11º CONGRESSO NACIONAL DE BIOMECÂNICA
A. P. G. Castro, C. Pina, P. R. Fernandes, C. Santos, J. Folgado & A. Amaro (Eds)
Sesimbra, Portugal, 21-22 de Fevereiro, 2025

SPATIALLY-RESOLVED GENE MECHANOREGULATION ANALYSIS DURING BONE FRACTURE HEALING UNDER MECHANICAL LOADING

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KEYWORDS: Correlative multimodal imaging, Micro-computed tomography, Preclinical model, Spatial transcriptomics, micro-Finite Element Analysis

1 INTRODUCTION

Bone fracture healing is a tightly regulated process that finely balances bone adaptation and regeneration, and is sensitive to local mechanical signals [1]. While this phenomenon has been extensively analysed at the tissue-level, the molecular mechanisms underlying this process remain incompletely understood. Since mechanobiological events in bone span multiple temporal and spatial scales, novel correlative multimodal imaging approaches are needed to investigate such complex hierarchical arrangements. Thus, we have introduced Spatial μProbe [2], a correlative multimodal imaging tool to integrate *in vivo* 3D micro-computed tomography (micro-CT) images with *ex vivo* 2D histological sections from the same sample. Using spatial transcriptomics (ST) data, the expression of individual genes can be correlated with the local mechanical environment computed by micro-finite element analysis (micro-FE). Here, we hypothesised that gene expression is spatially regulated by the local mechanical environment during fracture healing, with different responses in expression upon additional mechanical loading.

2 METHODS

The data used in this work was collected in a previous study: four 12-week-old female mice received mid-diaphyseal femoral osteotomies, followed by individualized cyclic mechanical loading via the external fixator (ML, n=2) or sham-loading (SL, n=2), from three weeks post-surgery onwards. Weekly *in vivo* micro-CT scans (10.5 μm, vivaCT 80) were performed until mice were euthanized (5 weeks post-surgery). Structurally intact sections (one per group) were processed with the 10x Genomics Visium ST protocol [3]. Micro-FE analysis was performed on binarized micro-CT scans (395 mgHA/cm^3) to compute the mechanical signal as effective strain (EFF) for weeks 4 and 5. 2D sections were positioned in 3D using Spatial μProbe, and EFF values were collected at each pixel inside bone of the ST section (micro-CT from week 5, Fig. 1A). Using iStar [4], super-resolution ST data was produced at micro-CT resolution (Fig. 1B). Gene expression was described as a function of the mechanical signal for selected genes such as *Sparc*, encoding Osteonectin, which is a protein involved in bone formation and mineralisation, and *Ctsk*, a lysosomal cysteine proteinase involved in bone remodelling and resorption.

3 RESULTS

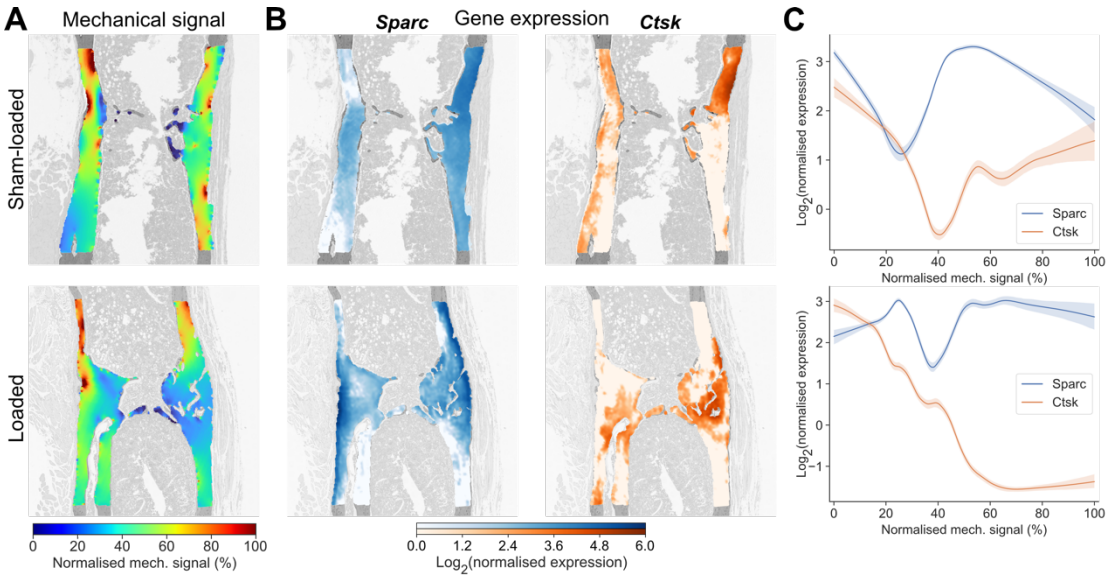

Figure 1 – A) Mechanical signal (week 5) mapped to the 2D-3D registered ST section for the SL and ML samples. B) Super-resolution expression maps of *Sparc* and *Ctsk* for the SL and ML samples. C) Mean gene expression curves after bootstrapped LOWESS and their 95% confidence intervals for both samples.

Our data showed that *Sparc* preserved similar expression profiles in SL and ML samples, with the ML sample maintaining a higher expression at high mechanical signals (>60%), concordant with the stronger anabolic stimulus induced. Conversely, upon mechanical loading, *Ctsk* expression decreased with increasing mechanical signals and was strongly reduced in regions of high mechanical signals (>60%). This result coincided with low likelihood of resorption events in regions of high strain [1], whereas in a less mechanically-driven healing environment (SL sample), regions with high local mechanical signals had limited influence on *Ctsk* expression.

4 DISCUSSION

Our results indicate that gene expression is controlled during fracture healing, with supra-physiological loading producing visibly distinct responses during healing in comparison to the sham-loaded case. Through the identification of mechanical signal thresholds where the expression of key markers changes consistently in response to supra-physiological mechanical loading, we aim to identify functionally and therapeutically relevant strain targets. In conclusion, Spatial µProBe using super-resolution ST data can be used investigate the complex mechanical regulation of gene expression, which can help our understanding of multiscale bone mechanobiology in ageing, disease and treatment.

ACKNOWLEDGEMENTS

Support from ERC-2016-ADG-741883, MSCA-IF-2020-101029062, IZCOZ0198152

REFERENCES:

[1] F. A. Schulte *et al.*, "Local Mechanical Stimuli Regulate Bone Formation and Resorption in Mice at the Tissue Level,", *Plos One,* vol. 8, no. 4, Apr 24 2013, doi: ARTN e62172 10.1371/journal.pone.0062172.

[2] F. C. Marques *et al.*, "A Correlative Multimodal Imaging Approach for Spatial Transcriptomics Mechanoregulation Analysis," 2023 28th Congress of the European Society of Biomechanics, Maastricht, The Netherlands, 2023, p. 742.

[3] E. Wehrle, *et al.*, 'Protocol for preparing formalin-fixed paraffin-embedded musculoskeletal tissue samples from mice for spatial transcriptomics', STAR Protocols, vol. 5, no. 2. Elsevier BV, p. 102986, Jun. 2024. doi: 10.1016/j.xpro.2024.102986.

[4] D. Zhang et al., 'Inferring super-resolution tissue architecture by integrating spatial transcriptomics with histology', Nature Biotechnology, vol. 42, no. 9. Springer Science and Business Media LLC, pp. 1372–1377, Jan. 02, 2024. doi: 10.1038/s41587-023-02019-9.