Peri-Implantitis versus Periodontitis: Functional Differences Indicated by Transcriptome Profiling

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Background: Periodontitis and Periimplantitis are oftentimes discussed as one entity, which is reflected by therapeutical as well as by scientific approaches. It is unclear, to which extent the similarity of the clinical characteristics is attributed to similarities in the underlying disease mechanisms.

Purpose: The main objective of the study is to display if or how different periimplantitis and periodontitis are on the mRNA level, representing a high-resolution map of disease-associated events.

Materials and Methods: Aiming to describe the pathophysiological mechanisms *in vivo*, primary gingival tissue from 7 periimplantitis patients, 7 periodontitis patients and 8 healthy controls was employed in order to generate genome wide transcriptome profiles.

Results: On the basis of quantitative transcriptome analysis, we could show that periimplantitis and periodontitis exhibit significantly different mRNA signatures. Additionally we present a disease associated mRNA profile, which displays potential periimplantitis disease mechanisms. A gene ontology analysis revealed various pathways, supporting the hypothesis of periimplantitis being a complex inflammatory disorder with a unique pathophysiology. While in periimplantitis tissue the regulation of transcripts related to innate immune responses and defense responses were dominating, in periodontitis tissues bacterial response systems prevailed.

Conclusions: Taken together, our results suggest considering periimplantitis and periodontitis as disease entities with shared as well as with distinct features, which should be reflected on the therapeutical as well as on the scientific level.

KEY WORDS: inflammation, peri-implantitis, periodontitis

INTRODUCTION

Peri-implantitis is defined as an inflammatory process in the environment of dental implants, characterized by infection of soft tissue and loss of the surrounding bone, while lesions that only concern the peri-implant soft tissues are known as peri-implant mucositis.[1] Following the osseointegration process and the so established stability of the implants, long-term success depends on the absence of inflammatory responses. With a growing number of dental implants inserted, the potential number of sites for implant-associated diseases increases.[2]

In contrast to that, periodontitis is characterized by an inflammatory destruction of the supporting apparatus of the teeth, including periodontal ligament and alveolar bone. An infection of the soft tissues with no destruction of the surrounding bone is known as gingivitis. Bacteria play an essential role in periodontitis, but bacteria alone seem to be insufficient to explain disease appearance or progression; a susceptible host is also essential.[3]

Even though the Seventh European Workshop on Periodontology stated clearly that peri-implant diseases display unique features,[4] in clinical settings, peri-implantitis is often compared to periodontitis. Differences seem to exist in the extent and the composition of

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cells in the lesion as well as the progression rate. Similarly, recent studies indicating that peri-implant diseases exhibit exclusive characteristics[5] are widely disregarded. Moreover, both phenotypes are merged into one entity in the context of scientific approaches to investigate the disease etiology as well as in the context of therapy. While natural teeth have an epithelial connection via hemidesmosomes and collagen fibers anchored at the tooth, this compound finally gets lost by extraction of the teeth. The scar tissues around implants may weaken the defense opportunities of the soft tissues.[6]

Several reports agree that the confirmed risk factors for periodontitis are identical to those for peri-implantitis.[5] The risk of peri-implantitis increases with patients who smoke, or with those who have poor oral hygiene.[5] For patients with a preexisting periodontitis, the incidence rate is four to five times higher. The development of periodontitis and peri-implantitis is closely related to the formation of a biofilm containing pathogens. The microbiota associated with both diseases is rich in gram-negative bacteria.[5] In partly edentulous patients around implants periodontal bacteria are dominant,[1,7] suggesting a strong relationship between peri-implantitis and periodontitis. But beside the fact that these bacteria are present, they do not regularly induce peri-implantitis.[8] So it remains unclear which bacteria cause peri-implantitis induction and which bacteria cause peri-implantitis progression. In contrast to partly edentulous patients, no periodontitis-associated bacteria are present in fully edentulous patients before implant installation.[9] This may allow the conclusion that peri-implantitis also occurs without the typical periodontal flora. Interestingly, the failure rates in fully edentulous patients are two times as high in that collective compared to partly edentulous patients.[10] Due to the opportunistic characteristics of periodontitis and peri-implantitis, for both diseases, the therapeutic regimens are anti-infective. In both phenotypes, the main goal is the removal of granulation tissue, disinfection, and the maintenance of an infection-free oral cavity. As implant surfaces are screw-like and rough, the access and disinfection are more difficult so that surgical access may be required more frequently and at an earlier stage than in periodontal therapy.[5]

In the presented study, we for the first time systematically assess the molecular differences between peri-implantitis and periodontitis, providing an additional layer of differences between the two diseases. By using primary gingival soft tissue from peri-implantitis patients, periodontitis patients, and healthy individuals and employing genome-wide mRNA expression profiling, this approach aims to present signatures which are unique and/or common to both phenotypes. Keeping the limitation of this exemplary ex vivo setup in mind, the subsequent pathway analysis of the identified transcripts may help to further understand the pathophysiology of these infections, encouraging scientific approaches which target the two diseases as unique entities. A long-term goal is to generate a high-resolution picture of disease mechanisms, enabling the development of new specific therapeutic approaches.

