

The Impact of Peri-implantitis on the Proteome Biology of Crevicular Fluid: A pilot study

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Introduction

Peri-implantitis affects up to 20 % of all dental implants.^{1, 2} However, the clinical maintainance of implants can be challenging, since the current diagnostic tools (including probing depth evaluation, bleeding on probing and x-ray examinations) only allow to detect peri-implantitis in late stages when significant bone loss already occoured.³ The peri-implant crevicular (PICF) has been proposed to be a source for new diagnostic markers. A variety of inflammatory cytokines has been identified in the PICF, yet these only provide low specificity and sensitivity for peri-implant diseases and most studies available examine a priori selected biomarkers (n=1-10) from the group of proinflammatory immune modulators^{4, 5}.

Mass spectrometry (MS) based proteomics enables quantification and identification of almost all proteins in the medium allowing for multibiomarker approaches and non-targeted exploratory essays. The PICF proteome has not been investigated by comparing healthy and diseased implants nor has the proteomic composition found in PICF been compared to the composition of gingival crevicular fluid.

Objective of the Study

The main goal was to analyze and compare the proteomic composition of the crevicular fluid of healthy and diseased implants and of healthy teeth by performing mass spectrometry-based proteomics.

Therefore, the aim of the present study was to

- Gain insights into the disease biology of peri-implantitis;
- Identify potential biomarkers for detection of peri-implant diseases.

Materials and Methods

In this case-control study, PICF and GCF samples were collected from healthy implants, healthy teeth and implants with peri-implantitis. Samples of different conditions were harvested in the same patients to guarantee better comparability.

Ethics consent was given by the ethics committee of the University of Freiburg, Germany.

Inclusion Criteria

Patients with at least one peri-implantitis affected implant were included. Periimplantitis was defined as follows:

- Peri-implant pocket depth $\geq$ 6 mm;
- Radiological bone loss $\geq$ 3 mm;
- Bleeding on probing and / or suppuration.

Patients were excluded in case of:

- Diabetes mellitus;
- Immunosuppression / immunosuppressive medication;
- Present malignancies of the head and neck;
- Oral mucosal disease;
- Bisphophonate of antiresorptive agent intake;
- Untreated periodontal disease or gingivitis;
- Bruxism;
- Inadequate oral hygiene.

