused not only by aging, but also by lifestyle, physical trauma, and can be hereditary.^[2] As disc degeneration advances, muscle atrophy in the spine, particularly in the multifidus muscle, can occur.^[3] This atrophy is a critical factor in the progression of severe spinal disorders, including low back pain, impaired neurological function, proximal junctional kyphosis, and decreased physical function.^[4] Consequently, the demand for spinal interbody implants has risen, with Titanium (Ti) emerging as the primary material of choice due to its exceptional biocompatibility and mechanical reliability.^[5–7]

Recent advances in additive manufacturing have enabled the production of implants that enhance both primary stability and implant longevity.^[8,9] Among these innovations, 3D-printed spinal interbody

S.-H. Lee, B. D. Stoeckl, E. Y. Zhang, R. L. Mauck, S. C. Heo
McKay Orthopaedic Research Laboratory
Department of Orthopaedic Surgery
University of Pennsylvania
36th Street and Hamilton Walk, Philadelphia, PA 19104, USA
E-mail: lemauck@pennmedicine.upenn.edu;
heosc@pennmedicine.upenn.edu

A. Kiapour
Department of Neurosurgery Massachusetts General Hospital
Harvard Medical School
Boston, MA 02461, USA

B. D. Stoeckl, E. Y. Zhang, R. L. Mauck, S. C. Heo
Department of Bioengineering
University of Pennsylvania
210 South 33rd Street, Philadelphia, PA 19104, USA

M. R. Begley
Department of Mechanical Engineering
University of California
Santa Barbara, Engineering II Building 1355, Santa Barbara, CA 93106, USA

J. Oldham, L. Harrison, J. Hunt
4WEB Medical
2801 Network Blvd, Frisco, TX 75034, USA

 The ORCID identification number(s) for the author(s) of this article can be found under <https://doi.org/10.1002/adhm.202501856>

© 2025 The Author(s). Advanced Healthcare Materials published by Wiley-VCH GmbH. This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](#) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

DOI: 10.1002/adhm.202501856

implants with an advanced structural design have emerged as a promising solution for spinal fusion procedures. These new generation implants feature an open architecture design consisting of interconnected trusses that possess high-strength, lightweight characteristics and provide ample porosity for bone growth.^[10]

An additional factor that may contribute to the success of truss implants lies in the possibility of engineered bioactive surface topography, which can be achieved through additive manufacturing. In one commercial system (Truss Implant Technology from 4WEB Medical), the manufacturing process creates a hierarchical surface roughness with macro-, micro-, and nano-scale features that closely resemble the dimensions and morphology of resorption lacunae formed by osteoclastic activity.^[11] By replicating the surface characteristics of native trabecular bone, this rough Ti surface of the truss implant creates a favorable environment for bone remodeling that facilitates local bone growth factor expression, fosters osteoid deposition, and promotes bone formation.^[12,13]

To optimize implant design, it is essential to understand the mechanical microenvironment at the implantation sites within the body. The intervertebral disc represents a typical heterogeneous structure that simultaneously provides both flexibility and load-bearing support, transmitting compression, tension, and shear stress through the spine, along with two facet joints.^[14] These physiologic external forces that act on the implant can induce minute deformations, leading to microscopic tensile and compressive strains on the implant surface,^[15] depending on the specific implant design. For instance, the 4WEB truss implant harnesses a unique spatial geometry that forms a scaffold of interconnected linear elements or struts, resulting in a 3D truss structure.^[16–18] This truss architecture distributes forces throughout the construct in response to physiological loads, optimizing load transfer through the strategic arrangement of truss unit cells.^[10,19] As a result, some elements in this implant experience compression, while others are subjected to tension, effectively balancing the distribution of forces. The size and arrangement of triangular structures within the truss system can also be precisely tuned to modulate and optimize the balance between tensile and compressive forces,^[19] allowing for the execution of patient-specific designs that optimize load distribution.

Given that the 4WEB truss implant generates a heterogeneous distribution of compressive and tensile strains among the structural elements under physiological loading conditions, and considering that these microscale deformations can influence the differentiation of adherent cells,^[15] this study explored how surface characteristics coupled with applied mechanical loading and biomimetic environment affect impact the osteogenic, myogenic and angiogenic differentiation of human mesenchymal stem cells (hMSCs) through RNA-sequencing. We discovered that the surface features of the implant regulate cell adhesion and clustering while also providing a favorable surface for osteogenic differentiation of hMSCs. Furthermore, when hMSCs were seeded on the rough Ti surfaces of the truss implant and subjected to

physiologically relevant media conditions and dynamic loading, these stimuli significantly amplified the expression of osteogenic, myogenic, and angiogenic markers, surpassing the effects of surface features alone. This finding suggests a synergistic interaction between topography (surface features), the mechanical environment (strain distribution enabled by the truss design), and the biochemical environment (osteogenic conditioned media) on hMSC differentiation.

By investigating the mechanical microenvironment and the unique structural characteristics of the 4WEB truss implant, this research enhances our understanding of its biomechanical properties and potential to promote bone remodeling in spinal fusion surgeries. Ultimately, these insights could contribute to the development of improved implant designs and surgical techniques, leading to enhanced patient outcomes.

2. Results and Discussion

The Electron Beam Melting (EBM) technology is an additive manufacturing method that converts metal powders into 3D materials, forming hierarchical surface asperities from the micro-scale to the nano-scale.^[15] To investigate the effects of surface roughness, we fabricated Ti test coupons using electron beam melting (EBM) with defined build parameters. Three groups were used: 1) Smooth Ti surface (Smooth), and two 3D-printed (3D) rough surfaces including 2) 3D1, produced with a 50 μm build theme, resulting in moderate surface roughness, and 3) 3D2, fabricated with a 90 μm build theme, yielding greater hierarchical surface roughness. The selection of 3D1 and 3D2 surface configurations was based on the standard build themes provided by the commercial EBM system (50 μm and 90 μm layer thicknesses, respectively), which allowed us to generate reproducible and well-characterized hierarchical surface roughness representative of clinically relevant implants.

Both the 3D1 and 3D2 test coupons exhibited evident surface roughness, with surfaces composed of bead-like structures. At higher magnification, finer levels of roughness were observed on the beads, confirming the formation of hierarchical surface roughness (Figure 1A; Figure S1A, Supporting Information). The average roughness values (R_a) of the 3D1 and 3D2 samples were $50 \pm 4 \mu\text{m}$ and $65 \pm 6 \mu\text{m}$, respectively (Figure 1B; Figure S1B, Supporting Information).

Further analysis of the surface topography through 3D scans and 2D contour plots showed the presence of irregular 3D peaks and valleys on both the 3D1 and 3D2 titanium surfaces (Figure 1C,D). Notably, the 3D2 substrate exhibited maximum height and depth values of 124.1 and 165.7 μm , respectively. To further quantify surface roughness, several parameters were measured across the Smooth, 3D1, and 3D2 groups. The arithmetical mean height (S_a) was $0.67 \pm 0.04 \mu\text{m}$ for the Smooth group, $33.05 \pm 3.90 \mu\text{m}$ for the 3D1 group, and $32.32 \pm 2.99 \mu\text{m}$ for the 3D2 group (Figure 1E). Additionally, the maximum height (S_z) values were $21.06 \pm 4.71 \mu\text{m}$ for the Smooth group, $242.83 \pm 25.24 \mu\text{m}$ for the 3D1 group, and $255.81 \pm 18.61 \mu\text{m}$ for the 3D2 group (Figure S1C, Supporting Information). The Kurtosis (S_{ku}) values were 2.75 ± 0.37 for 3D1 and 3.40 ± 0.18 for 3D2 (Figure 1F), indicating that while both 3D1 and 3D2 exhibited similar surface roughness, the 3D2 surface exhibited a sharper change in height distribution.

