Voxel-Programmed Micromechanical Heterogeneity Directs Durotaxis and Early Osteogenic Priming in µMATCH Hydrogels

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INTRODUCTION: Native musculoskeletal extracellular matrix (ECM) is a dynamic, spatially heterogeneous environment that transmits mechanical cues critical to development, repair, and disease progression. While ECM stiffness regulates cell migration, cytoskeletal organization, and lineage commitment, most in vitro models employ spatially uniform, macroscopic heterogeneity, or static substrates, failing to replicate the dynamic micromechanical heterogeneity that cells experience in vivo. Although a few systems provide either spatial (e.g., bulk gradient) or temporal (e.g., stimuli-responsive) control, none enable high-resolution spatiotemporal tuning of stiffness at the single-cell scale. *Here, we present µMATCH (Micro-Architected Tissue-Mimetic Construct with Heterogeneity), a hydrogel platform that integrates optical fiber-based interferometric nanoindentation of native tissue with voxel-wise grayscale visible light digital light processing (gVL-DLP) bioprinting.* Using the mouse lumbar disc as a pilot tissue model due to its defined radial stiffness gradient, we mapped native stiffness profiles (1-20 kPa) across the nucleus pulposus to inner annulus and encoded them into GelMA-PEGDA scaffolds *at 35 µm voxel resolution* by locally modulating blue light intensity voxel-by-voxel (range: 16-32 mW/cm²) (**Fig. 1a**).

METHODS: µMATCH gradient scaffolds were benchmarked against uniform-modulus controls printed at 32 mW/cm². We quantified: (i) crosslinking conversion by FTIR (methacrylate C=C absorbance), (ii) spatiotemporal micromechanics by optical fiber-based interferometry nanoindentation, and (iii) degradation kinetics in acellular enzymatic media incubated at 37 °C. For *in vitro* cell studies, mechanosensitive pre-osteoblasts (MC3T3-E1) were centrally seeded to assess viability, radial migration (durotaxis), cytoskeletal organization, live single-cell stiffness, and alkaline phosphatase (ALP) activity over time. All the statistical analysis were performed in Prism v.10 using unpaired two-tailed t-tests (two groups, single time point) and one-/two-way ANOVA (factors: condition, time) with Tukey's post-hoc (α=0.05).

RESULTS: Acellular µMATCH reproduced the target T0 radial modulus (~1-20 kPa), then stiffened by T3, followed by a decline by T7 (**Fig. 1b**). The T0-to-T3 increase is consistent with sol-fraction washout and network consolidation (removal of unreacted/short chains reduces local swelling and densifies the covalent network probed microscopically. Whereas the T3-to-T14 decrease reflects degradation-mediated softening (PEGDA ester hydrolysis with media-dependent GelMA proteolysis). FTIR showed that increasing local light intensity reduced the methacrylate C=C absorbance at T0, indicating greater initial crosslink conversion (**Fig. 1c**). ***Cell behavior and local remodeling.*** µMATCH induced pronounced durotaxis: MC3T3-E1 redistributed toward an intermediate-stiffness annulus, an effect absent on uniform-intensity controls (**Fig. 2a**). By T10, live single-cell nanoindentation in cell-dense µMATCH regions exceeded controls ($p < 0.0001$) despite µMATCH's lower initial mean modulus (**Fig. 2b**), indicating localized cell-mediated compaction and remodeling enabled by spatial heterogeneity. By T14, modulus decreased in both groups with degradation, yet µMATCH remained stiffer than controls ($p < 0.0001$). Osteogenic readouts showed an early ALP surge at T3 on µMATCH relative to controls and a decline by T14, whereas controls increased from T3-to-T14 ($p < 0.01$) (**Fig. 2c**), consistent with accelerated osteogenic initiation and earlier resolution on µMATCH.