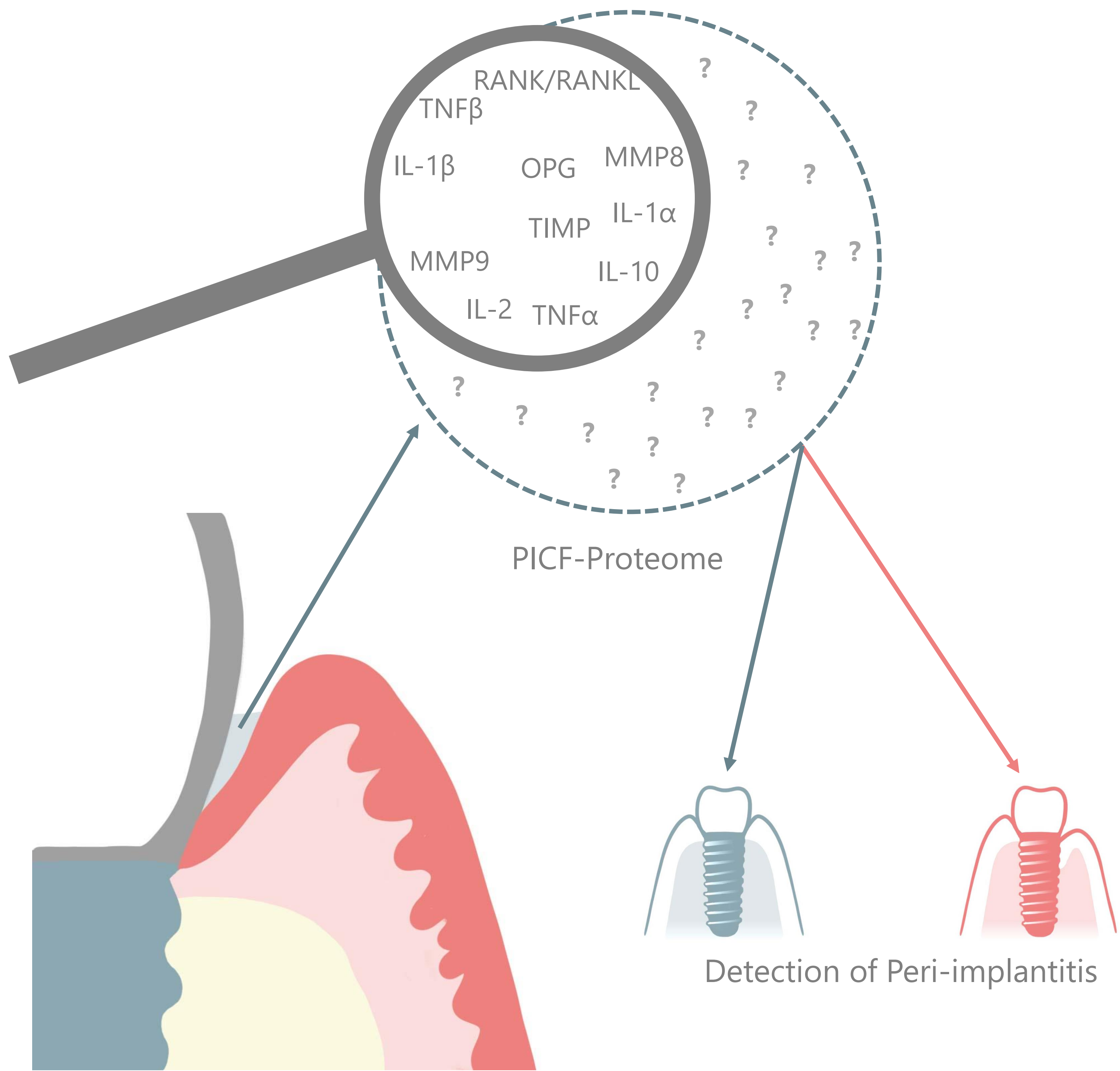


Figure 1: The peri-implant crevicular fluid (PICF) was proposed as a potential source for biomarkers of peri-implantitis and mucositis. With the help of antigen-based assays approximately 100 cytokines and proteins have been identified in the PICF, yet these only show low sensitivity and specificity for peri-implant diseases. Furthermore, the true proteomic composition of PICF remains unknown.

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Methods

38 samples of healthy and diseased implants and of periodontally healthy teeth were harvested from 13 patients. Sterile paper tips were placed for the 30 seconds in the mesio-buccal sulcus and subsequently frozen away at -80°C until samples processing.

Samples were processed using a unique protocol, including denaturation and tryptic cleavage of proteins. Samples were analyzed on a liquid chromatography tandem mass spectrometer (LC-MS/MS) in a data independent approach (data independent acquisition; DIA). DIA enables higher proteome coverage and better reproducibility in exploratory proteomics.^{6, 7}

Raw-data was processed in DIA-NN. Here, not only human proteins were identified and quantified, but also bacterial proteins and cleaved peptides.

Statistical analysis was performed in R, applying partial least squares regression (PLS-DA) and models for microarray data (*LIMMA*). Proteins were considered differentially expressed if the adjusted p-value was below 0.05 and the protein intensity was above +/- 50%. A gene ontology-analysis was applied subsequently to find up and downregulated biological pathways.