R. L. Mauck, S. C. Heo
Translational Musculoskeletal Research Center
Corporal Michael J Crescenzo VA Medical Center
3900 Woodland Ave, Philadelphia, PA 19104, USA

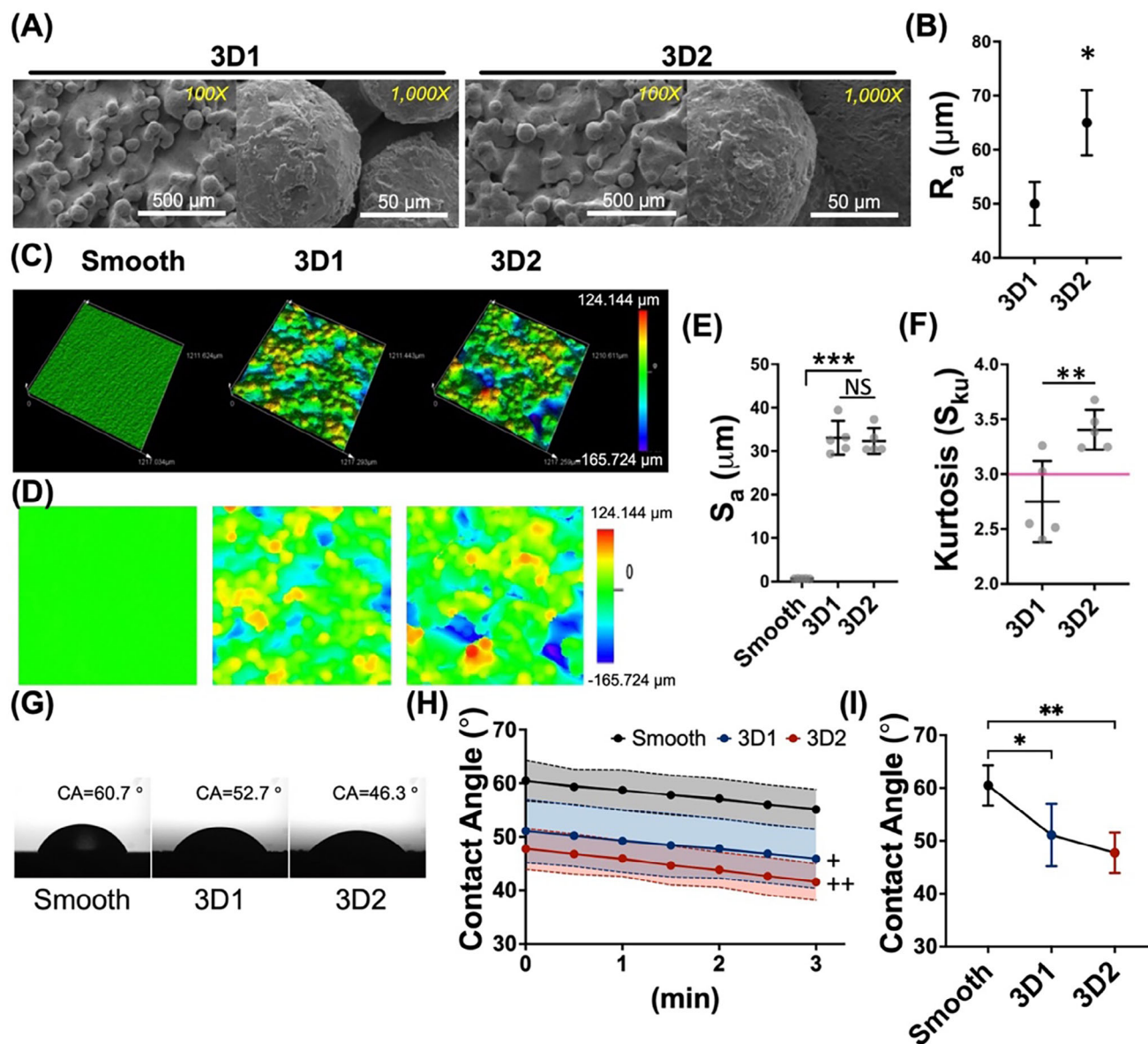


Figure 1. Characterization of surface roughness and topography of titanium substrates: A) FE-SEM images of fabricated 3D1 and 3D2 Ti surfaces. B) Arithmetic mean deviation (R_a ; $n = 3$). C) 3D laser scan microscope images. D) 2D contour plot of the surface features. E) Arithmetical mean height (S_a) measurements ($n = 5$) and F) Kurtosis values (S_{ku} ; $n = 5$; Red line = 3.0, $S_{ku} > 3.0$: high peaks/deep valleys, $S_{ku} < 3.0$: lack peaks/deep valleys). G) Representative images from the wettability test and H) contact angle measurements over time ($n = 4$). I) Quantification of contact angle changes over time ($n = 4$; *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, +: $p < 0.05$ versus angles at each time points, ++: $p < 0.01$ versus angles at each time points).

Surface roughness and total surface area influence wettability by trapping air bubbles, which in turn can significantly affect cellular responses.^[20] Initial contact angles (CA) were measured to be $60.5^\circ \pm 3.8$ for Smooth, $51.1^\circ \pm 5.9$ for 3D1, and $47.7^\circ \pm 3.8$ for 3D2 (Figure 1G,H). Over time, the contact angle decreased in all groups. After 3.0 mins, the CA was $55.1^\circ \pm 3.7$ for Smooth, $45.9^\circ \pm 5.5$ for 3D1, and $41.6^\circ \pm 3.4$ for 3D2. The relative rate of reduction in CA compared with the initial values was -8.9% for Smooth, -10.2% for 3D1, and -12.9% for 3D2 (Figure 1I). These topographical features likely contribute to the enhanced hydrophilicity observed in 3D2, as evidenced by its lower contact angle compared with 3D1. This trend suggests a relative in-

crease in surface free energy, indicating that surface roughness can modulate wettability. Prior studies have shown that the combination of greater surface asperity sharpness (reflected in higher S_{ku} values) and improved wettability may synergistically enhance protein adsorption and initial hMSC adhesion.^[21]

To investigate how surface topography regulates hMSC behaviors, cells were seeded on test coupons, and quantitative analysis revealed distinct hMSC responses to varying surface topographies (Figure 2A). On smooth surfaces (Smooth), hMSCs exhibited a large spread area with abundant stress fibers on Day 1. In contrast, on the rougher surfaces (3D1 and 3D2), hMSCs displayed a smaller spread area (Figure 2B; Figure S2,

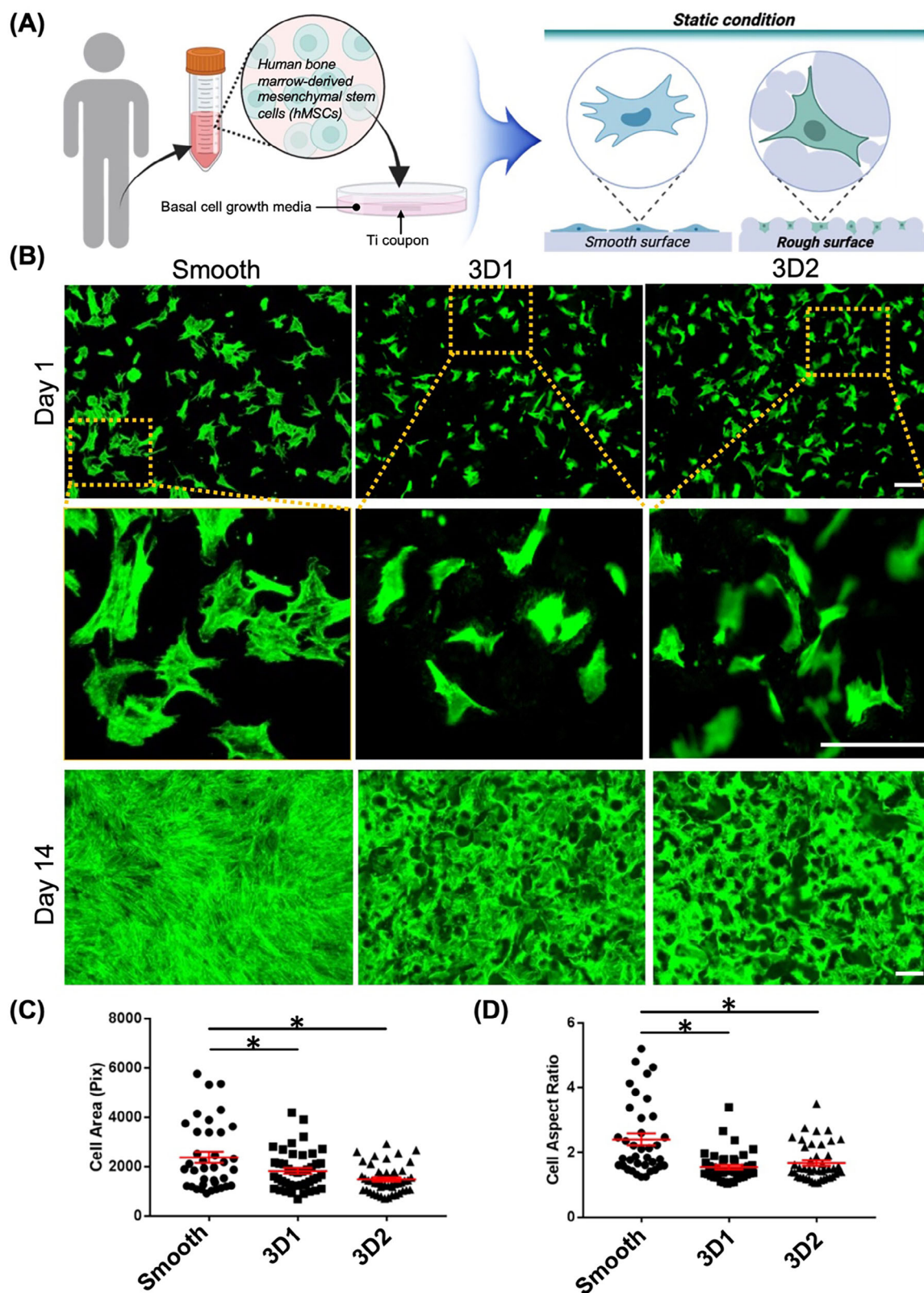


Figure 2. Effects of titanium surface features on hMSC behavior: A) Schematic representation of the in vitro static culture setup. B) Actin staining of human mesenchymal stem cells (hMSCs) cultured on titanium surfaces at Day 1 and Day 14 (Scale Bar: 100 μm). C) Quantification of cell area and D) aspect ratio ($n > 30$ technical replicates, male 23 y.o.; *: $p < 0.05$).

