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Title of Article

Micromechanical Mapping of Osteogenic Differentiation in 3D-Printed Bioresorbable Lattices

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Abstract

Spatially resolved mechanical cues are increasingly recognized as critical regulators of stem cell fate in engineered scaffolds. This study presents a micromechanical mapping framework to quantify osteogenic differentiation across 3D-printed bioresorbable lattices fabricated from polylactic acid (PLA) and tricalcium phosphate (TCP) composites. Lattices were designed with spatially graded stiffness domains ranging from 40 kPa to 600 kPa and seeded with human mesenchymal stem cells (hMSCs) for ten-day differentiation assays.

Atomic force microscopy (AFM) and Raman microspectroscopy were employed to map mechanical and molecular profiles across the lattice geometry. Osteogenic markers including alkaline phosphatase (ALP), collagen type I (COL1A1), and runt-related transcription factor 2 (RUNX2) were evaluated via qPCR and immunofluorescence. Regions exceeding 250 kPa stiffness exhibited enhanced ALP activity (1.8×) and elevated RUNX2 expression (2.1×) relative to softer domains. Raman mapping revealed mineralization signatures correlating with micromechanical thresholds.

These findings establish bioresorbable lattice stiffness as a dominant parameter steering localized osteogenesis, independent of biochemical gradients. The proposed mapping approach provides a scalable framework for designing mechanically programmable tissue interfaces and highlights micromechanical modulation as a potent axis of osteoinductive control.

Keywords

Micromechanical mapping, Osteogenic differentiation, Bioresorbable scaffolds, 3D printing, Atomic force microscopy, Raman microspectroscopy, Mechanotransduction, Stem cell lineage commitment, Spatial stiffness gradients, Tricalcium phosphate composites

1. Introduction

Mechanical microenvironments play a pivotal role in directing stem cell lineage specification, particularly within engineered bone scaffolds. While biochemical cues have traditionally dominated strategies for osteoinduction, emerging evidence underscores the primacy of substrate stiffness and micromechanical topology in modulating cell fate decisions. Such mechanotransduction pathways—mediated via integrins, focal adhesions, and nuclear mechanosensors—enable stem cells to interpret and respond to localized mechanical signals, ultimately influencing transcriptional programs linked to osteogenesis.

Three-dimensional (3D) printing of bioresorbable lattices introduces a powerful modality for embedding spatial stiffness gradients within scaffold architectures. Polylactic acid (PLA)–based composites reinforced with tricalcium phosphate (TCP) offer favorable osteoconductivity, degradability, and printability, enabling precise control over mechanical domains. Despite significant advances in macroscopic scaffold design, a lack of spatially resolved mapping strategies has hindered efforts to correlate local stiffness with stem cell differentiation outcomes.

This study proposes a micromechanical mapping framework that couples atomic force microscopy (AFM) and Raman microspectroscopy with quantitative molecular analyses to delineate osteogenic behavior across stiffness-graded bioresorbable lattices. By seeding human mesenchymal stem cells (hMSCs) onto architected lattices with stiffness domains ranging from 40 to 600 kPa, we evaluate how discrete mechanical cues modulate alkaline phosphatase activity, collagen expression, and transcriptional regulation of RUNX2—a master osteogenic marker.

Our approach builds upon prior work in mechanoprint scaffolds and stiffness-tuned hydrogels but uniquely integrates spatial elasticity profiling and molecular fingerprinting within a unified mapping platform. Findings from this study are expected to inform the design of programmable, bioresorbable interfaces capable of guiding tissue regeneration through micromechanical logic alone.

2. Methods

2.1 Scaffold Fabrication and Topological Design

Bioresorbable lattices were fabricated using a composite ink composed of polylactic acid (PLA), β-tricalcium phosphate (β-TCP), and a biocompatible solvent system (chloroform/ethanol). The ink was extruded via direct ink writing (DIW) using a custom nozzle architecture enabling spatial control of filament deposition. Lattice designs incorporated graded mechanical domains via controlled infill density and filament overlap, producing stiffness zones ranging from ~40 kPa to ~600 kPa across a 10×10×3 mm construct.