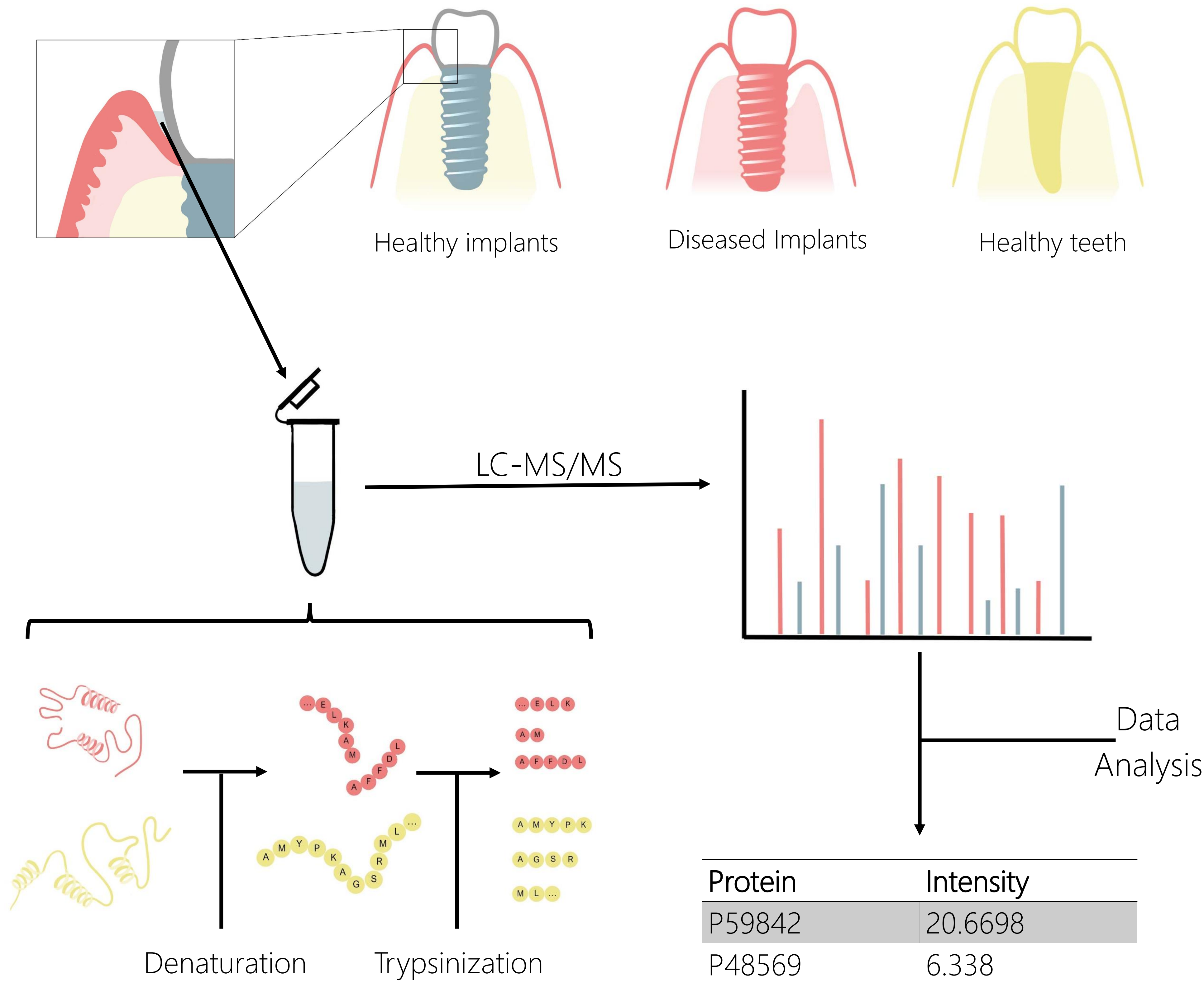


Figure 2: Workflow of sample harvesting and processing. PICF was collected from healthy and diseased implants and healthy teeth. Subsequently, samples were processed in a unique protocol including denaturation of proteins and protein cleavage with trypsin. Analysis was performed with untargeted liquid chromatography tandem mass spectrometry (LC-MS/MS)