Supporting Information). Quantification of cell area and aspect ratio further supported these observations, showing a significant reduction (over 25%) in both parameters on the 3D1 and 3D2 surfaces compared with cells on smooth titanium ($p < 0.05$) (Figure 2C,D).

By Day 7 and Day 14, cells fully covered the smooth surface. However, cell proliferation occurred mainly around the surface, avoiding the protruding peaks of 3D1 and 3D2. This indicates that surface roughness influences cell attachment, morphology, and proliferation throughout the culture period (Figure 2B; Figure S2, Supporting Information). The rougher topographies reduced cell spreading and altered cellular organization, resulting in smaller sizes. These findings highlight the role of surface asperities in regulating hMSC morphology and organization, with rough surfaces eliciting a distinct cellular response compared with cells on smooth surfaces. hMSCs exhibited rapid expansion on both smooth and rough surfaces over 21-day culture period, with no significant differences in proliferation between the surface types under either basal growth media (BM) or osteogenic media (OM) conditions (Figure S2B,C, Supporting Information).

From these findings, hMSCs on 3D2 surfaces exhibited unique clustering behavior within the surface roughness, and cytoskeletal alignment suggested active mechanotransduction in response to topographic cues. These observations indicate that 3D2 surfaces provide a microenvironment conducive to regulating stem cell fate through enhanced cell binding and actin remodeling. In addition, 3D2 demonstrated the most distinct biophysical characteristics, including sharper 3D architecture as evidenced by the highest kurtosis value and increased surface wettability. These features are known to support protein adsorption and focal adhesion formation, which amplify mechanotransduction signaling.^[21] Moreover, 3D2 closely mirrors the surface condition of our clinical implant prototype, enhancing its translational relevance. Based on these features, in this study, we selected 3D2 to investigate how optimized surface topography modulates transcriptional responses under both static and dynamic loading conditions.

Next, to understand how the surface roughness influences transcriptional profiles in hMSCs, we conducted a comprehensive transcriptome analysis with 3 different hMSC donors (#1–#3) cultured on Smooth or 3D2 Ti surfaces under BM or OM media conditions (Figure 3A). In BM, principal component analysis (PCA) revealed a clear separation between the two groups (Smooth vs 3D2) (Figure 3A). Heatmaps of differential gene expression showed an upregulation of osteogenic genes in the 3D2 group, including decorin (DCN), RUNX family transcription factor 2 (Runx2), Collagen type I alpha 1 chain (COL1A1), Osteopontin (OPN), Fibronectin 1 (FN1), Biglycan (BGN), and Secreted protein acidic and cysteine-rich (SPARC), compared with the Smooth group (Figure 3B). Additionally, genes involved in cell motility and tissue remodeling, such as Adrenoceptor alpha 2A (ADRA2A), Chitinase 3-like 1 (CHI3L1), Integrin subunit beta 8 (ITGB8), ATP binding cassette subfamily C member 3 (ABCC3), and Kinesin family member 26B (KIF26B), as well as those related to calcium metabolism like FAM20A were highly upregulated (Figure S3A, Supporting Information). Gene ontology (GO) analysis further revealed enrichment of biological processes such as angiogenesis and scavenger receptor activity in the 3D2 group,

while genes related to muscle and cardiac cells were enriched in the Smooth group (Figure 3C).

When the same experiments were performed under OM conditions, the PCA plot showed greater differentiation between the two groups and the heatmaps of differential gene expression showed that key osteogenic markers, including Runx2, COL1A1, COL1A2, Bone morphogenetic protein 2 (BMP2), FN1, Osterix (OSX), and Sclerostin (SOST), were generally upregulated in the 3D2 group compared with the Smooth group (Figure 3D,E). Notably, the upregulation in the 3D2 group under OM conditions was more pronounced compared with the BM condition. Additionally, genes associated with growth inhibition (NOTUM, BMP, and activin membrane-bound inhibitor (BAMBI)) and immune response regulation (Lysozyme (LYZ), CCAAT/enhancer-binding protein delta (CEBPD)) were significantly upregulated (Figure S3B, Supporting Information). GO analysis indicated significant upregulation of genes related to osteogenesis, such as osteoblast differentiation and ossification, while genes associated with cardiac muscle tissue, actin cytoskeleton, and contractile fibers were downregulated (Figure 3F). Our 3D2 surface exhibited significantly higher roughness values (R_a and S_a) and enhanced kurtosis (S_{ku}), as well as increased wettability compared to other surfaces. Previous studies have highlighted the influence of titanium surface roughness on MSC behavior. For instance, Lewallen et al.^[22] reported that moderately rough titanium surfaces limited osteogenic differentiation of hMSCs, while Deligianni et al.^[23] showed that increased surface roughness on Ti-6Al-4 V alloys reduced initial cell attachment and altered protein absorption. In contrast, our 3D surface exhibited significantly higher R_a values (≈ 50 – $65 \mu\text{m}$) and S_a values (≈ 32 – $33 \mu\text{m}$), along with elevated kurtosis and wettability. These features likely created a distinct microenvironment that promoted cytoskeletal organization and osteogenic gene expression under mechanical loading. These comparisons suggest that the biological response to roughness might not be solely dependent on roughness magnitude, but also on the architectural complexity and physicochemical properties of the surface.

Taken together, this suggests that the 3D2 surface, under both BM and OM conditions, provides a topological microenvironment that supports osteogenic differentiation more effectively than smooth surfaces. Based on these findings, the 3D2 surface was selected to analyze the potential synergy between topographical cues and dynamic loading or BMP-2 treatment.

A series of mechanical tests were conducted to assess the bending properties of the fabricated 3D2 Ti truss element (Figure 4A). A three-point bending test was performed with a span distance of 30 mm. In this test, a force of 40 N was applied when the displacement reached $\approx 0.35 \text{ mm}$, and the bending modulus in the linear-elastic region of the 3D2 truss element was calculated to be 123.3 N mm^{-1} (Figure 4B). To assess the mechanical reliability of the 3D2 truss element under repeated deformation, a fatigue test was performed with a repeated dynamic loading of 0.35 mm displacement. The maximum load during the iterations reached a saturation point of 37.15 N (Figure 4C), confirming that the displacement remained within the linear-elastic region and was suitable for repeated dynamic loading experiments.

Finite element modeling was employed to analyze strain distribution across test coupons with varying surface roughness grades. Each coupon was loaded until the peak strain at the cen-