MATERIAL AND METHODS

Cohort Setup

From 22 consecutive patients (11 men, 11 women, median age: 49, age range: 14–78), recruited at the University Hospital of the Christian-Albrechts-University Kiel, gingival/peri-implant soft tissue samples (one per patient) were harvested. Seven patients were suffering from peri-implantitis (probing depth ≥5 mm, radiographic bone loss around implants exceeding 3 mm, implants in function >1 year). The inflamed soft tissue around the implants was removed during open surgical debridement to treat peri-implantitis according to established study protocols.[11]

Gingival samples from periodontitis patients served as a second patient collective. Eligible patients were older than 18 years, were nonsmokers, and did not have any history of systemic periodontal therapy. According to self-reported smoking history, patients were categorized as current, former, and never-smokers. Never-smokers were patients who had never smoked in their lives. Patients who had quit smoking for at least 5 years were looked upon as former smokers. All other patients were classified as current smokers and excluded from the study.[12] Patients with systemic conditions, which may entail a periodontitis as a manifestation of a systemic disease,[13] were excluded.

The specimens were obtained in open surgical debridement, by a specialized surgeon with the assistance of an operation microscope, while gingival tissue from patients without any clinical infection served as control individuals. These patients were operated, that is, with wisdom teeth removals. The protocol was in accordance to the World Medical Association

TABLE 1 Patient and Control Groups Employed in the Study

	Healthy individuals	Peri-implantitis patients	Periodontitis patients
n	8	7	7
Gender (f/m)	2/6	2/5	7/0
Median age	32	57	49
Age range	14–78	38–71	33–51
Smoker (yes/unclear/no)	0/4/4	1/0/6	0/5/2

f, female; m, male.

Declaration of Helsinki and approved by the local ethics committee (reference: B275/05). Prior to surgical intervention, all patients gave written informed consent to participate in the study. Patients with periodontitis were identified according to the 1999 classification.[14] It is classified as localized if the affected sites are 30% or less and generalized if there are more than 30% affected sites.

Severity is based on the amount of clinical attachment loss (CAL) and is designated as mild (1–2 mm CAL), moderate (3–4 mm CAL), or severe (≥5 mm CAL). Group characteristics are presented in Table 1.

RNA Extraction and Transcriptome Analysis

Total RNA was extracted from biopsies, processed as previously described,[15] and hybridized to Affymetrix Human Gene 1.0 ST arrays following the manufacturer's guidelines. Data were normalized using RMA (R, Bioconductor) and signals that showed equal or lower expression than the 5th percentile of all exon signals were categorized as absent, thus excluded from further analysis. Differences between experimental groups were assessed using the Mann–Whitney *U*-test (software: NAG statistical tools, The Numerical Algorithms Group, UK), while *p* values were corrected for multiple testing using the Benjamini–Hochberg method.[16] Genes with corrected *p* values ≤0.05 were considered significantly differentially expressed. The false discovery rates (FDR) of the fold changes (based on the ratios of the medians of the experimental groups) were assessed using Westfall and Young permutation[17] with K = 5000 permutations. An FDR ≤ 5% was considered acceptable. The effect frequency was calculated as the percentage at which the selected regulation was observed in all patients (e.g. ABCC9 was upregulated in 100% of all the peri-implantitis patients when compared to healthy controls). Cluster analysis was conducted using TIBCO Spotfire 3.2.1 (TIBCO, Palo Alto, CA, USA) applying the hierarchical clustering (correlation, unweighted pair group method with arithmetic mean). Principle component analysis was performed using TIBCO Spotfire 3.2.1. Gene ontology (GO) analysis was carried out as previously published[18]; enrichment *p* values were corrected for multiple testing using the Benjamini–Hochberg method,[16] while GO terms were retrieved from http://www.geneontology.org. As a second attempt, gene ontology terms associated to regulated transcripts were categorized and their absolute counts were compared between peri-implantitis and periodontitis.