Results

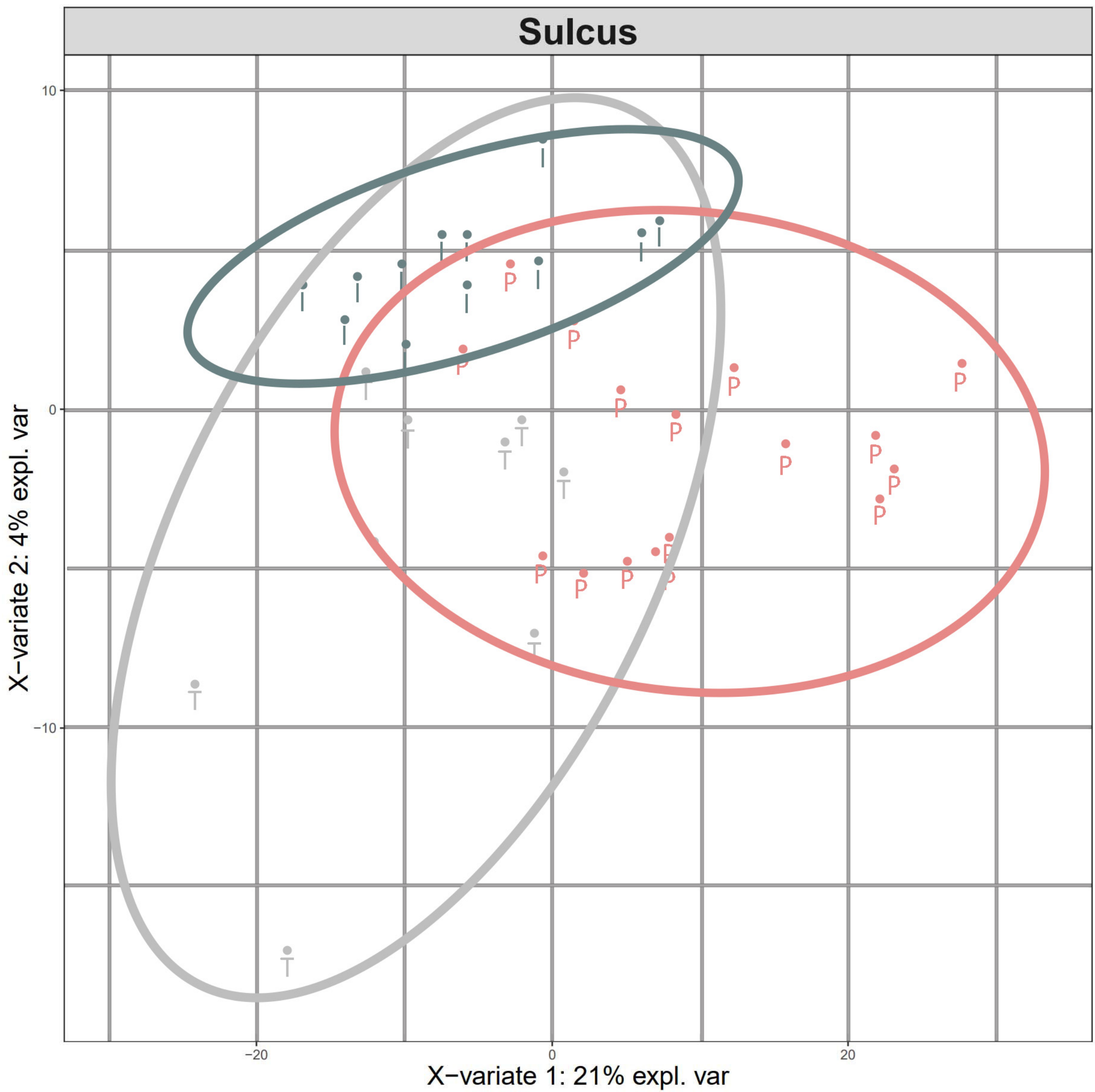


Figure 3: PLS-DA of samples of peri-implantitis (P), healthy implants (I) and healthy teeth (T) demonstrates proteomic differences between diseased implants and healthy conditions.

More than 2300 unique proteins were identified and quantified across all samples. PLS-DA indicated proteomic differences between diseased and healthy implants and strong similarities between the healthy conditions.

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Biological Processes

Gene-ontology analysis was applied to identify biological processes up- or downregulated in peri-implantitis. Here, mainly inflammatory processes, as “humoral immune response” or “cell killing” were found increased in diseased implants.