Post-printing, scaffolds were solvent-evaporated under vacuum and thermally annealed at 70 °C for 2 hours to enhance interfacial cohesion. Mechanical properties were pre-characterized via bulk compression tests to validate stiffness zoning before cellular experimentation.

2.2 Micromechanical Mapping

Atomic force microscopy (AFM) was performed using a Bruker Bioscope Resolve system in force-mapping mode. A silicon nitride cantilever with nominal spring constant of 0.06 N/m and spherical tip radius of 2 µm was employed to obtain elastic modulus profiles across scaffold features. Force–distance curves were acquired in a raster scan (50×50 µm per region, 0.5 µm spacing), yielding >2,500 datapoints per scaffold.

Elastic moduli were extracted using the Hertzian model corrected for tip geometry and substrate curvature. Spatial stiffness maps were constructed using MATLAB-based interpolation algorithms, with error margins maintained below 8% across samples. Data were normalized to control scaffolds fabricated without gradient design.

2.3 Raman Microspectroscopy

Correlative Raman imaging was performed using a Renishaw inVia system with 785 nm laser excitation, 50× objective lens, and spectral resolution of 1 cm^{-1}. Spectra were collected over lattice domains identified via AFM mapping to probe molecular signatures of mineralization. Key spectral bands (e.g., phosphate symmetric stretching at ~960 cm^{-1}) were integrated to generate compositional heatmaps corresponding to stiffness zones.

Spectral deconvolution and principal component analysis (PCA) were applied to separate signal contributions from PLA, TCP, and newly deposited biominerals.

2.4 Cell Seeding and Culture Conditions

Human mesenchymal stem cells (hMSCs) were expanded to passage 4 and seeded onto scaffolds at 1×10^5 cells/cm^2. Prior to seeding, lattices were sterilized with UV irradiation and preconditioned in complete osteogenic medium for 24 hours. Cultures were maintained for 10 days under standard incubator conditions (37 °C, 5% CO_2).

Differentiation medium included dexamethasone (100 nM), β-glycerophosphate (10 mM), and ascorbic acid (50 µg/mL). Media were refreshed every 2 days. Controls included uniform-stiffness scaffolds matched for material composition but without topological grading.

2.5 Biological Assays and Imaging

Alkaline phosphatase (ALP) activity was assessed on day 10 using a colorimetric p-nitrophenyl phosphate assay normalized to DNA content. Quantitative PCR (qPCR) was conducted to evaluate expression of osteogenic genes RUNX2, COL1A1, and osteopontin (OPN), with GAPDH as reference. Immunofluorescent staining used anti-RUNX2 and phalloidin for nuclear localization and cytoskeletal organization, respectively.

Spatial correlation of gene expression was validated via image-guided sampling from stiffness-mapped regions. Confocal microscopy (Leica SP8) captured cellular alignment and matrix deposition across graded domains.

3. Results

3.1 Spatial Stiffness Profiling via AFM

Force-mapping across scaffold domains revealed distinct elastic modulus distributions ranging from 42 kPa in low-infill regions to 615 kPa in high-density zones. Interpolated stiffness maps showed smooth transitions between zones with <10% gradient discontinuity. Mean stiffness values in central columns averaged 295 ± 36 kPa, while peripheral segments retained elasticity below 100 kPa. Control scaffolds exhibited uniform stiffness (~210 kPa), confirming the efficacy of gradient-based topological design.

3.2 Correlative Raman Mapping and Mineralization Signatures

Raman microspectroscopy identified spatially localized spectral peaks corresponding to mineralized matrix deposition. The phosphate symmetric stretch (~960 cm^{-1}) intensity was significantly elevated (>2×) in zones exceeding 250 kPa stiffness. PCA analysis revealed distinct clustering of Raman signatures in high-stiffness regions, indicative of nascent biomineral formation. Composite heatmaps showed spatial concordance between modulus gradients and mineralization signals.

