Bridging the Gap: A 3D Mechanoregulation Framework Revealing the Spatial Dynamics of Bone Healing

Ahmad Hedayatzadeh Razavi[1,2], Elizabeth Vickery[1], Alireza Mirahmadi[1], Shubham Laiwala[1], Nazanin Nafisi[1,2], Arthur Klujian[1], Matus Vician[1], Ani Jelalian[1], Mohammad Javad Shariyate[1], Edward Rodriguez[1], Ara Nazarian[1,2]
[1]Musculoskeletal Translational Innovation Initiative, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, 02215, USA
[2]Department of Mechanical Engineering, Boston University, USA

INTRODUCTION: Successful bone repair relies on the precise coordination of mechanical stability and biological signals. Recent advances in biology and imaging show that cellular activities (such as differentiation, migration, and proliferation) are strongly influenced by mechanical cues through complex mechanotransduction pathways. Callus formation and the ability to predict its development are vital for bone fracture healing, since the callus acts as a temporary bridge that stabilizes broken bone, and its progression signals successful recovery. However, most models consider only one aspect or assume that callus formation is uniform, missing key spatial patterns of healing. Mechanobiological modeling reveals that tissue repair is a result of interactions between cellular behaviors and their mechanical environment. Research has shown that damaged tissue directs cells and resources to areas with the strongest mechanical signals, reinforcing regions to maximize stability. Although optimization and finite-element models can simulate these processes, many overlook how callus formation starts and spreads over the bone surface. To address this, and building on our validated two-dimensional mechanoregulation framework, we have developed a three-dimensional hybrid model that combines finite-element mechanics, mesenchymal stem cell migration, vascular diffusion, and data from experimental histology and μCT imaging. This new approach helps reveal how mechanical and biological factors combine to determine where and how bone bridging begins and progresses during the healing process.

METHODS: An *in-vivo* rat femur osteotomy model (n = 34, 13-week-old female Sprague–Dawley rats) was used for calibration and validation. Animals were euthanized weekly from postoperative weeks 1–6 (n = 4/time-point; n = 10 at week 6). Rat femoral fractures (2 mm osteotomy, 4-screw fixation) were reconstructed from μCT scans, segmented slice-by-slice to extract regional bone area (B.Ar) across five anatomical zones, and used to initialize biphasic FE simulations in ABAQUS v25. Each element's material properties evolved according to coupled modules for (i) mechanical strain and fluid flow, (ii) vascular oxygen diffusion, and (iii) MSC migration solved in Python with diffusion, chemotaxis, and mechanosensitive guidance (**Figure 1**). To interpret the 42-day, high-dimensional simulation output, each of the ~7.5×10^5 callus elements was assigned a mechanobiological fingerprint comprising tissue fractions, mechanical stimuli, and four dynamic stiffness features (ΔE, t_{50}, AUC_norm, monotonicity ρ). Features were standardized, reduced via PCA, and embedded with UMAP to visualize emergent healing phenotypes. K-means clustering (k = 4) identified distinct trajectory patterns reflecting regional mechanical–biological regimes (**Figure 3B**). Model outputs were calibrated to μCT-derived B.Ar using isotonic and linear regression anchored at Day 28, while a Random Forest regressor (300 trees) predicted final stiffness gain (ΔE) from early (≤ day 14) mechanical–biological features.

RESULTS: The model accurately reproduced the spatial–temporal sequence of fracture healing observed in μCT and histology (**Figures 3A-B**), demonstrating early periosteal expansion, mid-stage cartilage formation, and late-stage endochondral bridging. Histological quantification confirmed distinct differentiation pathways at the fracture site and periosteum: hematoma and fibrous tissue dominated the first week, followed by progressive cartilage and woven bone formation, with the periosteum exhibiting a faster onset of ossification (**Figure 2, 3A**). Regional μCT validation demonstrated a strong correlation between simulated stiffness and bone area ($\rho = 0.98$, $p < 0.01$). The Random Forest achieved an R^2 of 0.984 and an MAE of 0.30, identifying the early immature-bone fraction and the fibrous-to-cartilage transition as the dominant predictors of long-term stiffening. Four distinct healing trajectories emerged (quiescent, early modest, gradual-consolidator, and rapid-ossifier) **(Figure 3B),** each corresponding to unique mechanical-biological regimes. Notably, the classical MR algorithm demonstrated diminished accuracy in distal, low-activity regions, indicating that while MR remains highly predictive at the fracture gap, far-field behavior may depend on additional biological factors not currently captured in models.