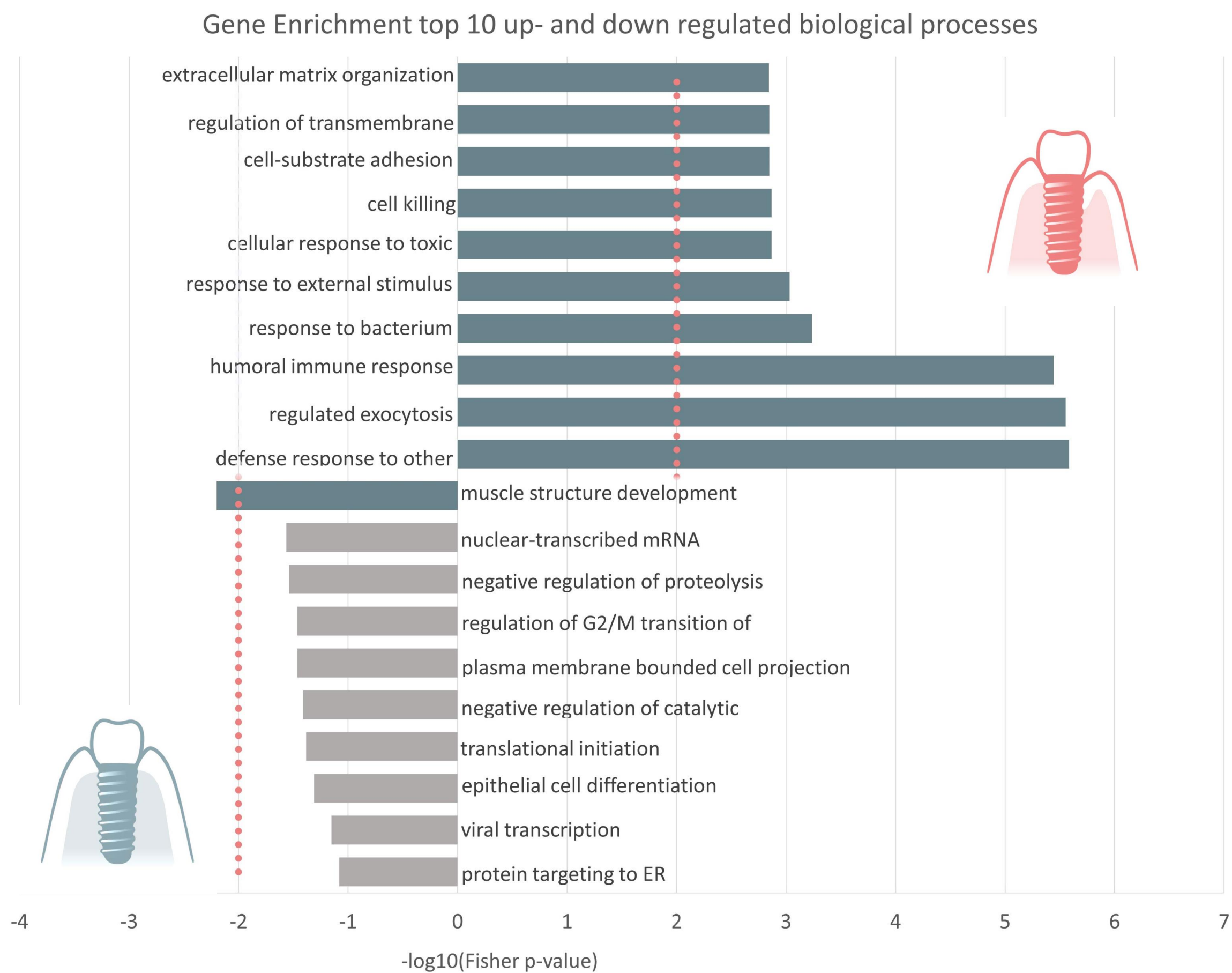


Figure 4: Gene Ontology Analysis identified up- and down-regulated biological pathways in peri-implantitis.

Bacterial Proteins

325 different proteins of bacterial origin were identified. Overall, concentration of bacterial proteins were low in all conditions with higher concentrations in the peri-implantitis subgroup. Most proteins were detected only in single samples. These findings are in accordance with microbiological studies indicating a complex and inhomogeneous bacterial colonization of the per-implant crevice.⁸

LIMMA-Analysis corroborated proteomic differences between peri-implantitis and healthy conditions. Comparing healthy implants and healthy teeth, LIMMA revealed no differentially expressed proteins. For peri-implantitis vs. healthy implants 59 proteins were found upregulated in case of inflammation and 31 upregulated in healthy implants. Enriched proteins in peri-implantitis mainly included inflammatory proteins, interestingly including a variety of enzymes secreted by neutrophils.