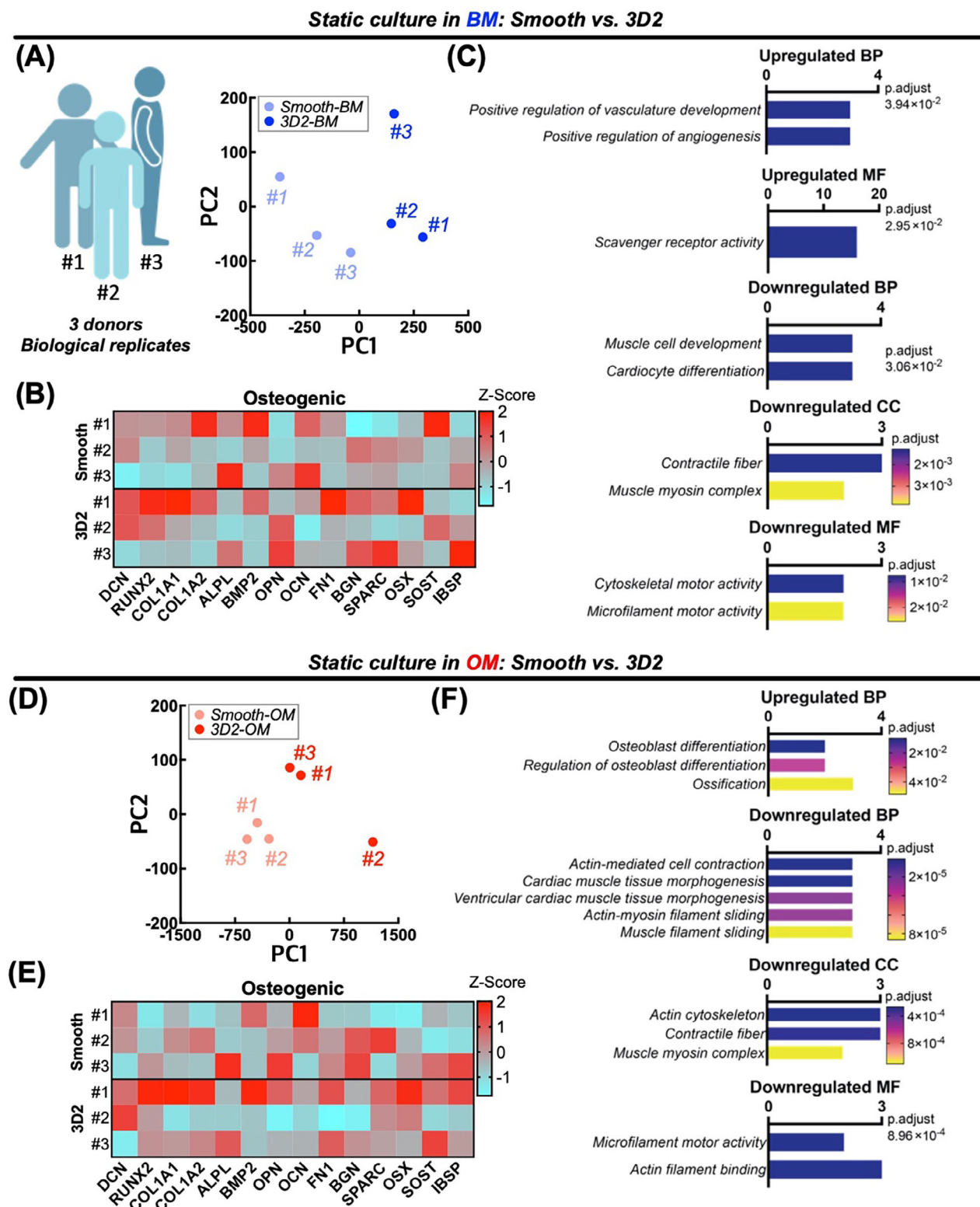


Figure 3. Transcriptional analysis comparing hMSCs on Smooth coupons (Control) versus 3D2 coupons (Treatment) in basal growth media (BM) or osteogenic media (OM) via RNA sequencing ($n = 3$ biological replicates): A,D) PCA graphs illustrating the separation between groups under basal media (BM) and osteogenic media (OM) conditions, respectively; B,E) Z-score heatmaps of osteogenic gene expression profiles for the two conditions; and C,F) Gene Ontology (GO) analysis of differentially expressed genes, highlighting differentially regulated biological processes (BP), molecular functions (MF), and cellular components (CC) under BM and OM conditions.

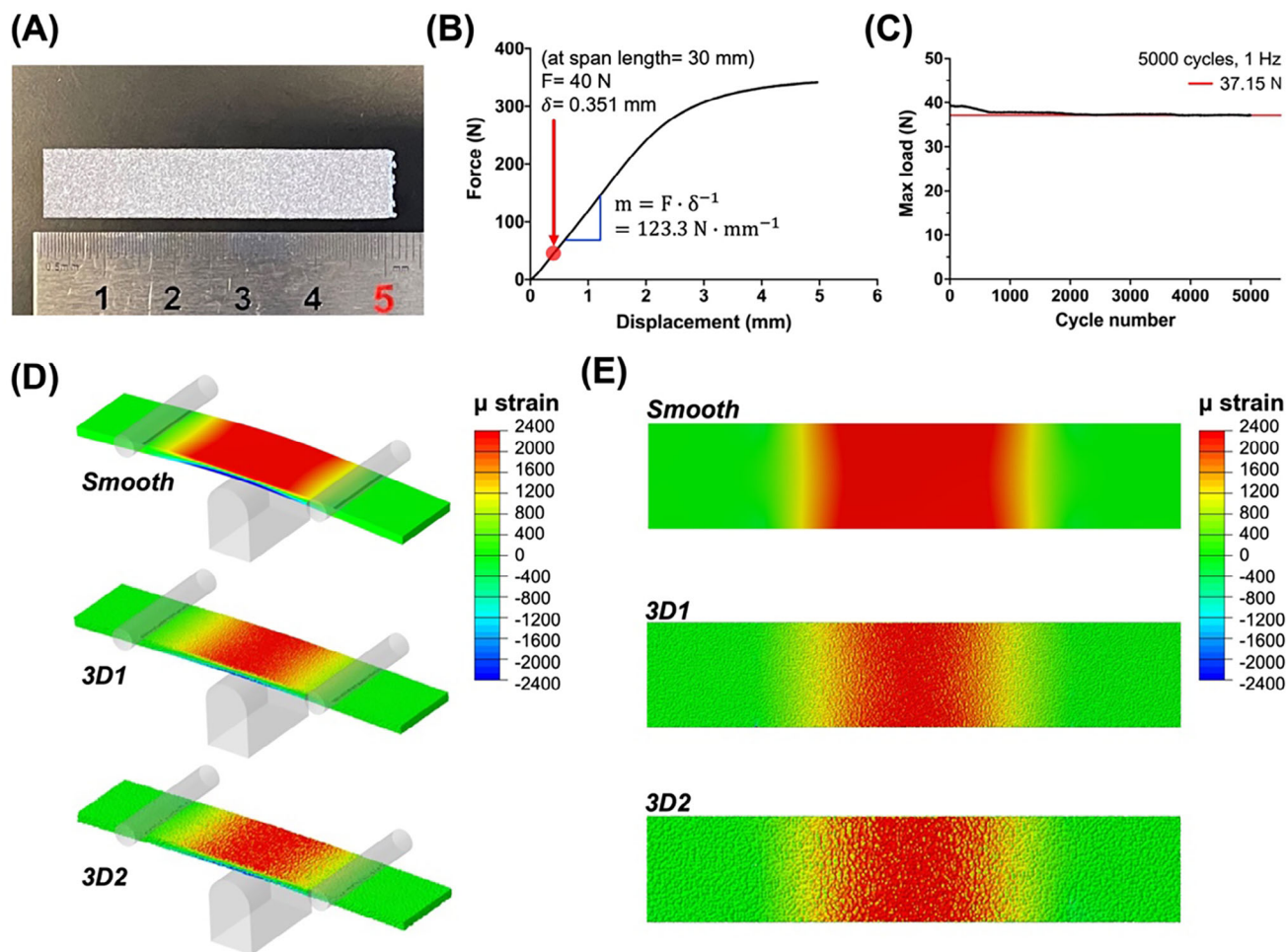


Figure 4. Numerical analysis of bending and strain in 3D-printed Ti truss elements: A) Fabricated 3D2 Ti coupon; Three-point bending test results: B) Force-displacement graph under static bending condition, C) Maximum load graph under bending fatigue condition; Numerical analysis: D) 3D sample image under bending strain, E) Contour plot on the sample surface.

ter reached 2400 μ strain (Figure 4D,E), with the corresponding loads recorded and compared. The simulation revealed a peak surface strain of ≈ 2400 μ strain localized at the central region of the truss structure, which corresponds to the primary cell-seeding area. This spatial overlap confirms that the cells were exposed to the peak strain zone during dynamic loading. The applied strain magnitude (≈ 2400 μ strain) falls within the physiological range known to promote osteogenic differentiation,^[24] suggesting that the observed transcriptional changes are consistent with mechanosensitive responses to loading. In addition, computational analysis showed that the surface topography, particularly in the 3D2 coupon, led to a slightly broader strain distribution compared with the smoother surface (Figure 4E). This suggests that the surface microstructure plays a role in affecting strain distribution, with rougher surfaces like the 3D2 coupon exhibiting more distributed strain compared with controls, which showed strain patterns closer to pure bending. These findings suggest that the fabricated 3D2 truss element exhibited favorable bending properties within the linear elastic range and that the surface roughness of the truss element influenced the distribution of strain during bending.