RESULTS

Transcriptome Profiling Results

The microarray data (raw and normalized) were processed according to MIAME guidelines and submitted to Gene Expression Omnibus[19] and is accessible through GEO Series accession number GSE33774 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE33774). In total, 23,376 of 33,297 transcripts were categorized as present. Applying the cutoff criteria (corrected *p* value ≤0.05, FDR ≤ 5%) resulted in 137 transcripts when comparing peri-implantitis to healthy individuals (comparison I), 46 transcripts when comparing periodontitis patients to healthy individuals (comparison II), and no transcripts when comparing peri-implantitis to periodontitis directly (comparison III). All transcripts originating from the three comparisons showed an effect frequency of 100% in the comparison, which represented the transcript selection criteria. However, 136 of the transcripts identified in comparison I were unique features of peri-implantitis, as they were not significantly regulated between periodontitis and healthy individuals. Only one transcript was found to be significantly regulated in both phenotypes (*TRIB1*, tribbles homolog 1, Drosophila). In the same context, a set of 77 transcripts which show a FDR ≤ 5% but no significant *p* value in comparison III was generated. Taken together, 208 transcripts were categorized as significant in at least one of the comparisons (see Supporting Information Table S1).

Cluster Analysis Results

Based on the generated candidate transcript sets, two different clusters were created: First, a cluster based on

208 transcripts identified as being significantly differentially expressed in at least one comparison in all samples was created (Figure 1). The resulting horizontal dendogram represents the similarity between the three phenotypes (healthy controls, peri-implantitis, and periodontitis) while displaying a separation with two outliers (two periodontitis patients clustering with the peri-implantitis group). All healthy individuals represent a distinct cluster. The vertical dendogram shows a separation in four different regulatory categories of transcripts: i) transcripts downregulated in peri-implantitis but not in periodontitis when compared to healthy individuals (21 transcripts); ii) transcripts downregulated in both peri-implantitis and periodontitis when compared to healthy individuals (14 transcripts); iii) transcripts upregulated in both peri-implantitis and periodontitis when compared to healthy individuals (44 transcripts); and iv) transcripts upregulated in peri-implantitis but not in periodontitis (129 transcripts).

In a second cluster analysis, 27 transcripts separating peri-implantitis from periodontitis were identified (Figure 2). The presented subset of transcripts consists of top 27 transcripts, ranked by their *p* value resulting from the comparison between peri-implantitis and periodontitis. Based on these transcripts, each phenotype represents a distinct cluster.

Principle Component Analysis Results

To test whether the identified set of 208 transcripts represents a unique molecular signature for peri-implantitis and/or periodontitis, a principle component analysis was performed, resulting in a clear separation of all patients originally recruited for this study (Figure 3).

GO Analysis Results

Three different GO analyses were performed, based on each of the comparisons listed earlier. In comparison I, all transcripts that were significantly regulated between peri-implantitis patients and healthy individuals were selected. Of those, only characterized transcripts were subjected to GO analysis (in total 124). In comparison II, all characterized transcripts, which were differentially expressed between periodontitis and healthy individuals (33), were subjected to a GO analysis. Since in comparison III not enough transcripts were differentially expressed between peri-implantitis and periodontitis patients, 81 transcripts with a p value <0.05 prior to the correction for multiple testing were subjected to the GO analysis. The three comparisons resulted in 10, four, and five enriched biological processes, respectively (Figure 4), where immune responses represented the most prominent signal.

In a categorical GO analysis, where biological process groups were counted for terms prominent in peri-implantitis, remarkable differences are displayed in the profile when comparing peri-implantitis and periodontitis: While in peri-implantitis immune system associated terms dominate, periodontitis shows apoptosis and proliferation as dominant features (Figure 5).

DISCUSSION

Peri-implantitis and periodontitis, two inflammatory diseases of the oral cavity with a bacterial background, share various clinical characteristics. As a result of these similarities, studies addressing disease manifestation and progression as well as therapeutic approaches are oftentimes merged. This view is supported by the shared risk factors between periodontitis and peri-implantitis.[2,20] Additionally, a periodontitis background represents a risk indicator for peri-implantitis.[21–23] Various studies allow concluding how the similarity of the two phenotypes is attributed to similar disease mechanisms: In partially edentulous patients, bacterial colonization occurs within 30 minutes after implant placement.[24] Bacteria around infected implants are similar to those found in periodontitis which include members of the red complex species: *Porphyromonas gingivalis, Treponema denticola*, and *Tanerella forsythia*.[25] In the same context, a study comparing the accumulation of biofilm and the host response of the soft tissues in humans revealed no differences between gingivitis and peri-implant mucositis.[26] In contrast to that, the uniqueness of peri-implant diseases is oftentimes neglected.[4]

Initial studies attempting to unravel the molecular pathophysiology of periodontitis[27] and experimental gingivitis[28] using transcriptome profiling not only demonstrated the validity of this approach, but also could successfully present a high-resolution insight into disease-associated biological processes. Both studies suggest that gingival tissue transcriptome profiling may further help to identify disease relevant mechanisms, ultimately leading to a better understanding of disease manifestation and progression.

