Spatial Transcriptomics in Mechanomics: New Horizons in Exploring the Mechano-regulation of Bone Regeneration.

Neashan Mathavan[1], Amit Singh[1], Francisco Correia Marques[1], Denise Günther[1], Gisela Kuhn[1], Esther Wehrle[1, 2*], Ralph Müller[1*]
[1]Institute for Biomechanics, ETH Zürich, Zürich, Switzerland, [2]AO Research Institute Davos, Davos Platz, Switzerland
Email: ram@ethz.ch * *equal contribution*

Disclosures: No conflicts of interest to disclose.

INTRODUCTION: In the late 19th century, the German orthopedic surgeon Julius Wolff established the fundamental principle of bone mechanobiology by describing the dynamic nature of bones and their remarkable ability to adapt to their mechanical environment. This fundamental principle underscores the critical importance of the mechanical environment to the regenerative capacity of bone. Mechanical stimuli can either enhance or impair the regenerative process. At its core, the mechanobiology of bone regeneration is governed by the response of cells at the fracture site to physical stimuli. However, it has proven extremely difficult to investigate the transduction of mechanical stimuli exerted at the organ level to site-specific cellular responses at the molecular level. Mouse loading models and *in vivo* imaging techniques (time-lapsed micro-computed tomography, micro-CT) are well-established within the field. Coupled with multi-scale *in silico* modelling (micro-finite element analysis, micro-FE), morphological changes at the tissue scale can be associated with the mechanical environment. Recent advances in spatially resolved "omics" technologies now permit the comprehensive mapping of molecular pathways and cellular function within the spatial context of complex tissue architectures. To characterize mechano-transduction of physical stimuli at the molecular level and cell-driven responses to changes in the local mechanical environment, we propose the multiplexed use of *in vivo* micro-CT imaging, micro-FE modelling and spatial transcriptomics in a mouse femur defect loading model. Our aim is to demonstrate a spatial transcriptomics-based "mechanomics" strategy to identify the molecular mechanisms governing the mechanobiology of bone regeneration.

METHODS: Female 12-week-old bone cell reporter (BCR) mice (n = 4) received mid-diaphyseal femoral defects (0.68 ± 0.04 mm) stabilized with an external fixator (license number: ZH229/2019; Kantonales Veterinäramt Zürich, Zurich, Switzerland). ***In vivo* imaging:** *In vivo* micro-CT imaging of the fracture site was performed weekly in all mice (weeks 0-5; vivaCT 80, 10.5 µm resolution). Following bridging of the fracture site at 3 weeks post-surgery, the mice received either individualized cyclic mechanical loading via the external fixator (n = 2, 9-16N, 10Hz, 3000cycles; 3x/week) or sham-loading (n = 2). All mice were euthanized at 5 weeks post-surgery. **Spatial transcriptomics:** Spatially resolved transcriptomics analyses were performed on explanted femurs (n = 1 per group) using the Visium Spatial Gene Expression for formalin-fixed paraffin-embedded (FFPE) protocol (10x Genomics) [1]. Sequencing data was processed using the SpaceRanger analysis pipelines (10x Genomics) to spatially map the whole transcriptome. Further downstream statistical analyses and visualization were performed in R using Seurat and Bioconductor packages. ***In silico* modelling:** Micro-FE analyses based upon the *in vivo* micro-CT images were used to simulate axial compression and generate tissue-scale 3D maps of the mechanical environment. Visium histological sections were visually aligned within the 3D maps of the mechanical environment to correlate spatially resolved gene expression profiles with their local *in vivo* mechanical environments (Paraview). Maps of each mechanical environment were sub-divided into regions of high / low strain and the corresponding gene expression profiles were analyzed. To characterize gene expression profiles at sites of high and low strain, the co-efficient of variance (CV) was used to identify genes of functional significance across all spots within a region.