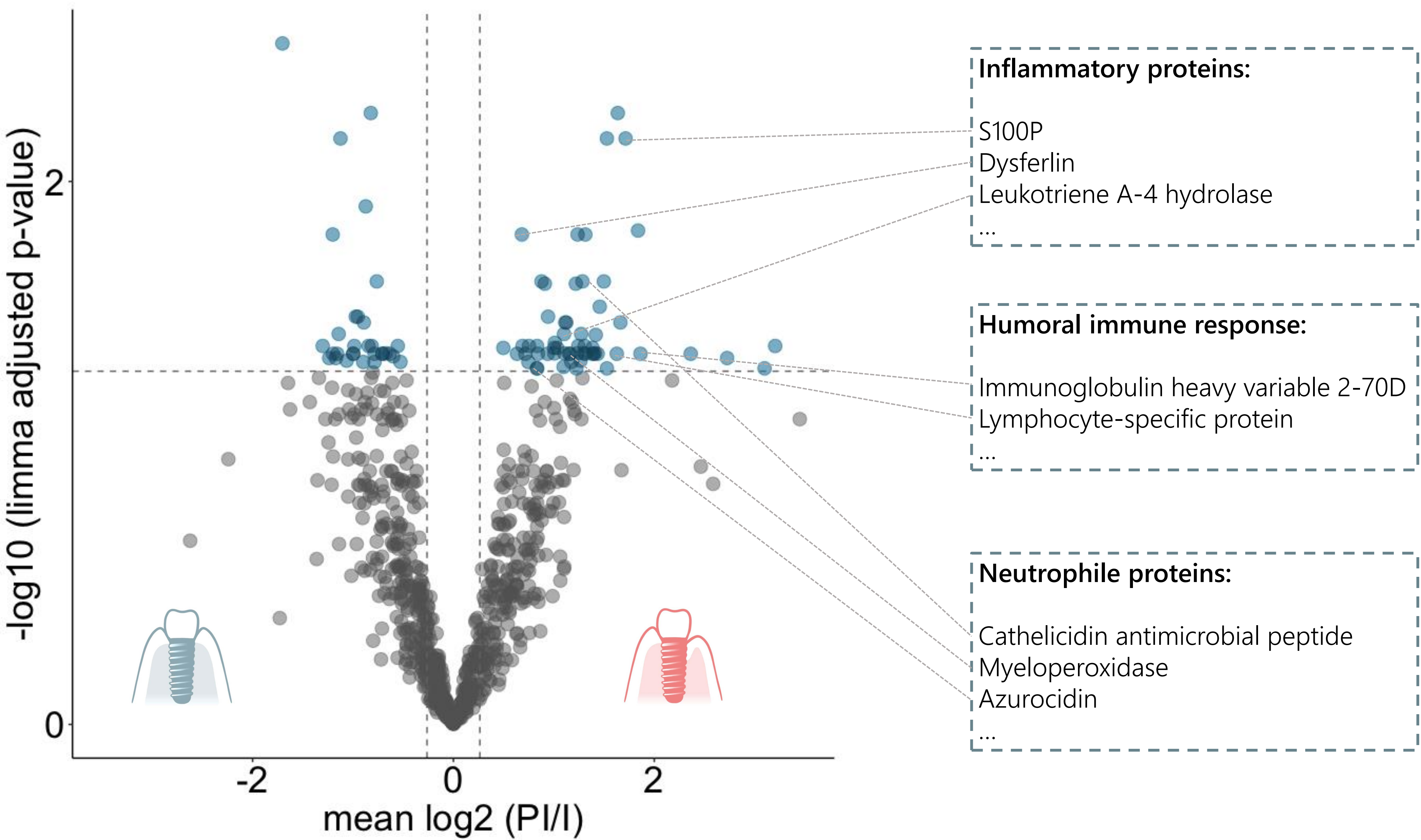


Figure 5: Volcano plot of LIMMA results comparing healthy and diseased implants. 31 proteins were found enriched in healthy implants (left side), 59 were higher in case of inflammation.

