High Depth Proteomics of Synovial Fluid Reveals Osteoarthritis Biomarkers

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Introduction

- Osteoarthritis (OA) is the most common form of arthritis with no disease-modifying therapies.
- Synovial fluid (SF) serves as a valuable liquid biopsy for biomarker discovery.
- SF proteomics is challenging due to extreme dynamic range in protein concentrations[1].
- Enrich-iST enrichment technology combined with timsUltra AIP sensitivity enables deep SF coverage.
- **Goal:** Maximize SF proteome and identify OA-associated proteins.

Methods

- **Samples:** 10 µL SF from healthy (n=4) and OA (n=4) patients
- **Sample Prep:** Enrich-iST kit (PreOmics) (Figure 1)
- **LC:** EVOSEP Eno, 30 SPD method, PepSep column (15 cm × 150 µm × 1.5 µm)
- **MS:** Bruker timsUltra AIP, CaptiveSpray Ultra source, dia-PASEF mode; IM 0.75–1.3 V·s/cm², 100–1,700 m/z
- **Data Analysis:** Spectronaut 20 (directDIA+ mode), Human UniprotKB reviewed database; statistical analysis in R and Python

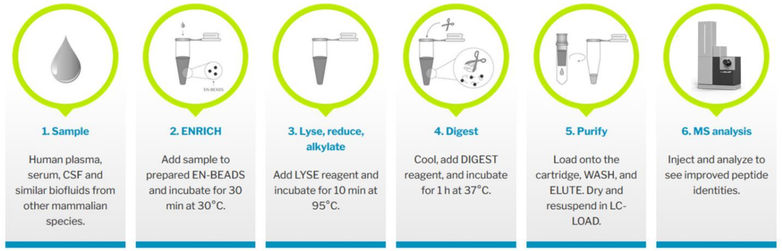

Figure 1: Sample preparation steps for the PreOmics Enrich-iST protocol.

Results

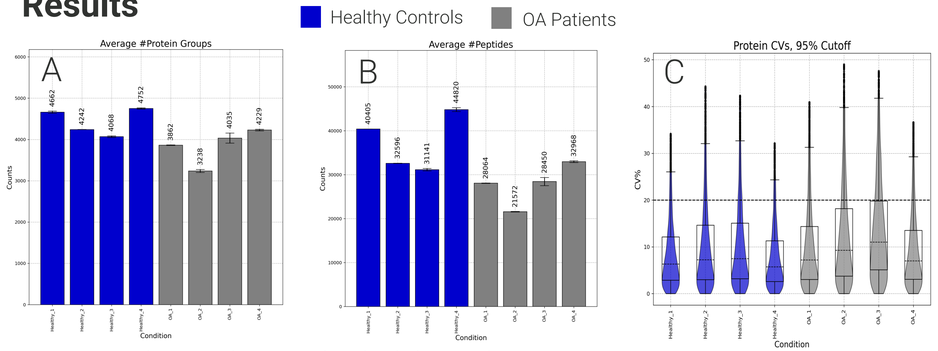

Figure 2. Enrich-iST enrichment combined with the timsUltra AIP system enabled very deep protein profiling of synovial fluid from knee joints. ~4,800 protein groups, ~45,000 peptides were identified with excellent reproducibility (median CVs ≤10%).

- **Coverage:** ~4,800 protein groups and >45,000 peptides per sample — to the authors knowledge the most extensive SF proteome coverage reported without fractionation or depletion (Figure 2A and B).
- **Reproducibility:** Median CVs ≤10% across technical replicates (Figure 2C).
- **Separation:** PCA analysis shows clear separation between controls and OA (Figure 3A).
- **Differential Expression:** Numerous proteins significantly up or downregulated in OA, including matrix metalloproteinases (MMPs) implicated in cartilage degradation (Figure 3B).
- **Pathway Enrichment:** Inflammation, cytokine production, and immune response pathways altered in OA (Figure 3C).

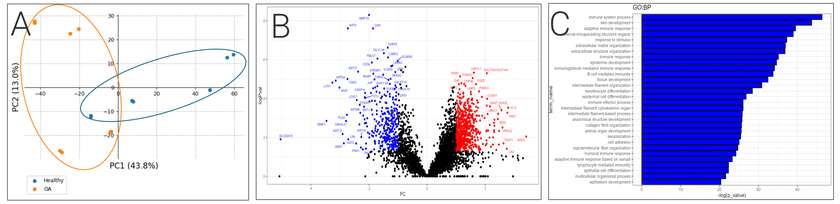

Figure 3: (A) Principal component analysis (PCA) of synovial fluid proteomes clearly separates osteoarthritis (OA) patients from healthy controls, demonstrating distinct molecular signatures. (B) Volcano plots from t-test statistical comparisons highlight numerous proteins significantly up- or downregulated in OA. (C) Gene Ontology enrichment analysis reveals key biological processes altered in OA, including extracellular matrix remodeling, inflammation, and immune response.

Conclusion

- The Enrich-iST kit and timsTOF Ultra AIP identified ~4,800 protein groups and 45,000 peptides in synovial fluid, achieving record coverage without fractionation.
- The method showed strong reproducibility, with median CVs at or below 10% for proteins and peptides.
- PCA analysis distinguished OA patients from healthy controls based on proteomic profiles. Numerous OA-associated proteins were significantly up- or downregulated in OA compared to controls.
- MMPs were notably changed, implicating them in cartilage degradation and OA development.
- The approach reveals potential OA biomarkers and therapeutic targets.

Technology

1.Peffers, M. J., Smagul, A., & Anderson, J. R. (2019). Expert Review of Proteomics, 16(4), 287–302.

COI Statement: MW and DA are employees of Bruker Scientific LLC. CE is an employee of PreOmics

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