Multi-omics profiling of bone regeneration in diabetes reveals association of increased mast cell activity with healing impairment

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) and bone healing

- The systemic metabolic disorder T2DM also affects the bone metabolism and poses an increased risk for impaired bone defect healing[1,2].
- However, molecular mechanisms and dynamics of compromised healing progression in diabetic patients are not yet fully understood.
- Consequently, current treatment options for complex bone injuries in these patients lack personalized treatment strategies.

AIM

Mapping the dynamics between T2DM and impaired bone healing

- by analysing the molecular profiles of different scaffold-supported bone healing progressions using multi-omics techniques
- to identify diabetes-specific alterations leading to impaired bone healing
- in order to develop functionalized PCL scaffolds that target these dysregulated processes to support the endogenous bone regeneration.

METHOD OPTIMISATION

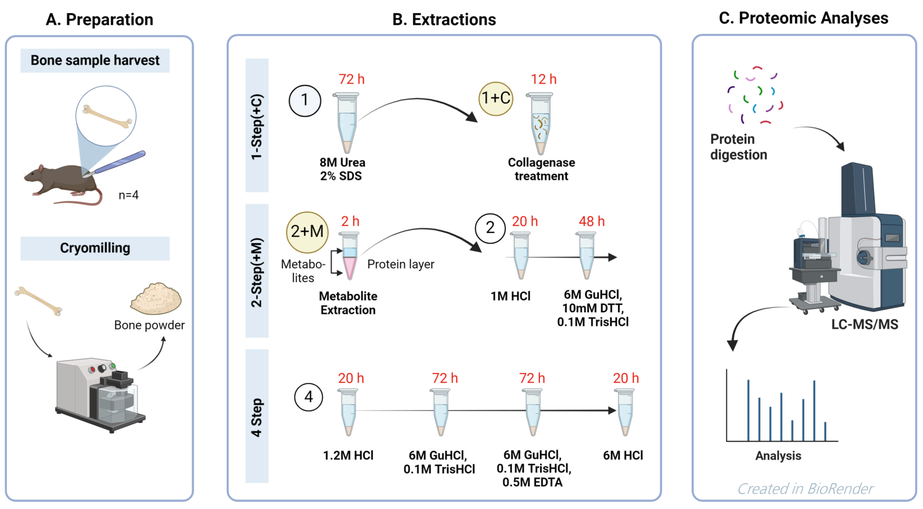


Figure 1. Schematic summary of study design for method comparison for efficient bone proteome extraction and subsequent analysis by LC-MS/MS using a timsTOF PRO 2.

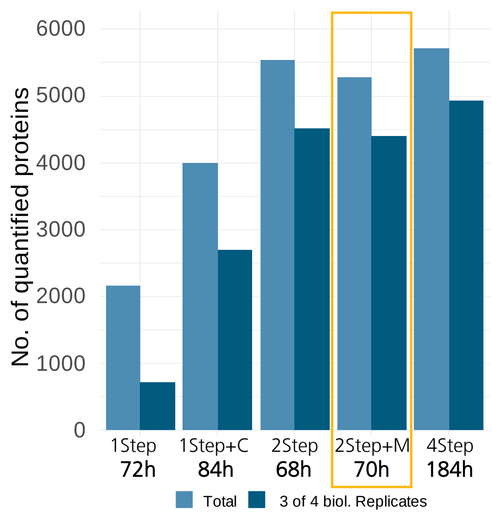


Figure 2. Number of quantified and reproducibly quantified proteins per method.

2-Step+M: Novel streamlined multi-biomolecule extraction protocol

- ✓ Short extraction time (< 3 days)
- ✓ Great proteome depth (4,393 reproducibly quantified proteins)
- ✓ Reduced collagen content by sequential extraction (no collagenase)
- ✓ Compatibility with upstream metabolite and lipid extraction[3] (2Step+M)