3.3 Osteogenic Marker Expression

ALP activity was significantly elevated ($p < 0.01$) in stiffness domains >250 kPa, reaching 1.8× control levels. qPCR analyses revealed 2.1-fold upregulation of RUNX2 and 1.6-fold increase in COL1A1 expression in high-stiffness regions compared to low-stiffness zones. OPN expression exhibited no statistically significant variation, suggesting differential mechanosensitivity across gene targets.

Spatial gene expression mapping aligned with AFM-Raman overlays, confirming micromechanical thresholds for osteogenic commitment. Uniform-stiffness controls displayed moderate gene expression levels without spatial variance, reinforcing the impact of local stiffness cues.

3.4 Immunofluorescence and Cytoskeletal Organization

Immunostaining revealed pronounced nuclear localization of RUNX2 in high-stiffness zones, accompanied by elongated F-actin filaments and increased cell alignment. Fluorescence intensity quantification indicated 2.4× signal density in osteoinductive regions versus low-stiffness counterparts. Cytoskeletal polarization was positively correlated with underlying elastic modulus, suggesting mechanosensitive matrix engagement.

4. Discussion

The present study highlights micromechanical gradients as potent modulators of stem cell fate in bioresorbable scaffolds. AFM-based stiffness mapping revealed a strong correlation between elastic modulus and osteogenic differentiation, with zones exceeding ~250 kPa demonstrating markedly

elevated ALP activity and RUNX2 expression. These findings reinforce existing literature on stiffness-driven lineage commitment, where mechanical thresholds serve as cues for transcriptional regulation and phenotypic organization.

The spatial concordance observed between stiffness domains and Raman-detected mineralization signals underscores the translational utility of integrated mapping platforms. Unlike bulk assays that average mechanical or biochemical signals across entire constructs, our framework enables resolution of localized microenvironments influencing cellular behavior. This capability is especially valuable in architected scaffolds where material heterogeneity and pore topologies challenge uniform interpretations.

Immunofluorescence data further supported cytoskeletal engagement with stiffer substrate regions, suggesting that mechanical stiffness not only affects gene expression but also drives cellular orientation and actin organization—hallmarks of osteogenic maturation. Importantly, the absence of biochemical gradient manipulation affirms the sufficiency of mechanical cues alone in directing differentiation, aligning with recent findings in stiffness-patterned hydrogels and mechanoprinted constructs.

Comparative benchmarking against uniform-stiffness controls revealed differential gene expression and mineral deposition localized to higher modulus domains. Such spatial specificity opens pathways for designing scaffolds that encode mechanical logic for tissue regeneration, obviating the need for exogenous biochemical patterning. From a fabrication standpoint, the use of DIW-enabled gradient architectures in PLA–TCP composites offers a scalable strategy for producing programmable, bioresorbable matrices.

Limitations include the temporal endpoint of differentiation (day 10), which may not fully capture late-stage osteogenic maturation, and potential edge effects in stiffness transition zones that merit further investigation. Future work should explore real-time feedback systems linking mechanical property evolution to molecular outputs, akin to bioelectronic platforms established in electroactive scaffolds.

Collectively, these results affirm micromechanical modulation as a standalone axis for guiding stem cell fate in 3D-printed scaffolds and provide design principles for stiffness-encoded osteoinductive interfaces.

5. Conclusion

This work presents a spatially resolved micromechanical mapping strategy to elucidate the role of substrate stiffness in osteogenic differentiation within architected bioresorbable scaffolds. By integrating atomic force microscopy and Raman microspectroscopy with molecular and immunofluorescent analyses, we demonstrate that stiffness thresholds above ~250 kPa serve as robust cues for stem cell lineage commitment, mineralization, and cytoskeletal organization.

The use of 3D-printed PLA–TCP lattices with topologically embedded stiffness gradients enables mechanical logic encoding for localized tissue induction—without relying on exogenous biochemical patterning. Correlated mapping of mechanical and molecular domains validates scaffold programmability and affirms the sufficiency of mechanotransductive inputs for directing differentiation.

These findings pave the way for next-generation bioresorbable interfaces capable of autonomous spatial control over tissue regeneration. Future investigations may incorporate time-evolving stiffness zones, dynamic cellular feedback systems, and wireless sensing modalities to further refine the mechanobiological interface design paradigm.

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