Mass Spectrometry-Based Proteomics of Polymethyl Methylacrylate-Embedded Bone

Annika Topitsch[1,2,3], Tim Halstenbach[1,4], Annekathrin Kopp[5], Annette Lindner[4], Susanne Nahles[5], Oliver Schilling[1], Tobias Fretwurst[4,6] and Katja Nelson[4]

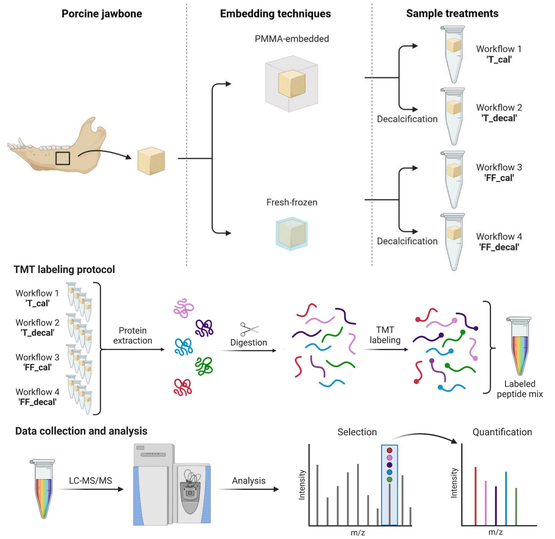
[1]Institute for Surgical Pathology, University Medical Center Freiburg, Germany
[2]Spemann Graduate School of Biology and Medicine (SGBM), University of Freiburg, Germany
[3]Faculty of Biology, University of Freiburg, Germany
[4]Department of Oral and Maxillofacial Surgery, University Medical Center Freiburg, Germany
[5]Department of Oral and Maxillofacial Surgery, Charité Berlin, Germany
[6]Department of Oral Medicine, Infection and Immunity, Harvard School of Dental Medicine, Boston, USA

Introduction

Polymethyl methacrylate (PMMA) embedding media are widely used for the preservation of hard tissues such as bone and teeth. Various studies have shown the applicability of PMMA-embedded specimens for histological and immunohistochemical investigations [1-3]. However, for further research on the cellular and molecular mechanisms in bone, in-depth and high-throughput analysis techniques are essential. One such technique is mass spectrometry (MS)-based proteomics, which is used to identify and comparatively quantify the proteins in a biological sample. At present, clinical proteomics largely focuses on soft tissues or body fluids, while the use of bone as a sample is not well established [4-6]. This study is the first to answer the question: **Are PMMA-embedded bone specimens suitable for MS-based proteomics?**

Methods

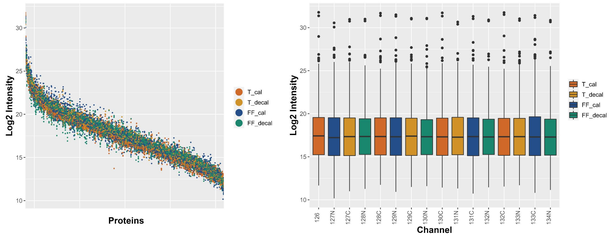
Samples: Porcine jawbone cut into 5 mm cubes
Embedding: Technovit 9100 or fresh-frozen in liquid nitrogen
Treatment: Decalcification in HCl for 17 days for half of the samples
Protein extraction: Grinding using Precellys MK28 beads; sonication; incubation at 95°C in SDS
Digestion: Reductive alkylation using TCEP and CAA; digestion with trypsin protease
Labeling: Chemical labeling using isobaric tandem mass tags (TMTs)
Measurement: Data-dependent acquisition on a Q Exactive Plus mass spectrometer