To further explore the combined impact of dynamic physiologic strain and surface topography on hMSC osteogenic responses, a custom bioreactor system was developed (Figure 5A,B). This system applied 3-point bending to hMSC-seeded 3D2 surfaces, with a cyclic deformation of 350 μ m at 1 Hz (Figure 5C). Cyclic tensile strain at 1 Hz has been reported to enhance the expression of osteogenic markers such as Runx2 and ALP in hMSCs.^[25,26] We selected this frequency as it approximates the physiological strain levels experienced during moderate physical activity, such as walking, and is known to activate mechanosensitive signaling pathways including RhoA/ROCK and YAP/TAZ.^[27,28] Future studies will also explore a broader frequency range to determine optimal loading conditions for bone-specific differentiation. Accordingly, our study aimed to model a moderate mechanical stimulus that enables investigation of both mechanotransductive signaling and downstream differentiation-related gene expression. Cyclic loading was applied once (DLx1; Figure S4A, Supporting Information), or three times (DLx3; Figure 5D) daily for three consecutive days in basal cell growth media (BM) or in osteogenic media (OM). The application of cyclic strain did not negatively affect cell via-

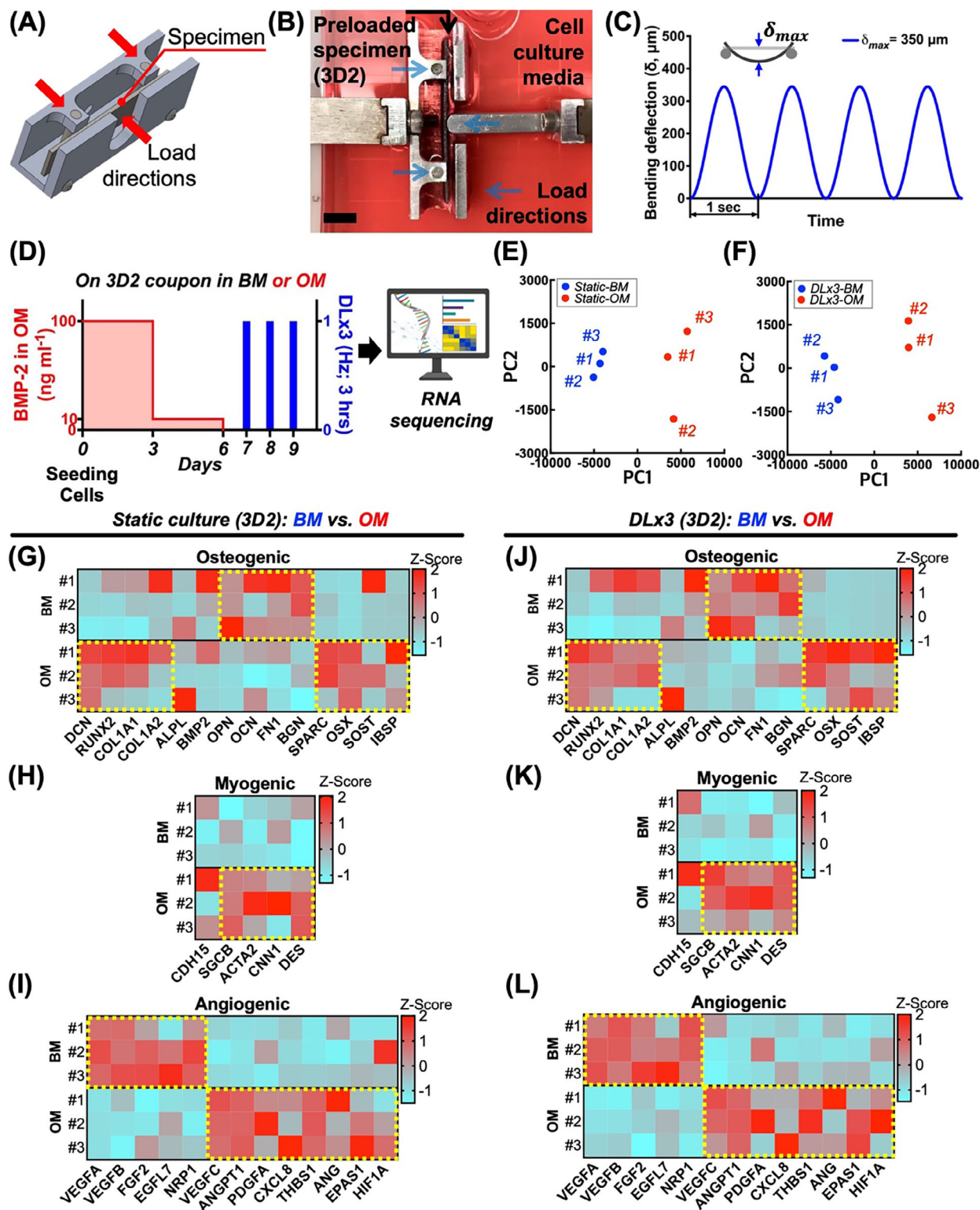


Figure 5. Synergistic effects of surface topography and mechanical strain on hMSC osteogenic response: A) Schematic representation of the three-point bending system. B) Image of a loaded 3D2 coupon in the custom bioreactor system (Scale bar: 10 mm). C) Bending deflection frequency (Cyclic loading at 1 Hz). D) Culture timeline for long-term stimulation (DLx3: 3 h per day). Transcriptomic analysis of BM versus OM by RNA sequencing ($n = 3$, biological replicates): PCA analysis under E) static and F) three-times dynamic loading (DLx3) conditions. Z-score heatmaps of osteogenic (G: Static, J: DLx3), myogenic (H: Static, K: DLx3), and angiogenic (I: Static, L: DLx3) gene expression (Yellow dotted lines are calculated as the z-score total; Figure S6A–C, Supporting Information). $n = 3$ biological replicates.

bility, confirming the suitability of this mechanical stimulation for assessing cell response to mechanical loading (Figure S4B, Supporting Information).

In a first set of studies, differential gene expression between the BM and OM environments was analyzed when hMSCs underwent DLx1 stimulation. PCA analysis showed a clear separation between the two groups (Figure S4C, Supporting Information). DLx1 stimulation led to the upregulation of early osteoblast-related genes, such as OPN, FN1, BGN, and SPARC, in the BM condition, while mature osteoblast-related genes, including Runx2, OSX, SOST, and integrin-binding sialoprotein (IBSP)^[29] were upregulated in the OM condition (Figure S4D, Supporting Information). Interestingly, myogenic genes, such as Cadherin 15 (CDH15), sarcoglycan beta (SGCB), and Actin alpha 2, smooth muscle (ACTA2), showed significant upregulation in the OM condition under stimulation (Figure S4E, Supporting Information).

This short-term dynamic stimulation also enhanced the expression of angiogenic markers, including Vascular endothelial growth factor A (VEGFA), VEGFB, Fibroblast growth factor 2 (FGF2), EGF-like domain multiple 7 (EGFL7), and Neuropilin 1 (NRP1) in the BM condition, whereas in OM the mechanical stimulation upregulated VEGFC and Angiopoietin 1 (ANGPT1) (Figure S4F, Supporting Information). Gene Ontology (GO) analysis further revealed that genes related to osteoblast differentiation, skeletal system development, and muscle tissue development were upregulated in the OM condition compared with the BM condition (Figure S4G, Supporting Information). These findings suggest that the short-term dynamic loading (DLx1) in growth media promotes early osteoblast differentiation, while dynamic culture in an osteogenic media environment may accelerate osteogenic and myogenic differentiation of hMSCs.

Given that the short-term dynamic stimulation enhanced hMSC differentiation, we next investigated how longer-term dynamic stimulation (i.e., DLx3) regulated hMSC differentiation under the same media culture conditions (Figure 5D). Under both static and DLx3 conditions in BM or OM environments, PCA analysis revealed a significant separation between the two groups (Figure 5E,F). First, in static culture (in the absence of dynamic loading) on 3D2 Ti coupons, heatmaps of differential gene expression in the BM group showed upregulation of early osteoblast markers (e.g., OPN, OCN, FN1, and BGN), while mature osteoblast markers (e.g., Runx2, SPARC, OSX, and IBSP) were upregulated in the OM conditions (Figure 5G). In addition, myogenic gene expressions (e.g., SGCB, ACTA2, and DES) were upregulated in the OM condition (Figure 5H), as were angiogenic-related genes (Figure 5I). GO analysis indicated that osteogenic genes, including those related to osteoblast differentiation, bone mineralization, and myofibril formation, were significantly upregulated in the OM condition, while genes associated with transcription by RNA, cell motility, ECM, and growth factor activity were downregulated (Figure S5A, Supporting Information). These findings suggest that extended BMP-2 treatment under static culture conditions significantly enhanced osteogenic, myogenic, and angiogenic gene expression on the 3D2 surface.

In the three-times dynamic loading culture condition (DLx3), the regulation of osteogenic, myogenic, and angiogenic genes

was similar to that observed under static culture conditions (Figure 5J–L). However, dynamic stimulation further pronounced the differences and patterns in gene expression between the groups (Figure 5J; Figure S6A, Supporting Information). GO analysis revealed that dynamic loading in OM conditions led to a significant upregulation of pathways related to protein phosphorylation, osteoblast differentiation, cardiac muscle tissue development, collagen-containing ECM, actin binding, and growth factor binding compared with BM conditions (Figure S5B, Supporting Information). While spinal fusion implants are primarily designed to promote bone regeneration, this myogenic activation may have clinical significance. Muscle atrophy, especially of the paraspinal muscles such as the multifidus, is a common complication in spinal pathology and post-surgical recovery.^[30,31] The observed myogenic response suggests that mechanical loading may influence the peri-implant microenvironment, potentially supporting muscle preservation or muscle-tendon interface regeneration. These findings raise the possibility that strain-responsive implant designs could provide broader therapeutic benefits by simultaneously supporting musculoskeletal integration.