Proteolytic Activity in Peri-implantitis

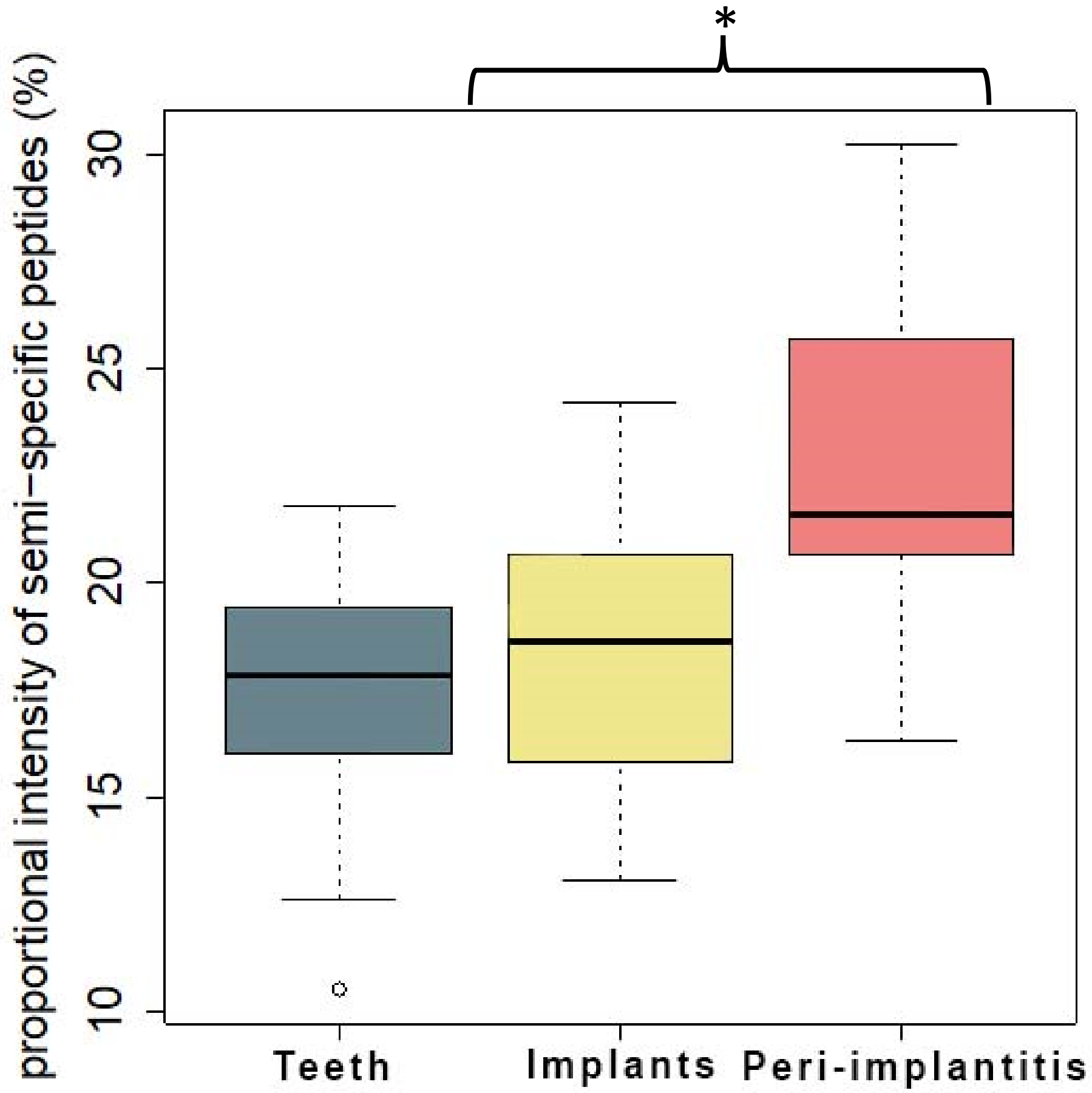


Figure 6: Proteolytic activity was significantly higher in case of peri-implant inflammation.

Proteolytic enzymes play a central role in inflammations and processes of tissue destruction. For peri-implantitis multiple proteases were found enriched in the PICF, including leukotriene A-4 hydrolase, matrix metalloproteinase (MMP)-8, MMP-9 and cathepsins. By assessing the intensity of degraded (non-tryptic) peptides, increased proteolytic activity was found in peri-implantitis when compared to healthy teeth or implants. Cleavage patterns point to inflammatory proteases like cathepsin G or neutrophil elastase.