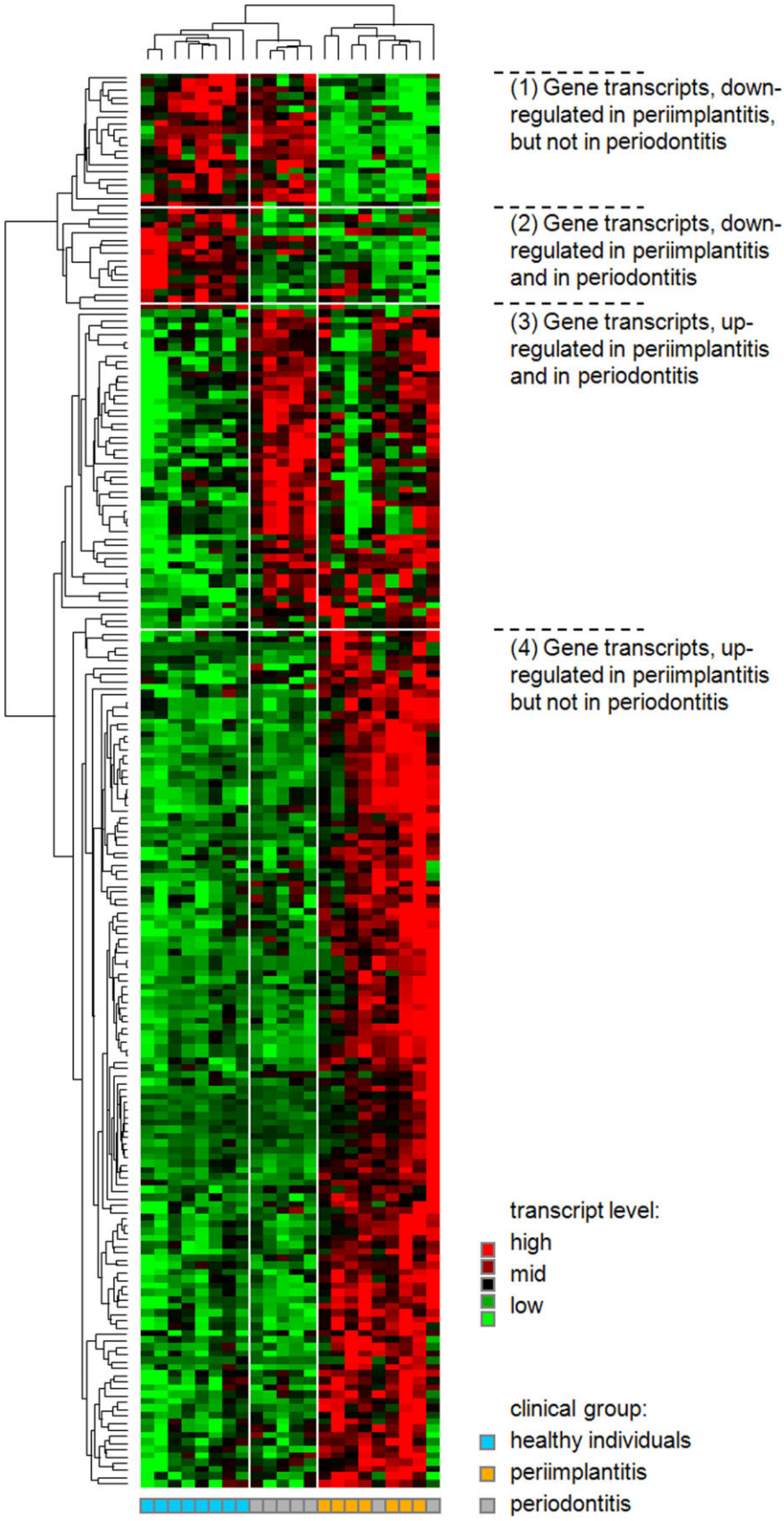

Figure 1 Molecular phenotype cluster of peri-implantitis, periodontitis, and normal controls. Samples are organized in columns, transcripts are organized in rows. The vertical dendogram displays similarities between transcripts, while the horizontal dendogram displays similarities between samples. The heat map is colored according to the relative expression of a transcript; for better readability of the results, z-score normalized values are displayed. In total, 208 transcripts are displayed which names are presented in Supporting Information Table S1.