Additionally, repetitive mechanical loading induces a transcriptional shift in hMSCs from a proliferative, undifferentiated phenotype toward osteogenic lineage commitment through mechanobiological signaling.^[32] Specifically, the upregulation of Cofilin 1 (CFL1) and Myosin regulatory light chain 9 (MYL9) indicates enhanced actin filament turnover and increased cytoskeletal contractility, supporting dynamic remodeling of cell architecture and the propagation of mechanical signals (Figure S7A,B, Supporting Information).^[31] Concurrently, increased expression of Ras homolog family member A (RhoA) suggests activation of upstream mechanotransduction pathways. Notably, despite RhoA upregulation, Rho-associated coiled-coil protein kinase 1 (ROCK1) expression was significantly downregulated. This decoupling may be mediated by Rho family GTPase 3 (RND3/RhoE), which potentially helps regulate cytoskeletal tension and facilitates efficient force adaptation during load-induced cell differentiation.^[33–35] Collectively, these findings outline a finely tuned mechanoadaptive signaling cascade that primes hMSCs for osteogenesis and may enhance bone regeneration within mechanically active environments. This mechanistic insight also aligns with the broader upregulation of osteogenic, myogenic, and angiogenic gene expression observed under dynamic culture conditions.

In this study, we explored the influence of titanium surface topography, mechanical strain, and biochemical cues on human MSC differentiation. Our goal was to gain insight into how these mechanical and micro-environmental features could promote bone remodeling and fusion, to improve outcomes following spinal fusion surgeries. To achieve this, we began with a comprehensive characterization of the surface roughness and topography of the titanium substrates. Our findings revealed that the rough titanium surface produced by additive manufacturing promoted cell adhesion and clustering, enhancing the osteogenic differentiation of hMSCs. Additionally, we investigated gene expression patterns associated with osteogenic differentiation, finding that rough surfaces supported the expression of osteoblast markers, a favorable topographical advantage for osteogenesis.

We also evaluated the influence of mechanical stimulation on hMSC osteogenic differentiation in combination with these surface topographies. The mechanical inputs induced by dynamic loading of the rough titanium surface of the truss implant, combined with OM conditions, significantly amplified the expression of osteogenic markers, as well as myogenic and angiogenic markers in hMSCs. This finding suggests a synergistic effect between the material microenvironment (surface properties), biochemical microenvironment (media conditions), and mechanical microenvironment (strain distribution enabled by the truss design) on hMSC differentiation. Our results emphasize the importance of considering both surface properties and biochemical/mechanical stimuli to promote osteogenic differentiation and bone formation. Additionally, enhanced angiogenesis could support larger areas of bone formation^[36] and muscle tissue regeneration.^[37] In this study, since the 3D2 surface alone significantly enhances osteogenic differentiation compared to smooth titanium, the focus of this study was to evaluate the synergistic effect of combining this biomimetic surface with physiological cyclic loading. In addition, this choice was intentional to reflect the clinically relevant conditions of our implant system, which incorporates this specific rough surface topography. However, we also acknowledge that including a dynamically loaded smooth surface control would further isolate the contribution of mechanical stimulation alone. As such, future studies will incorporate a full factorial design including both smooth and rough surfaces under static and dynamic conditions to more precisely dissect the individual and interactive roles of topography and mechanical strain in directing hMSC fate.

Overall, this study provides compelling evidence supporting the potential of unique structural attributes of an implant comprised of a truss architecture, which contribute to favorable biomechanical properties and the ability to promote bone remodeling. The combination of bioactive surface topography and the delivery of strain, within ranges known to be therapeutic, significantly enhances osteogenic activity, potentially accelerating bone formation and fusion. These findings advance our understanding of implant biomechanics and cellular responses, paving the way for improved implant designs and surgical techniques that may enhance patient outcomes in spinal fusion surgeries. To our knowledge, this is the first implant that has been shown to be therapeutic through its function. Although this study focuses on transcriptional profiling to investigate early mechanobiological responses, we acknowledge that gene expression alone does not fully represent functional outcomes. Our primary objective was to delineate how surface topography and dynamic loading regulate mechanotransductive signaling pathways in hMSCs. Functional assays such as matrix mineralization, alkaline phosphatase (ALP) activity, and osteocalcin (OCN) production are essential for evaluating osteogenic potential more comprehensively. These efforts will validate whether the observed gene-level changes translate into bone-forming activity and support the future development of functional implant systems. Furthermore, it is essential to note that our in vitro evaluations were conducted using human MSCs, which may not fully represent the complex cellular and biological responses occurring in vivo. Therefore, further investigations using animal models will be necessary to validate our findings. Additionally, long-term studies are required to assess the implant's performance over extended periods and its ability

to achieve successful spinal fusion. Future research should focus on evaluating the implant's integration with native bone, assessing its stability and long-term biomechanical behavior, and investigating the implant's therapeutic properties.

3. Conclusion

This study confirms that the roughened titanium surface achieved through additive manufacturing on the 4WEB implant promotes osteogenesis more effectively than a smooth titanium surface. The enhanced roughness of the implant surface creates a favorable microenvironment that facilitates the osteogenic differentiation of human mesenchymal stem cells (hMSCs), even in the absence of osteoinductive media components. This highlights the intrinsic bioactivity of the implant surface and its potential to promote bone formation. Furthermore, our study demonstrates that dynamic mechanical loading, as would be expected through the 4WEB truss implant under in vivo physiological conditions, further enhances osteogenic, angiogenic, and myogenic gene expression in hMSCs, compared with static implants without surface modifications. While previous studies have separately explored the impact of rough surface topography or mechanical loading on osteogenic differentiation, our study uniquely integrates these factors using a 3D-printed titanium truss implant. This approach allowed us to elucidate synergistic mechanobiological effects that simultaneously enhance osteogenic, angiogenic, and myogenic differentiation pathways in hMSCs, thus providing novel insights into multi-lineage differentiation control through combined surface and mechanical cues. This finding indicates that the synergistic effects of the surface features of the 4WEB implant, combined with its truss architecture, may promote effective bone formation.

Overall, our findings demonstrate that a structurally optimized 3D-printed implant surface, when combined with physiologically relevant dynamic loading, can significantly enhance mechanosensitive osteogenic, myogenic, and angiogenic gene expression in human mesenchymal stem cells. These findings highlight the potential of integrating biomechanical design principles into implant development to direct favorable cellular responses, offering a foundation for future in vivo and translational studies.

4. Experimental Section

Fabrication of Titanium Coupons with Controlled Surface Roughness by Electron Beam Melting Technology: A proprietary metal 3D printing technology to fabricate a Ti structure has been introduced in the previous studies.^[15,38] Rectangular test coupons, representative of a single truss element or strut, with three different levels of Titanium (grade 23 Titanium alloy, Ti 6Al-4 V ELI) surface conditions were fabricated using EBM (4WEB Medical Inc., USA). The test coupons had dimensions of 2' × 0.4" × 0.05" (L × W × H).

Textured Surface Characterization: Surface analysis was performed in both two and three dimensions to compare the characteristics of the fabricated surfaces. 2D surface characterization was performed using scanning electron microscopy (SEM; FEI Quanta 250, ThermoFisher, USA), and surface profiles were determined via image analysis (Image J; NIH, USA) of brightfield micrographs, where average roughness values (Ra) were determined according to an Equation (1) in ASME B46.1.

$$R_a = \frac{1}{l} \int_0^l |z(x)| dx \quad (1)$$

where “l” is the evaluation length, and “z(x)” corresponds to the profile height function.

3D topography profiles on the surface were measured using a 3D measuring laser microscope (OLS5100; Olympus, Japan) in the laser field mode with objective lenses MPLAPON20 × LEXT, and a working distance of 0.60 mm.

In the areal method parameters, Equation (2) was used to define the arithmetical mean height (S_a).^[39]

$$S_a = \frac{1}{A} \iint_A |z^2(x, y)| dx dy \quad (2)$$

where “A” is the scanned area and “z” indicates surface height in positions x and y.

Maximum height (S_z) was defined as the sum of the maximum peak height (S_p) and the maximum valley depth (S_v) per the following Equation (3).^[40]

$$S_z = S_p + S_v \quad (3)$$

To analyze the sharpness of the roughness profile, the Kurtosis (S_{ku}) value was defined from the following Equation (4).^[39,41]

$$S_{ku} = \frac{1}{S_q^4} \left[\frac{1}{A} \iint_A z^4(x, y) dx dy \right] \quad (4)$$

where “ S_q ” indicates the root mean square roughness. A total of five samples per group were analyzed to investigate the surface characteristics.

