Spatio-temporal characterization of compositional and cellular properties in the murine fracture callus

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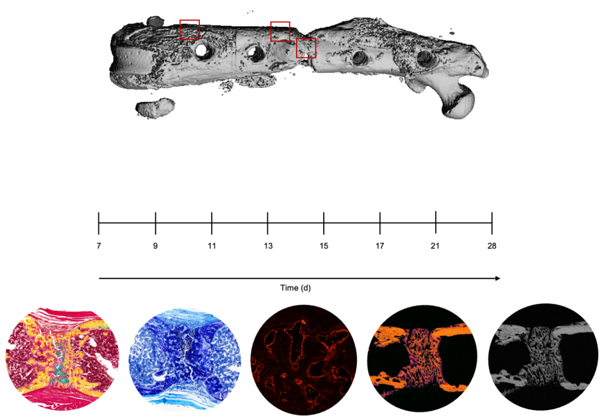
INTRODUCTION: Fracture healing is an evolutionarily conserved process that, under optimal conditions, results in scar-free regeneration. The murine femoral osteotomy with external fixation is a standardized model, but a comprehensive spatio-temporal characterization of callus mineralization and cellular dynamics has been lacking. The objective of this study was to systematically evaluate fracture healing at multiple time points and across distinct bone regions.

METHODS: A total of 84 female C57BL/6J mice underwent standardized femoral osteotomy stabilized by external fixation. Female mice were used to reduce biological variability and in accordance with 3R principles. Sham-operated controls were included. Animals were sacrificed at eight time points (day 7, 9, 11, 13, 15, 17, 21, 28). All experiments were approved by local authorities. Analyses included µCT, undecalcified histology, histomorphometry, immunofluorescence vessel staining, quantitative backscattered electron imaging (qBEI), and nanoindentation, focusing on callus, cortical bone adjacent to the fracture, and distant cortical bone.

RESULTS SECTION: µCT revealed progressive increases in bone volume and mineral density, peaking at day 21. Histology showed early cartilage expansion (day 9–13) with subsequent resorption and bone formation, leading to cortical bridging. Osteoblast and osteoclast indices peaked at day 15, corresponding to maximal calcein labeling at days 15–17. Vessel density, including type-H vessels, reached a maximum at day 13. Osteocyte lacunar density and area peaked at days 13–15 before declining. qBEI demonstrated persistent mineralization heterogeneity in the callus until day 28, when values approximated cortical bone. Unexpectedly, cortical bone adjacent to the fracture showed progressive demineralization and reduced hardness from day 21–28, while distant cortical bone remained stable.

DISCUSSION: This study provides a comprehensive multiscale view of murine fracture healing, linking structural, cellular, and compositional changes over time. Callus mineralization followed endochondral ossification, accompanied by transient peaks in osteoblast and osteoclast activity as well as vascularization, emphasizing the dynamic interplay between angiogenesis and bone formation. The pronounced but temporary increase in osteocyte lacunar number and size suggests an important mechanosensory function during matrix mineralization. The reduction in mineralization heterogeneity at late stages indicates a transition toward mature lamellar bone. Importantly, cortical bone adjacent to the fracture exhibited progressive demineralization and mechanical weakening despite successful callus consolidation, a finding consistent with clinical observations of refracture risk near previous fracture sites. These results highlight the need to further investigate calcium mobilization and remodeling mechanisms in adjacent cortical bone. Limitations include restriction to female mice and descriptive rather than mechanistic approaches, but the detailed spatio-temporal framework provided here establishes essential reference values for future interventional studies.

SIGNIFICANCE/CLINICAL RELEVANCE: These findings establish reference values for bone quality and cellular dynamics during fracture repair. Identification of cortical demineralization near the callus highlights a potential mechanism for refractures and supports the development of therapeutic strategies to preserve bone quality during late healing.



IMAGES AND TABLES:

Figure 1: Multiscale spatio-temporal assessment of fracture healing in the murine femoral osteotomy model.
Representative 3D µCT reconstruction of a mouse femur following standardized mid-diaphyseal osteotomy stabilized with an external fixator. Red boxes indicate regions of interest analyzed at the fracture callus and adjacent cortical bone. Schematic timeline of experimental endpoints from day 7 to day 28 post-osteotomy, illustrating the sequential evaluation of fracture healing. Overview of multiscale analyses applied to the callus: histology for microstructure, histomorphometry for bone cells, immunofluorescence for vascularization, quantitative backscattered electron imaging for mineralization, and nanoindentation for biomechanical properties.