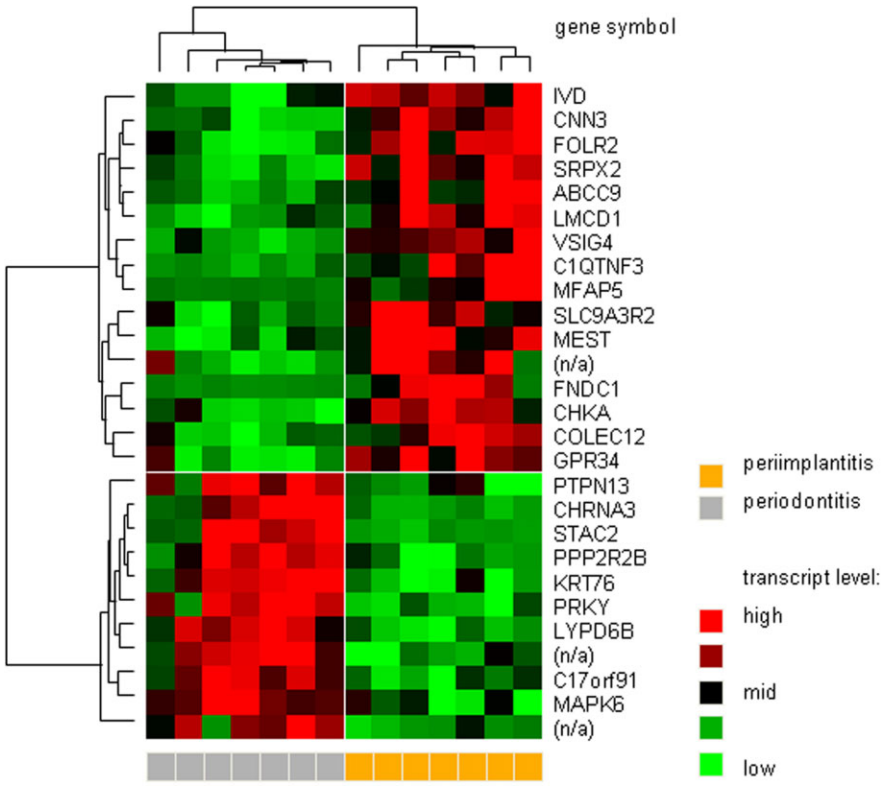

Figure 2 Transcript profile, differentiating peri-implantitis from periodontitis. Here, 27 transcripts with the best *p* values from comparison III (peri-implantitis vs. periodontitis) are presented. Samples are organized in columns, transcripts are organized in rows. The vertical dendogram displays similarities between transcripts, while the horizontal dendogram displays similarities between samples. The heat map is colored according to the relative expression of a transcript; for better readability of the results, z-score normalized values are displayed. Transcripts are labeled with gene symbols if available.

Aiming to describe molecular differences between peri-implantitis and periodontitis, affected tissue from both phenotypes was compared while employing non-affected gingival tissue from healthy individuals to control for molecular events without disease relevance. Naturally, without including additional controls (e.g. patients free of medication, smokers and nonsmokers, etc.), such a descriptive approach has limitations. Similarly, this setup does not allow identifying predisposing factors or causal effects, yet it remains a powerful tool to describe fundamental differences between phenotypes. In contrast to the limitations, the presented disease-associated transcripts exhibit high effect frequencies, which further support the approach chosen here.

Interestingly, the results presented in this study indicate only a small number of similarities between peri-implantitis and periodontitis: Only a single transcript was found to be significantly regulated in peri-implantitis and periodontitis (2.5-fold upregulated in peri-implantitis; 2.0-fold upregulated in periodontitis): *TRIB1* (tribbles homolog 1, Drosophila). The gene product function was described to be involved in the JNK-cascade and in the negative regulation of lipopolysaccharide-mediated signaling pathways.[29] In this context, its functional link to peri-implantitis remains unclear. In contrast to overlap represented by a single transcript, the number of features unique to each group is essentially larger: 136 transcripts unique to peri-implantitis and 45 transcripts unique to periodontitis illustrate that these entities should be treated as distinct disease events.