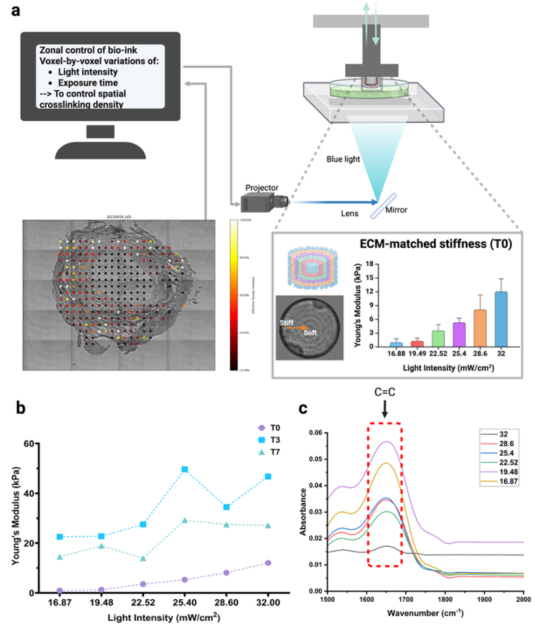

Fig. 1: *(a) µMATCH design & 3D printing using gVL-DLP. acellular mechanics. (b) Longitudinal micromechanical profiling using interferometric nanoindentation showed T0 to T3 stiffening, then degradation-driven softening. (c) FTIR results showing methacrylate C=C associated to each light dose at T0.*

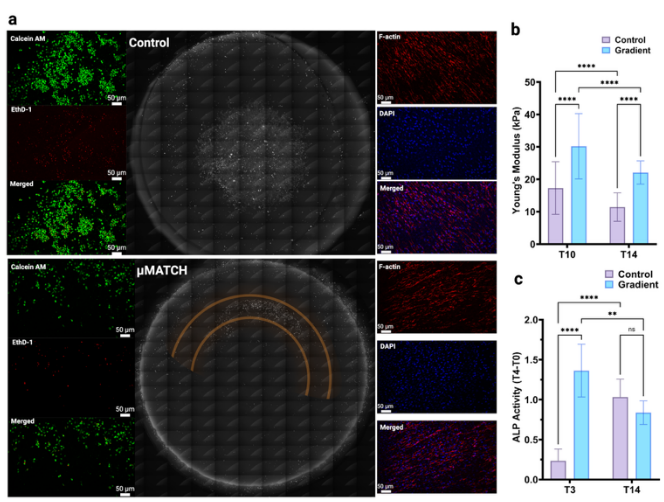

Fig. 2: *(a) MC3T3-E1 durotaxis toward an intermediate stiffness; absent on uniform-intensity controls. (b) Live single-cell nanoindentation showed local modulus higher at T10 in cell-dense µMATCH regions vs control. (c) ALP activity assay showed early surge at T3 on µMATCH, decline by T14.*

DISCUSSION: Voxel-programmed micromechanics at single-cell resolution direct where cells migrate and when they mature. Two coupled processes explain the response: an early network consolidation after sol-fraction washout and cell-driven local compaction within an intermediate-stiffness corridor, both offset by degradation-mediated softening thereafter. *Using live single-cell nanoindentation at serial timepoints, we provide a cell-resolved demonstration that engineered, tissue-mimetic micromechanical heterogeneity repositions cells (durotaxis) and accelerates early osteogenic priming. Because µMATCH decouples spatial heterogeneity from average modulus and allows independent tuning of gradient amplitude/slope (light-dose maps) and degradation time constants (ink chemistry), these data yield actionable design rules for mechano-signaling scaffolds.* We acknowledge that responses are cell-type dependent; our ongoing studies with chondrocytes and progenitor cells are extending these rules across rules across phenotypes.

SIGNIFICANCE: ***Light-programmable, voxel-scale biomechanical patterning provide a practical way to encode precise "where/when" cues in mechano-signaling scaffolds.*** Practically, this yields a simple design rule: encode an intermediate-stiffness corridor to concentrate cells (durotaxis) and accelerate early osteogenic priming. The approach is directly applicable to graded orthopaedic interfaces, such as osteochondral unit and vertebral endplate.