DISCUSSION: This integrative framework couples high-fidelity mechanics, biological transport, and data-driven prediction to transform fracture healing from a descriptive to a predictive system. The novelty lies in resolving healing spatially and temporally through three complementary modalities (histology, μCT, and finite-element modeling), enabling direct regional comparison. Histology revealed that the fracture gap and periosteal callus follow distinct tissue-differentiation pathways, showing that they cannot be modeled under a single mechanoregulation rule. μCT provided the time-lapsed morphology of bone formation, capturing the true temporal emergence of bridging, while the FE model linked these changes to their mechanical and biological drivers. Together, these methods portray healing as a coordinated, region-specific process. The findings highlight that while MR accurately predicts callus evolution near the fracture site, its assumptions require refinement in distal regions where early callus formation initiates. This multi-scale framework establishes a foundation for personalized, spatially informed strategies in fracture management.

SIGNIFICANCE: Healing is not uniform; it unfolds as a spatially coordinated dialogue between biology and mechanics. This work integrates high-fidelity simulations, machine learning, and experimental validation to decode that dialogue in 3D. By predicting region-specific healing trajectories and uncovering the reasons why periosteal and fracture-gap pathways diverge, the study provides a new predictive paradigm for assessing fixation strategies and designing patient-specific interventions that align with the bone's intrinsic regenerative logic.

REFERENCES:

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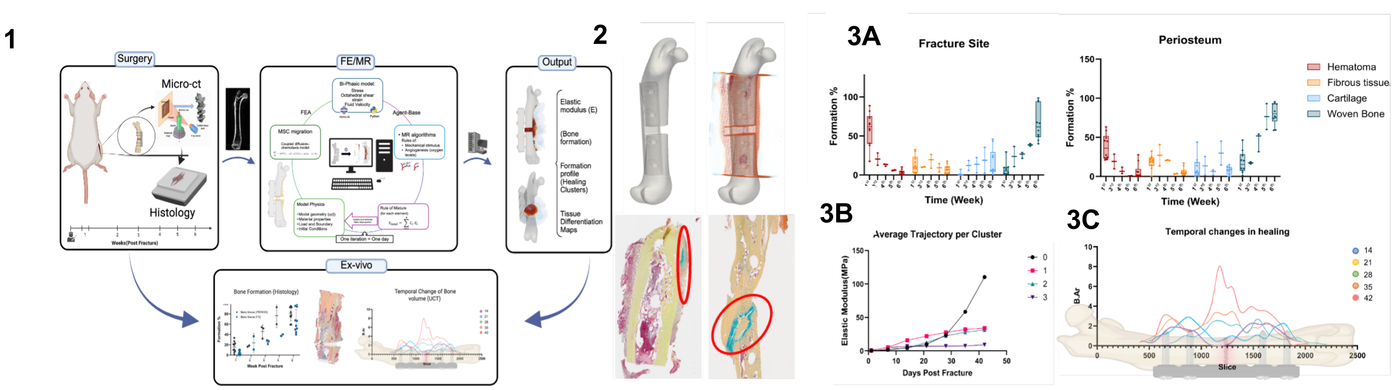

Figure 1. Integrated experimental–computational workflow: In-vivo rat femur osteotomy model with serial μCT and histology used for validation of the biphasic FE–mechanoregulation (FE/MR) model. **2. Model geometry and histological validation:** model output showing simulation regions (top) and representative histological sections (bottom) highlighting spatially distinct periosteal and fracture-gap tissue differentiation. **3. Histology-derived temporal tissue formation**: Quantified hematoma, fibrous tissue, cartilage, and woven bone formation. **4. Model-predicted healing trajectories and μCT validation**: Average cluster-based elastic-modulus trajectories (left) and μCT-derived bone-area (B.Ar) evolution across slices and days (right), illustrating region-specific temporal progression.