Correspondingly, an approach of categorizing GO terms into major groups displayed also a low degree of overlap: Only proliferation-associated transcripts represented a common feature. This further supports the hypothesis of substantially different biological mechanisms being responsible for both diseases, independent of their clinical similarities.

It is important to mention that we examined gingival soft tissue in this study. This tissue was chosen in

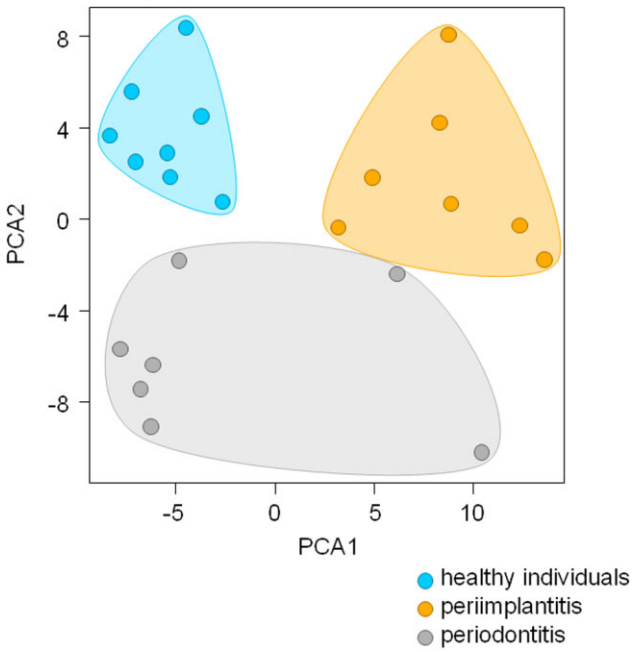

Figure 3 Principle component analysis separating peri-implantitis patients, periodontitis patients, and healthy individuals. The presented analysis is based on 208 transcripts, differentially expressed in at least one of the three comparisons. The two strongest components (PCA1 and PCA2) are plotted on the *x*- and on the *y*-axis; samples are color coded according to their phenotype.

concordance with previous studies[30] since this region either acts as a barrier against bacteria or allows their intrusion. The mucogingival border is the first and probably most important barrier. Our results suggest that this region reacts in a completely different way in peri-implantitis compared to periodontitis. These differences may be explained by the anatomy, which is very different comparing the scar tissue in peri-implantitis with the specialized fibers inserting the surface of the teeth. In peri-implantitis tissue, transcripts associated to innate immune responses and defense responses were dominating, while in periodontitis tissues, bacterial response systems prevailed.

As peri-implantitis is defined as an infection affecting also the bone, further studies have to evaluate whether the observed differences are also reflected in bone tissue. This has to be conducted on a genome-wide level in humans, to supplement the phenotypic data and observations from animal models collected in previous studies.[6,31,32] In this context, it is noteworthy that we identified SRGN (serglycin) as being significantly upregulated in peri-implantitis when compared to healthy individuals (2.9-fold upregulated, p value = 0.0311; FDR = 0,47%). This gene is known to inhibit bone mineralization in vitro.[33] In contrast, when comparing periodontitis tissue to healthy tissue, SRGN did not show significant differences. This supports the hypothesis i) that the involvement of bone in the pathophysiology of peri-implantitis represents a central difference to periodontitis and ii) that molecular manifestations of these effects can be monitored in soft tissue. Interestingly, the mRNA expression differences between peri-implantitis and periodontitis are primarily attributed to 27 transcripts. Among those are transcripts associated to cell death (*PPP2R2B*; protein phosphatase 2 (formerly 2A), regulatory subunit B, beta isoform), defense response to virus (*ABCC9*; ATP-binding cassette, sub-family C (CFTR/MRP), member 9), and innate immune response (*COLEC12*; collectin subfamily member 12). Assuming that these identified differences reflect features that differ between peri-implantitis and periodontitis, one could conclude that fundamental wound healing and immune system processes separate the two phenotypes. Naturally, as our observations are based on transcriptome data, they are not reflecting the effects of functional proteins. A validation of individual protein levels as well as experiments assessing their potential role will have to be carried out to further elucidate the clinical relevance of these findings. Nevertheless, the presented transcriptome data enables us to draw a first high-resolution picture of the uniqueness of peri-implantitis in comparison to periodontitis.

The hypothesis of fundamental differences is further supported by our observation that the biological processes associated to the transcripts identified exhibit only a minor degree of overlap: "Defense response" and "innate immune response" represented the most prominent terms associated to peri-implantitis. In contrast to that, these terms were absent in the periodontitis setting. In periodontitis, however, we identified the term "response to molecule of bacterial origin" which was represented by three transcripts, which were all absent in peri-implantitis. This type of analysis does not allow to conclude that bacterial factors do not play a role in peri-implantitis, as some mechanisms of this category are also found in the "innate immune response" term; however, this displays a further difference between peri-implantitis and periodontitis.