RESULTS: At week 5, BV/TV in the defect center was 40.9 ±0.8% in Control mice vs. 70.8 ±6.5% in Loaded mice – thus, cyclic mechanical loading induced larger callus / bone volume formation (Fig 1). Loading induced an increased rate of bone formation (0.73 ±0.2% per day in Control mice vs. 1.67 ±0.3% per day in Loaded mice) and a diminished rate of bone resorption (-0.71 ±0.2% per day in Control mice vs. -0.11 ±0.0% per day in Loaded mice) in the defect center. Differential gene analyses of bone regions at the fracture sites of Control and Loaded mice revealed significantly higher expression of markers of osteoblast differentiation and osteoblast activity in response to loading: *Col1a1*, *Bglap*, *Runx2* and *Alpl* (Fig 2). Further indicative of an augmented osteogenic response at the loaded fracture site, markers of mineralizing osteocytes: *Dmp1*, *Phex*, and mature osteocytes: *Mepe*, were also upregulated (Fig 2). In comparisons of gene expression profiles in regions of high / low strain at the Loaded fracture site, the top-ranked genes in regions of high strain included *Col1a1*, *Col1a2*, *Bglap* and *Sparc* (Fig 3). In contrast, the top-ranked genes in regions of low strain included *Mmp9*, *Ctsk*, *S100a8* and *Ncf1* (Fig 3).

DISCUSSION: Micro-CT-based bone morphometric analysis confirmed the effect of cyclic mechanical loading with a significantly enhanced osteogenic response producing much larger callus / bone volumes. Visualization of spatial gene expression at the fracture sites and differential gene expression analyses further underscored this osteogenic effect with upregulation of genes associated with endochondral ossification, matrix synthesis, mineralization and mechano-regulation. Genes without defined roles in bone regeneration also featured prominently in the differential gene expression analyses underscoring the transformative potential of spatial profiling technologies in unravelling molecular pathways and mechanisms. In associating molecular pathways at the cellular scale with their local mechanical *in vivo* environment within a single histological section, cells in regions of high strain were found to express genes involved in bone formation responses whereas cells in regions of low strain were found to express genes involved in bone resorptive responses. This finding is in agreement with the fundamental principle of Wolff's Law on the capacity of bone to functionally adapt to its mechanical environment. This demonstration within a single histological section of bone thus represents a significant achievement with the potential to develop a molecular based understanding of the mechano-regulation of bone regeneration. Further optimization of this strategy will consider improving the accuracy of the FE simulation and improving the alignment of the histological section within the micro-CT-derived mechanical environment.

SIGNIFICANCE/CLINICAL RELEVANCE: Insights into the mechano-regulation of bone regeneration will have implications for the broader translation of mechano-therapeutics to clinical settings and will potentially identify new strategies and novel mechano-responsive targets to enhance regeneration in compromised healing environments.

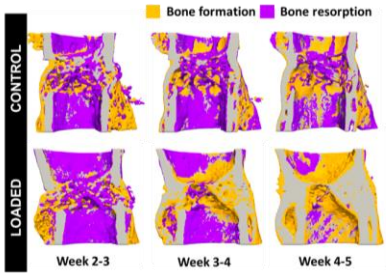

Fig 1. Volume rendering of sites of bone formation (orange) and bone resorption (purple) at the fracture site via registration of time-lapsed micro-CT images.

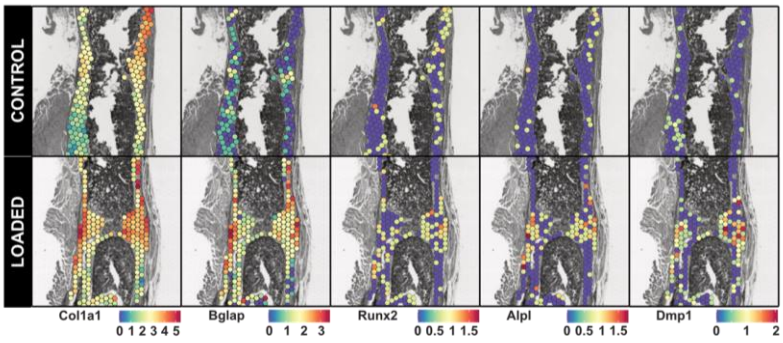

Fig 2. Spatially resolved gene expression of osteogenic bone markers.

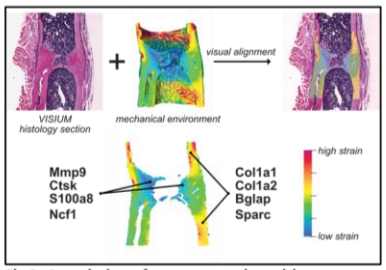

Fig 3. Association of gene expression with respect to the local *in vivo* mechanical environment.

REFERENCES: [1] Wehrle et al., ORS 2023.
ACKNOWLEDGEMENTS: EU funding: (ERC-2016-ADG-741883) and (101029062-MechanoHealing-MSCA-IF-2020).