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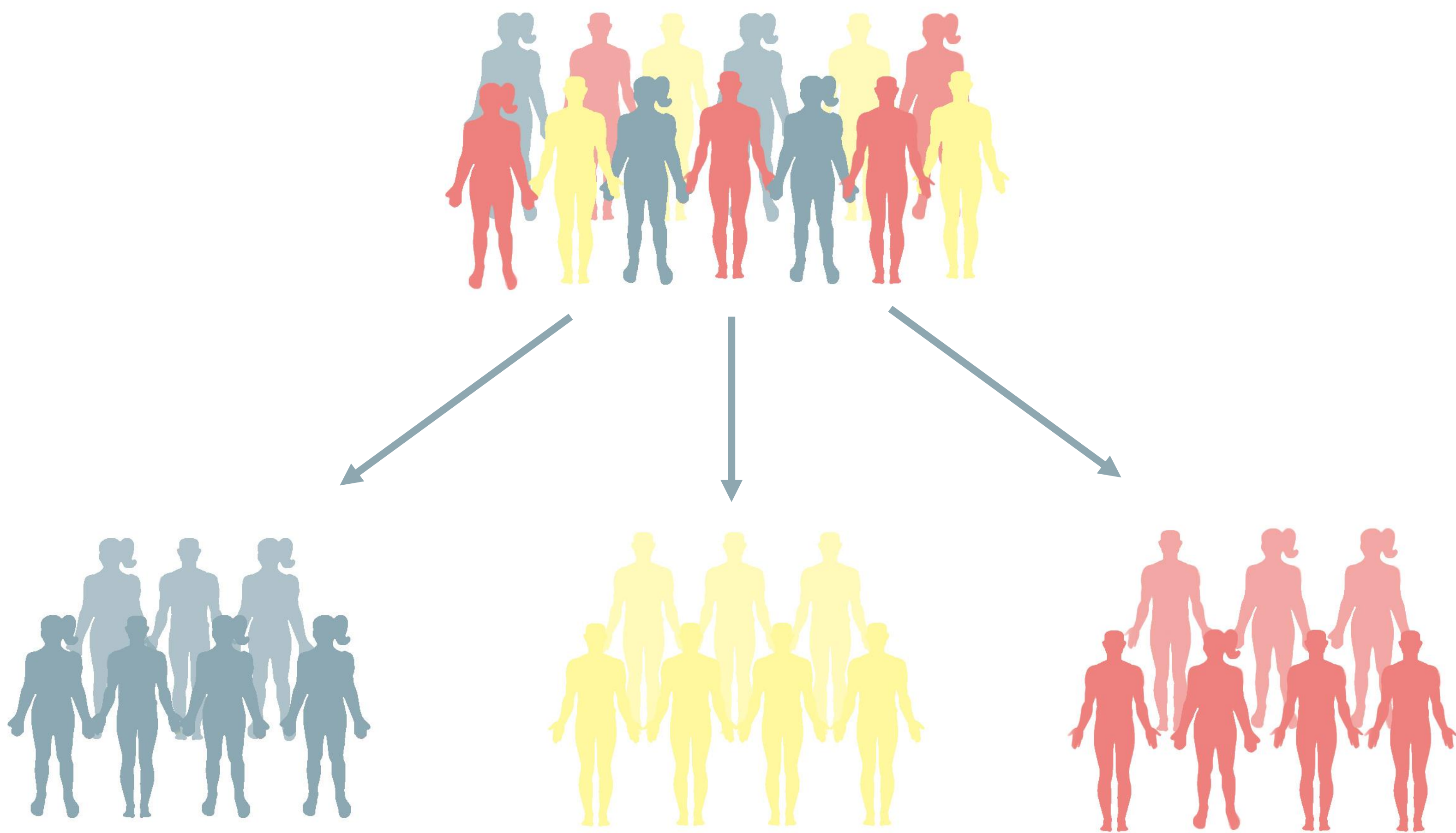
Discussion

The present study was the first to compare the crevicular fluid of healthy teeth, healthy and diseased implants in an exploratory proteomic fashion. Before, only about 100 different proteins had been identified in PICF.⁵ By applying mass spectrometry-based proteomics it was possible to quantify more than 2300 unique proteins, therefore decrypting the PICF composition to an even deeper level.

The data presented here indicates strong similarities in PICF proteome between healthy implants and healthy teeth. In case of peri-implantitis 59 proteins were found increased, hence indicating a specific proteomic fingerprint. These proteomic differences enable insight into disease biology:

- A complex variety of inflammatory processes is increased in peri-implantitis;
- Prevalence of neutrophile proteins may indicate that peri-implant inflammation is mediated by neutrophile activity;
- Proteolytic activity is increased in peri-implant inflammation. Inflammatory proteases like cathepsins or neutrophile elastase may play a part in tissue destruction in peri-implant disease;
- Bacterial proteins identified in the PICF point to a higher bacterial load in the peri-implant crevice in case of inflammation. In the recent literature peri-implantitis was discussed as a multifactorial with rather unspecific microbiological component, which is in accordance with the findings of the present study.

Outlook



As presented in this study, PICF proteomic data can provide patient- and site-specific insights into molecular alterations of the peri-implant biology. Therefore, proteins and protein clusters could not only serve as biomarkers for the (early) detection of peri-implantitis, but also for treatment monitoring and prognosis. Although mass spectrometry-based proteomics provides detailed biological information, that can be used for in-depth analysis of patients of diseased sites, its insights can also be transferred to more easily available, chairside POC test. Recent progress in biomedical technology also holds out the prospect of implementing proteome data into biosensors and so-called labs on a chip⁹, allowing even more precise, real-time monitoring of health and disease of dental implants. Therefore, the proteomic techniques and insights as presented in this study are a step towards personalized peri-implantitis diagnostics and handling, enabling the best treatment for each patient.

Literature

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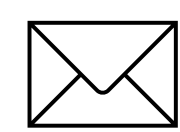
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