On a categorical level, our results show a high concordance with previous findings, showing that

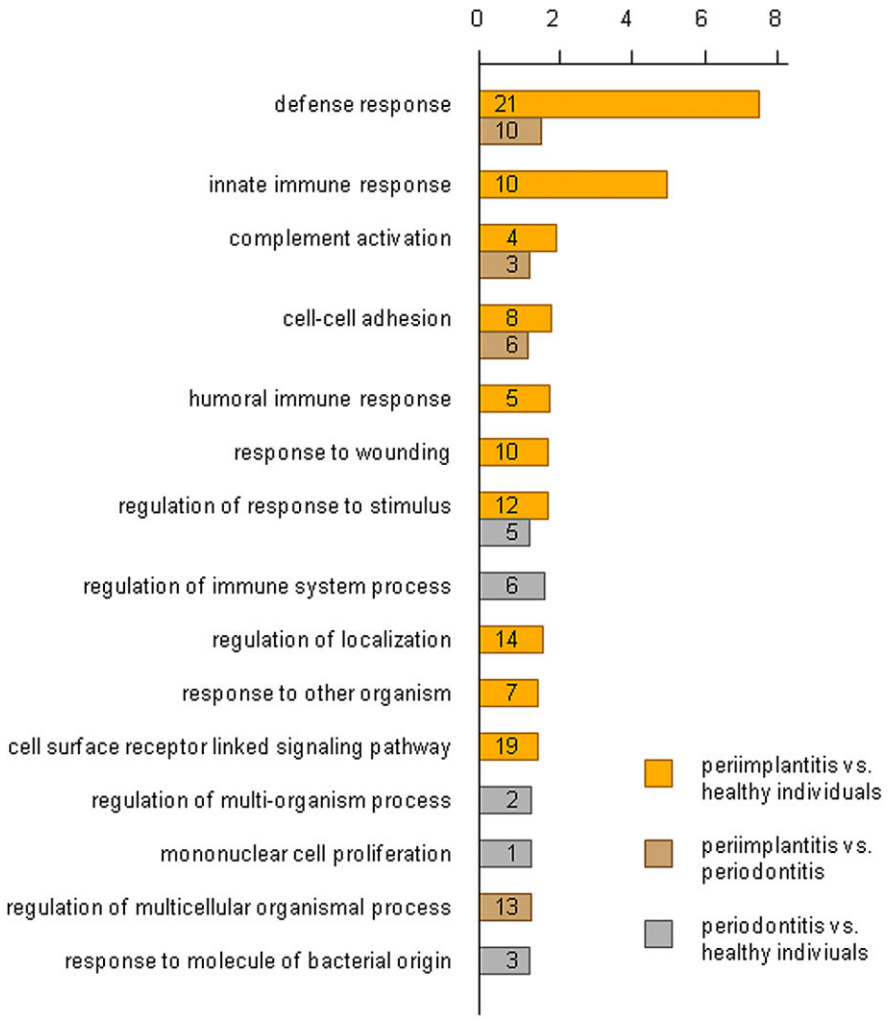

Figure 4 Biological processes, associated to transcripts regulated in peri-implantitis and periodontitis. Three separate gene ontology analysis approaches are displayed: Peri-implantitis vs. healthy individuals, periodontitis vs. healthy individuals, and peri-implantitis vs. periodontitis. Significance is plotted as –log(p), while the numbers displayed on the bars represent the number of transcripts observed associated to the corresponding gene ontology term. Only biological terms with a significant enrichment (when compared to the expected value, corrected for multiple testing) are displayed.

regenerative processes like wound healing, proliferation and apoptosis as well as defense and immune mechanisms may play an important role in gingival soft tissue-associated inflammations.[27,28] Despite the weakness of comparing results of different study setups, it is important to note that the categorical overlap of biological processes between peri-implantitis and periodontitis[27] seems larger than the overlap to experimental gingivitis,[28] further explaining why periodontitis and peri-implantitis are oftentimes summarized into one group.

There is emerging evidence that differences between peri-implantitis and periodontitis extend even to the microbiological level[34]: *Staphylococcus aureus*, which is not strongly associated with chronic periodontitis, has been identified in high levels in deep peri-implant pockets. In addition, the transmission of bacteria from teeth to implants in partially dentate patients was confirmed in studies investigating the dynamics of colonization,[35] thus indicating that the implant is required to trigger this event. Consequently, in a periodontitis scenario with the absence of an implant, colonization mechanisms will differ. This is in concordance with our results pointing toward differences in defense mechanisms against pathogens.