ANALYSIS OF MOLECULAR PROFILES

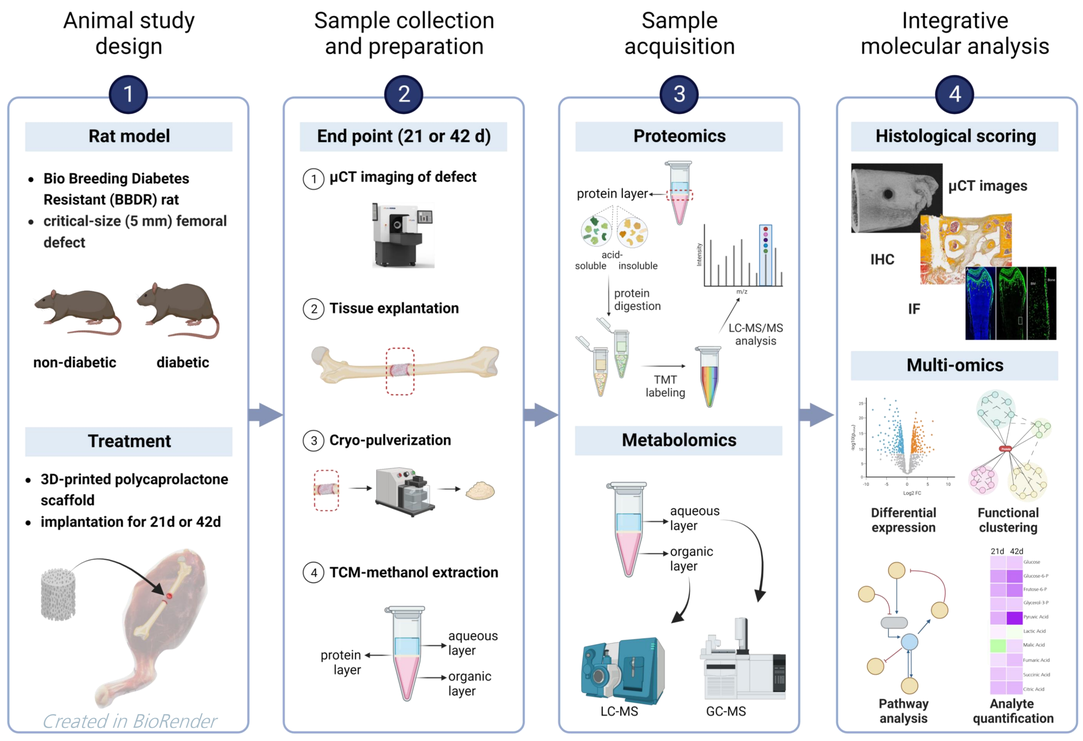
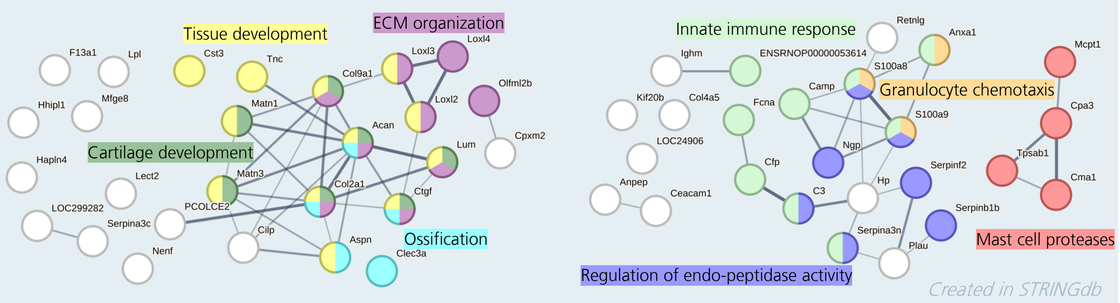


Figure 3. Schematic summary of study design for multi-omics animal study. Proteomic analyses, immunofluorescence (IF) staining done at Fraunhofer IZI, animal study, immunohistochemistry (IHC) staining, metabolomic and µCT analyses done at Charité Berlin.

Prolonged inflammatory state and increased mast cells presence in diabetic bone healing after 42 days



- Higher abundant DAPs involved in cartilage and/or ECM formation
- Example: Lysyl oxidases (Loxl) crosslink collagens in advanced state of bone regeneration

- Higher abundant DAPs typical for primary inflammation during healing
- Example: Chemokines recruiting innate immune cells or mast cell proteases that degrade ECM

Mast cells and mediator histamine significantly increased

- Mast cells appear 1.8x often in regenerated tissue of diabetic animals
- Increased histamine levels, a potent mast cell mediator for inflammation

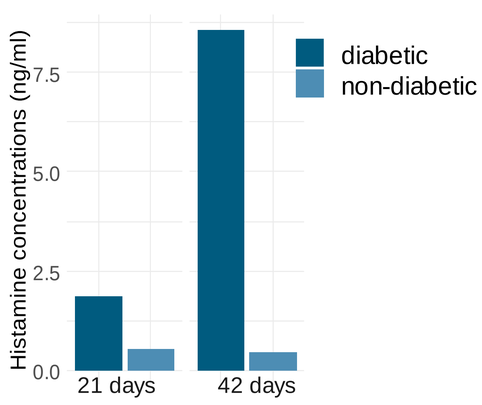
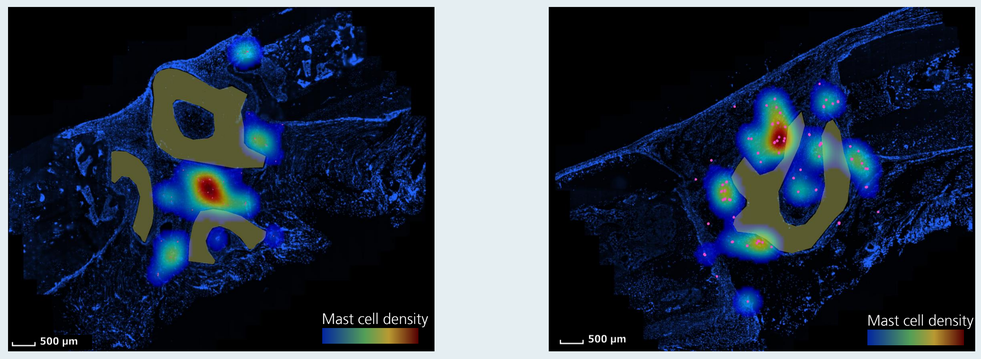


Figure 4 (left). Functional protein-networks of "Extracellular region"-annotated differentially abundant proteins (DAPs) in non-diabetic and diabetic animals at 42 days (top) and IF-stained Cpa3⁺/histamine⁺-mast cells in the regenerated area with PCL-scaffold.

Figure 5 (right). Histamine concentrations in PCL-supported regenerative bone tissue



CONCLUSION

- Study setup allows for multi-omics correlation of proteome, metabolome and imaging data of same specimens over a 42 days healing course and enables comprehensive description of proteomic state in diabetic bone healing
- Observed delayed bone healing in diabetic animals (not shown) correlates with results obtained from molecular analyses.
- Evidence from functional annotation analysis of DAPs, histological and metabolomic data point towards a dysregulated and active population of mast cells in diabetic bone healing.
- **Ongoing preclinical study will test interventions targeting mast cell activity to enhance regeneration.**

References
1 Marin C. *et al.* (2018), *Front. Endocinol.* 9:6
2 Jaber M. *et al.* (2022), *Front. Bioeng. Biotechnol.* eCollection
3 Dias D. *et al.* (2022), *Metabolites* 12(5):453

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