Results

1 Quantification of Proteins

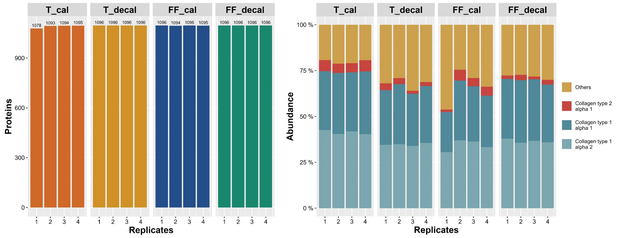
- Quantified proteins cover a wide range of abundances in all samples and replicates
- Homogenous distribution of intensities in TMT labeling channels



Left: Identified proteins ranked by logarithmic abundance. Right: Distribution of logarithmic abundances per TMT channel after median normalization. Data analysis was performed using the FragPipe software.

2 Identification of Proteins

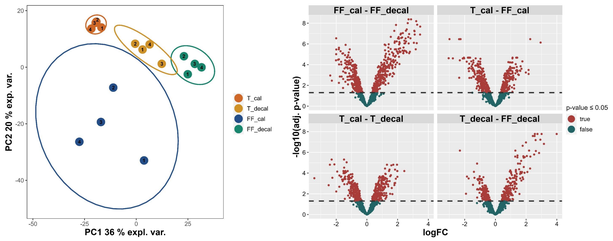
- 1096 proteins identified across all samples
  → similar numbers for Technovit and fresh-frozen samples
- Three most highly abundant proteins:
  Collagen type 1 alpha 1 and 2, collagen type 2 alpha 1



Left: Numbers of identified proteins per sample. Right: Percentage of the three most highly abundant proteins per sample.

3 Proteome Comparisons

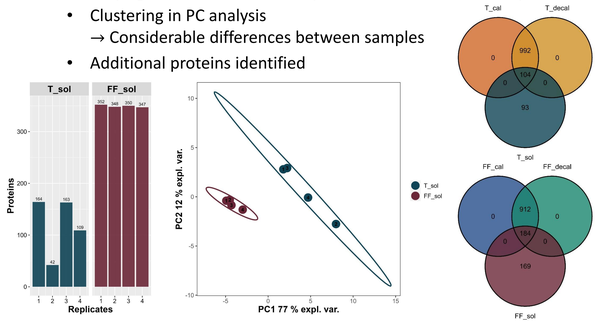
- Clustering in PC analysis → Considerable differences in proteome of Technovit and fresh-frozen samples
- High reproducibility and comparability within workflows
- Numerous significantly differentially abundant proteins



Left: Principal component (PC) analysis showing the clustering of workflow replicates. Right: Differential abundance analysis comparing the effects of embedding techniques and decalcification reveals substantial fold changes (FCs) of proteins that are significantly differentially abundant between workflows. Statistical analysis was performed in R using the limma package.

4 Effect of Decalcification

- Varying numbers of identified proteins within Technovit replicates
- Clustering in PC analysis
  → Considerable differences between samples
- Additional proteins identified



Left: Numbers of identified proteins per sample. Middle: PC analysis showing the clustering of workflow replicates. Right: Overlap of identified proteins of Technovit-embedded (top) and fresh-frozen (bottom) samples for all sample preparation workflows. Data analysis was performed using the MaxQuant software.

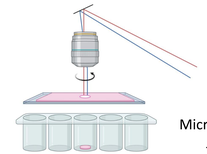
Conclusion

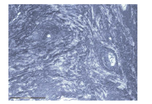
- High numbers of identified proteins, independent of embedding technique and sample treatment
- Both, embedding technique and sample treatment, affect proteome composition
- Consistent sample handling ensures high reproducibility and comparability
- Decalcification can increase the numbers of identified proteins, but is not recommended for Technovit-embedded samples

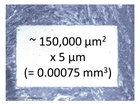
→ Yes, MS-based proteomics can be performed on PMMA-embedded bone specimens

Outlook

Laser microdissection of Technovit sections







Microscopic isolation of bone tissue from Technovit sections
→ Challenging task to detect low-abundant proteins

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