The use of primary tissue and the resulting heterogeneity in cell composition may introduce confounding factors in such an analysis. However, previous studies have shown that primary tissue represents a powerful tool to monitor complex disease mechanisms, which are the result of the interplay between different cell

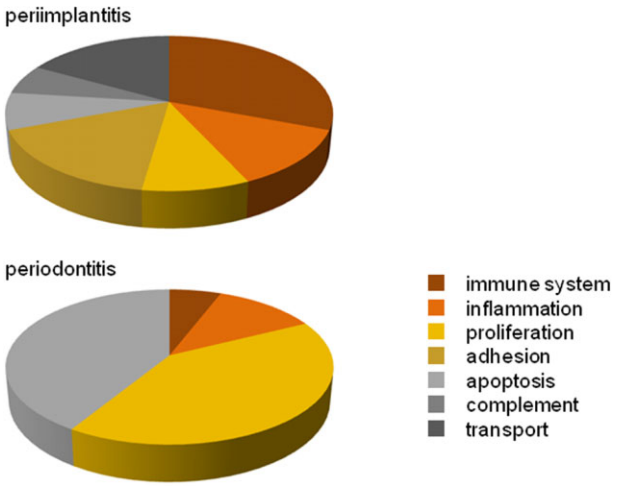

Figure 5 Biological term categories found in regulated transcripts. Categories, based on biological processes retrieved from http://www.geneontology.org, display differences between peri-implantitis and periodontitis. Seven categories, prominent in peri-implantitis (comparison I), are compared to periodontitis (comparison II). Pie charts represent a relative scaling, adding up to 100%.

types.[36,37] Moreover, altered cell compositions might in fact represent disease-relevant elements. In addition, nonparametric methods as applied in this study allow compensating the high variations observed in clinical settings. These variations were also observed on patient level, where in the hierarchical clustering of three phenotypes (peri-implantitis, periodontitis, and healthy controls), outliers were observed: Two periodontitis patients clustered with the peri-implantitis patients, indicating interindividual variation within these groups. It is tempting to speculate that apart from the observed differences, a degree of overlap exists, resulting in these outliers. In contrast to that observation, two other approaches showed a reduced variability: The cluster comparing peri-implantitis to periodontitis as well as the principle component analysis. This indicates that the variability is strongly dependent on the transcripts selected. It has been previously shown that different variations in transcript levels may be attributed to different regulatory mechanisms. Genes that are under strong genetic control show a significant lower variation than genes under weaker genetic (e.g. under environmental) control.[38] In the context of peri-implantitis, it has been discussed that a combination of triggering factors, such as genetic and environmental factors, may be required for disease manifestation.[2]

In the same context, a large number of other factors may contribute to i) disease mechanisms and ii) differences between peri-implantitis and periodontitis.[2] The current study setup does not allow to appropriately monitor such factors, for example, the influence of smoking or other environmental effects. Moreover, the results do not suggest that peri-implantitis and periodontitis do not share any pathophysiological mechanisms, especially on the level of disease susceptibility and manifestation. However, this exemplary approach creates a high-resolution picture of molecular differences which can serve as a starting point for further functional studies, which may be then conducted in a larger study group.

CONCLUSION

In conclusion, our results indicate that while peri-implantitis and periodontitis share clinical characteristics, they represent distinct entities. Keeping the limitations of a descriptive transcriptome analysis in mind, the results illustrate that approaches aiming to unravel the molecular mechanisms of peri-implantitis pathophysiology can only partially be addressed in a periodontitis setting. Ongoing studies in the field of peri-implantitis targeting disease mechanisms and interactions with the implant as well as with the microbiota will help to close the gap between clinical observations and biological models.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Table S1 All transcripts, regulated significantly in at least one of the three comparisons (peri-implantitis vs. normal controls, peri-implantitis vs. periodontitis, periodontitis vs. normal controls). Significance was determined using the Mann–Whitney *U*-test and corrected for multiple testing using the Benjamini–Hochberg correction. In total, 183 transcripts are presented (43 transcripts with no public reference and no gene symbol were omitted). Each column represents one transcript, and is displayed with its fold change (FC), false discovery rate (FDR), and its *p* value (P) and its effect frequency (F). A star (*) indicates, that this frequency corresponds to the condition, for which this transcript was originally selected due to its significance.