A water contact angle (CA) measurement was performed to confirm the effect of surface roughness on surface wettability. The fabricated coupons were rinsed with distilled water (DW) on a rocker for 30 min followed by a wash in 70% ethanol. The samples were dried in a fume hood for 2 days. The CA measurements on different substrates were performed using a Goniometer (Attension, Sweden). For all the measurements, 5 μ L of the DW was dispensed onto the substrate. Four coupons per group were measured at 23 degrees Celsius and 25% relative humidity.

Three-Point Bending Analysis: In a dynamic culture environment, fatigue occurs within the Ti coupon material due to repetitive deformation. Therefore, it is important to investigate the suitability of the specimen to be used in advance. Moreover, surface roughness can be a dominant factor in low fatigue performance.^[42] The 3D2 Ti coupon would likely be the most susceptible to fatigue failure, given that it has the highest surface roughness among the three coupons. Therefore, this formulation was used in mechanical performance evaluation.

The bending properties of the 3D2 coupon were evaluated through three-point bending tests. The tests were performed using an electromechanical universal testing machine (5542, Instron, USA) with a 30 mm span distance. The coupon was subjected to dynamic bending at 1 Hz over 5000 cycles with a target of 3000 μ strain at the surface.

Numerical Analysis of Test Coupon Bending Properties: To evaluate the micro-strain generated at the surface of the test coupons under a three-point bending load, finite element analysis (FEA) was used. The Finite Element (FE) model of the test coupon under a 3-point bending load was created using the 3D model of the smooth coupon with the dimensions of 50 mm × 10 mm × 1.25 mm (L × D × H). Three cylindrical supports with a diameter of 3.175 mm were designed, and two were placed at the bottom of the coupon, 30 mm apart (center-to-center), and the third one at the mid-length at the top of the coupon. The FE model of the test coupons with the simulated surface roughness of 50 microns and 90 microns was created using nTop software (nTop Inc., USA). The roughened coupon models were meshed using a linear tetrahedral volumetric mesh and exported for Finite Element Analysis (FEA). The meshed models were imported into the Abaqus FE modeling software (Simulia Inc., USA) for analysis. Next, a 3D-bending simulation on the smooth, 50 microns (3D1) and 90 microns (3D2) coupons was conducted by defining friction-based contact between the supports, actuator, and the outer surfaces of the coupon, fixing the lower supports in all degrees of freedom and applying a gradually

increasing vertical displacement with a maximum of 0.35 mm to the middle actuator. The material properties of grade 23 Titanium alloy, Ti 6Al-4 V ELI ($E = 113.8$ GPa, $\nu = 0.342$) were assigned to the plates. Following the completion of each analysis, the displacement that resulted in the peak microstrain of 1500 at the lower surface of the coupon was calculated, and the distribution of microstrain on each test coupon was compared among the designs.

In Vitro Cell Culture: hMSCs from three different donors were purchased from Lonza (Switzerland): 23 y.o. Male (donor #1), 21 y.o. Male (donor #2), and 25 y.o. Male (donor #3). Cells were expanded in basal growth media (BM; MEM alpha supplemented with 10% FBS and 1% penicillin/streptomycin) in a 5% CO₂ incubator at 37 °C for one week prior to experiments.

In Vitro Evaluation of Cell Morphology and Proliferation: The fabricated coupons underwent sterilization by rinsing with a 70% ethanol solution and exposure to UV light for 30 mins. For static in vitro culture, hMSCs were seeded onto the middle 20 mm region of 3 coupons (Smooth, 3D1, and 3D2 Ti surface) at a density of 5×10^4 cells (Passage 2). After allowing the cells to attach to the surfaces in the incubator for 2 h, the BM was added, and the cells were cultured in the incubator. Cell morphology was assessed by staining for F-actin (using Alexa Fluor 488 Phalloidin, Invitrogen, US) on 1, 7, and 14 days, and cell area and aspect ratio (>25 cells from $n = 3$ surfaces) on Day 1 were analyzed using Image J. Z-stack images were acquired with a confocal microscope on 1 and 7 days. The proliferation of hMSCs on each surface was assessed using the Alamar Blue assay ($n = 6$ samples per group; Invitrogen, US), with cells cultured in the basal growth media or osteogenic media (OM; MEM alpha medium supplemented with 10% FBS, 1% penicillin/streptomycin, 10 mm β -glycerophosphate disodium, 50 μ g mL⁻¹ L(+)-Ascorbic acid sodium salt, and 10 nm dexamethasone) for 21 days.

hMSC Cultivation and Cytotoxicity under In Vitro Dynamic Loading: For dynamic loading experiments, hMSCs were precultured for one week before being seeded on 3D2 coupons at a density of 1×10^5 cells (Passage 2), and mechanical stimulation was applied using a custom bioreactor system designed to deliver three-point bending to the test coupons (Figure 5A,B). The bioreactor system was sterilized with 70% ethanol and UV light for 30 min before each experiment. Dynamic loading was applied at a frequency of 1 Hz, a rate known to promote hMSC proliferation and migration.^[43,44] The protocol consisted of cyclic deformation (350 μ m displacement) applied to the center of the test coupon opposite the cell-seeded surface. Loading was applied once daily (DLx1), or three times daily (DLx3), each for 3-h cycles. Cytotoxicity following the DLx1 condition was assessed using a Live/Dead assay (L3224, Invitrogen, US), confirming that dynamic 3-point bending did not negatively impact cell viability in basal growth media.

In Vitro Dynamic Loading of hMSC-Seeded Test Coupons: To investigate the synergistic effects of osteogenic growth factors, hMSCs were precultured for one week before being seeded on 3D2 coupons at a density of 1×10^5 cells (Passage 2). Cells were cultured in either basal media (BM) or osteogenic media (OM) for seven days. For the DLx1 experiment, cells were treated with 100 ng mL⁻¹ Bone Morphogenetic Protein-2 (BMP-2) on day 0, followed by 10 ng mL⁻¹ BMP-2 in OM on day 2. From day 5 onward, OM without BMP-2 was used, and dynamic loading (1 Hz) was applied once for 3 h on day 7 (Figure S4A, Supporting Information). In the extended mechanical stimulation (DLx3) experiment, BMP-2 was administered at 100 ng mL⁻¹ on day 0, followed by 10 ng mL⁻¹ BMP-2 in OM on day 3. Starting from day 6, OM without BMP-2 was used for all subsequent media changes (Figure 5D). This BMP-2 dosing regimen and OM composition were consistently applied across both static and dynamic culture conditions to enable direct comparisons. In the DLx3 group, mechanical loading was applied on days 7, 8, and 9, with each session lasting 3 h.

RNA Sequencing: For in vitro static culture, hMSC cells were seeded onto the Ti coupons (either Smooth or 3D2). RNA was collected and purified using Direct-zol RNA Microprep Kits (Zymo Research, USA). RNA library preparation, including rRNA depletion and sequencing (150 bp paired-end), was performed by Genewiz (South Plainfield, USA). The sequence reads were processed using Trimmomatic v.0.36 to trim potential adapter sequences and low-quality nucleotides. The processed reads were

aligned to the Homo sapiens reference genome (available on Ensembl) with the STAR aligner v.2.5.2b. Unique gene hit counts were determined with featureCounts from the Subread package v.1.5.2. To normalize transcript expression levels, transcripts per million (TPM) values were calculated, and z-score values were determined. To achieve deeper insight into the functional profiling of significant genes, gene enrichment analysis was conducted to identify highly enriched biological processes (BP), molecular functions (MF), and cellular components (CC). This analysis was performed using the gProfiler web server (<https://biit.cs.ut.ee/gprofiler/gost>), with the significance threshold set using the Benjamini-Hochberg false discovery rate (FDR). Significant Gene Ontology (GO) terms were defined by an adjusted *p*-value of <0.05. For visualization, the significant GO terms were represented using the ggplot2 package in R. Heatmaps of z-score and GO graphs across the groups were generated with GraphPad Prism (San Diego, CA, USA).

Statistical Analysis: All data are presented as the mean $\pm$ standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA) with Tukey's post hoc testing with a 95% confidence interval via GraphPad Prism version 9 software. A *p*-value <0.05 was considered statistically significant.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgements

This research was supported by a sponsored research agreement from 4WEB Medical Inc. The authors also gratefully acknowledge the National Institutes of Health (K01 AR07787), the NSF Science and Technology Center for Engineering Mechanobiology (CMMI-1578571), and the Penn Center for Musculoskeletal Disorder Biomechanic Core for their support (P30 AR069619).

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

electron beam melting (EBM), mechanobiology, surface roughness, therapeutic strain, titanium implant

Received: April 10, 2025
Revised: June 26, 2025
Published online: July 14, 2025

- [1] H. Hwangbo, H. Lee, E. J. Roh, W. Kim, H. P. Joshi, S. Y. Kwon, U. Y. Choi, I.-B. Han, G. H. Kim, *Appl. Phys. Rev.* **2021**, *8*, 021403.
- [2] Y. Guo, H. Zhao, J. Lu, H. Xu, T. Hu, D. Wu, *J. Pain Res.* **2022**, *15*, 2879.
- [3] D. Sun, P. Liu, J. Cheng, Z. Ma, J. Liu, T. Qin, *BMC Musculoskelet. Disord.* **2017**, *18*, 167.

- [4] Z. Gong, D. Li, F. Zou, S. Liu, H. Wang, X. Ma, *J. Orthop. Surg.* **2022**, *17*, 490.
- [5] M. Abdel-Hady Gepreel, M. Niinomi, *J. Mech. Behav. Biomed. Mater.* **2013**, *20*, 407.
- [6] W.-M. Chen, Y. M. Xie, G. Imbalzano, J. Shen, S. Xu, S.-J. Lee, P. V. S. Lee, *Int. J. Precis. Eng. Manuf.* **2016**, *17*, 793.
- [7] J. W. Johnson, B. Gadowski, K. Labus, H. Stewart, B. Nelson, H. Seim, D. Regan, D. von Stade, C. Kelly, P. Horne, K. Gall, J. Easley, *JOR SPINE* **2023**, *6*, 1268.
- [8] K. Barri, Q. Zhang, I. Swink, Y. Aucie, K. Holmberg, R. Sauber, D. T. Altman, B. C. Cheng, Z. L. Wang, A. H. Alavi, *Adv. Funct. Mater.* **2022**, *32*, 2203533.
- [9] J. M. Haglin, A. E. Eltorai, J. A. Gil, S. E. Marcaccio, J. Botero-Hincapie, A. H. Daniels, *Orthop. Surg.* **2016**, *8*, 417.
- [10] S.-H. Lee, K.-G. Lee, J.-H. Hwang, Y. S. Cho, K.-S. Lee, H.-J. Jeong, S.-H. Park, Y. Park, Y.-S. Cho, B.-K. Lee, *Mater. Sci. Eng. C* **2019**, *98*, 949.
- [11] R. A. Gittens, T. McLachlan, R. Olivares-Navarrete, Y. Cai, S. Berner, R. Tannenbaum, Z. Schwartz, K. H. Sandhage, B. D. Boyan, *Biomaterials* **2011**, *32*, 3395.
- [12] W. Peng, L. Xu, J. You, L. Fang, Q. Zhang, *Biomed. Eng. Online* **2016**, *15*, 85.
- [13] P. J. Rao, M. H. Pelletier, W. R. Walsh, R. J. Mobbs, *Orthop. Surg.* **2014**, *6*, 81.
- [14] B. V. Fearing, P. A. Hernandez, L. A. Setton, N. O. Chahine, *JOR SPINE* **2018**, *1*, 1026.
- [15] J. P. Hunt, M. R. Begley, J. E. Block, *Expert Rev. Med. Devices* **2021**, *18*, 707.
- [16] M. P. Bendsøe, O. Sigmund, in *Topol. Optim. Theory Methods Appl.* (Eds.: M. P. Bendsøe, O. Sigmund), Springer Berlin, Heidelberg, **2004**, pp. 221.
- [17] C.-Y. Lin, T. Wirtz, F. LaMarca, S. J. Hollister, *J. Biomed. Mater. Res., Part A* **2007**, *83A*, 272.
- [18] G. G. Tejani, V. J. Savsani, V. K. Patel, P. V. Savsani, *J. Comput. Des. Eng.* **2018**, *5*, 198.
- [19] J. P. Caffrey, E. Cory, V. W. Wong, K. Masuda, A. C. Chen, J. P. Hunt, T. M. Ganey, R. L. Sah, *J. Biomech.* **2016**, *49*, 4090.
- [20] B. Xia, D.-H. Kim, S. Bansal, Y. Bae, R. L. Mauck, S.-J. Heo, *Acta Biomater.* **2021**, *128*, 175.
- [21] K. Xu, C. Mu, C. Zhang, S. Deng, S. Lin, L. Zheng, W. Chen, Q. Zhang, *Biomaterials* **2023**, *301*, 122268.
- [22] E. A. Lewallen, W. H. Trousdale, R. Thaler, J. J. Yao, W. Xu, J. M. Denbeigh, A. Nair, J.-P. Kocher, A. Dudakovic, D. J. Berry, R. C. Cohen, M. P. Abdel, D. G. Lewallen, A. J. van Wijnen, *Tissue Eng., Part A* **2021**, *27*, 1503.
- [23] D. D. Deligianni, N. Katsala, S. Ladas, D. Sotiropoulou, J. Amedee, Y. F. Missirlis, *Biomaterials* **2001**, *22*, 1241.
- [24] W. Peng, K. Cheng, Y. Liu, M. Nizza, D. A. Baur, X. Jiang, X. Dong, *Mater. Sci. Eng. C* **2021**, *124*, 112056.
- [25] G. Friedl, H. Schmidt, I. Rehak, G. Kostner, K. Schauenstein, R. Windhager, *Osteoarthritis Cartilage* **2007**, *15*, 1293.
- [26] J. Tan, X. Xu, Z. Tong, J. Lin, Q. Yu, Y. Lin, W. Kuang, *Bone Res.* **2015**, *3*, 15003.
- [27] Y. Yang, L. Xiao, Y. Wu, Y. Xu, Z. Xia, S. Wang, *Sci. Sports* **2023**, *38*, 760.
- [28] E. Kim, B. D. Riehl, T. Bouzid, R. Yang, B. Duan, H. J. Donahue, J. Y. Lim, *Front. Bioeng. Biotechnol.* **2024**, *11*, <https://doi.org/10.3389/fbioe.2023.1306002>.
- [29] G. S. Stein, J. B. Lian, A. J. van Wijnen, J. L. Stein, M. Montecino, A. Javed, S. K. Zaidi, D. W. Young, J.-Y. Choi, S. M. Pockwinse, *Oncogene* **2004**, *23*, 4315.
- [30] K. He, J. Head, N. Mouchtouris, K. Hines, P. Shea, R. Schmidt, C. Hoelscher, G. Stricsek, J. Harrop, A. Sharan, *Glob. Spine J.* **2020**, *10*, 657.

- [31] D. Pan, K. Liu, M. Huang, T. Sun, Z. Zhang, *Medicine (Baltimore)* **2024**, *103*, 37239.
- [32] A. O. Duty, M. E. Oest, R. E. Guldberg, *J. Biomech. Eng.* **2007**, *129*, 531.
- [33] P. Riou, S. Kjær, R. Garg, A. Purkiss, R. George, R. J. Cain, G. Bineva, N. Reymond, B. McColl, A. J. Thompson, N. O'Reilly, N. Q. McDonald, P. J. Parker, A. J. Ridley, *Cell* **2013**, *153*, 640.
- [34] A. Karystinou, A. J. Roelofs, A. Neve, F. P. Cantatore, H. Wackerhage, C. De Bari, *Arthritis Res. Ther.* **2015**, *17*, 147.
- [35] E. Seo, U. Basu-Roy, P. H. Gunaratne, C. Coarfa, D.-S. Lim, C. Basilico, A. Mansukhani, *Cell Rep.* **2013**, *3*, 2075.
- [36] H. A. Rather, D. Jhala, R. Vasita, *Mater. Sci. Eng. C* **2019**, *103*, 109761.
- [37] I. M. Olfert, O. Baum, Y. Hellsten, S. Egginton, *Am. J. Physiol.-Heart Circ. Physiol.* **2016**, *310*, H326.
- [38] A. Kiapour, E. Massaad, M. K. Kodigudla, A. Kelkar, M. R. Begley, V. K. Goel, J. E. Block, J. H. Shin, *J. Biomech.* **2023**, *155*, 111635.
- [39] P. Pawlus, R. Reizer, W. Zelasko, *Materials* **2020**, *13*, 4898.
- [40] H. Liu, Z. Tong, Y. Yang, W. Zhou, J. Chen, X. Pan, X. Ren, *J. Alloys Compd.* **2021**, *865*, 158701.
- [41] G. M. Krolczyk, J. B. Krolczyk, R. W. Maruda, S. Legutko, M. Tomaszewski, *Measurement* **2016**, *88*, 176.
- [42] D. Greitemeier, C. Dalle Donne, F. Syassen, J. Eufinger, T. Melz, *Mater. Sci. Technol.* **2016**, *32*, 629.
- [43] H. Y. Nam, B. Pingguan-Murphy, A. Amir Abbas, A. Mahmood Merican, T. Kamarul, *Biomech. Model. Mechanobiol.* **2015**, *14*, 649.
- [44] D. Kaspar, W. Seidl, C. Neidlinger-Wilke, A. Beck, L. Claes, A. Ignatius, *J. Biomech.* **2002**, *35*, 873.