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Biome-microbiome interactions in peri-implantitis: a pilot investigation

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Abstract

Background: Dental implants replace missing teeth in at least 100 million people, yet over one million implants fail every year due to peri-implantitis, a bacterially induced inflammatory disease. Our ability to treat peri-implantitis is hampered by a paucity of information on host-microbiome interactions that underlie the disease. Here, we present the first open-ended characterization of transcriptional events at the mucosal-microbial interface in the peri-implant crevice.

Methods: We simultaneously sequenced microbial and human mRNA from five pairs of healthy and diseased implants from the same patient and used graph theoretics to examine correlations between microbial and host gene expression in the peri-implant crevice.

Results: We identified a transcriptionally-active peri-implant microbiome surrounding healthy implants. Microbial genes encoding phenylalanine, tyrosine and tryptophan biosynthesis, cysteine, methionine, arginine, proline and histidine metabolism correlated to human genes encoding cell development, metabolism, morphogenesis, adhesion, gap junctions, cell-cell signaling and immuno-inflammatory pathways, suggesting a role for commensals in protecting epithelial integrity. In disease, we found 4–200-fold upregulation in microbial genes encoding biofilm thickness, heme transport and utilization, and gram-negative cell membrane synthesis. These genes correlated with mucosal zinc finger proteins, apoptosis, membrane transport, inflammation and cell-cell communication.

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Author contributions: CWL conducted all the clinical studies and sample processing; AJM designed and supervised the clinical studies, SMG was responsible for the transcriptomic metatranscriptomic analysis, SMD was responsible for all bioinformatics pipelines, HNN was responsible for the statistical oversight, PSK was responsible for overall study design, designed and supervised the sequence and data analysis. All authors participated equally in data analysis, interpretation of results and manuscript preparation.

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Conclusions: Within the limitations of a small sample size, our data suggest that microbial dysbiosis in the peri-implant sulcus might promote abandonment of host-bacterial transactions that dictate health and instead drives a move towards chronic programming of a non-healing wound.

Summary sentence:

Microbial dysbiosis in the peri-implant sulcus promotes abandonment of homeostatic host-bacterial transactions and creates a scenario compatible with chronic programming of a non-healing wound

Keywords

Implantitis; Bacteria; Whole genome sequencing; RNA

INTRODUCTION

Although dental implants are estimated to have a survival rate of 95% over a 10-year period, one in ten implants (and one out of five patients) develop peri-implantitis[1–3], a disease that is triggered by a disruption of host-microbial homeostasis in the peri-implant crevice. The histological, immunological and microbiological aspects of peri-implantitis have been extensively studied in separate investigations[4–9], however, the dynamics of host-microbial equilibrium in peri-implant health and disease have never previously been characterized.

Early investigations by our group and others investigated correlations between selected cytokines, bacterial species or genes in an effort to study host-bacterial interactions[10, 11]. However, these targeted analyses do not provide a global view of the events that occur in an environment. On the other hand, the transcriptome and the metatranscriptome, which are repositories of all genes expressed either by a species (for example, human) or a community (i.e., the microbiome) in a particular milieu, provide a real-time, in-depth view of these complex interactions, and has given rise to a novel field of study - interactomics[12, 13]. Thus far, only one published study has investigated the transcriptome of peri-implantitis[6]. However, the study used a microarray approach, which is necessarily limited to a predefined set of transcripts. In contrast to microarrays, RNA sequencing provides a low-bias, high-throughput, open-ended, quantitative approach to simultaneously characterize both human and bacterial gene expression[14]. A recent study using this approach has demonstrated the ability of this methodology to stratify healing outcomes following surgical intervention based on immune profiles[15].

The purpose of the present exploratory investigation, therefore, was to map all possible interactions in the peri-implant sulcus using dual-transcriptomics and network analysis, and to extricate patterns of microbial-mucosal transactions that govern peri-implant homeostasis or cause peri-implantitis.

MATERIALS AND METHODS

Subject Recruitment:

Approval for this study was obtained from the Institutional Review Board at The Ohio State University (Protocol Number:2014H0170). Informed consent was obtained from dentate adults with multiple non-splinted, tooth-bounded implants in function for at least 1 year. Exclusion and inclusion criteria, as well as criteria for case selection are detailed in Supplemental File 1. The study and reporting conform to STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines[16].

Sample Collection:

Two implants in two different quadrants were selected in each of 5 individuals using the criteria for health and disease as delineated by the 2018 Classification of Periodontal and Peri-Implant Diseases and Conditions[17]. Microbial and peri-implant sulcus samples were collected from one healthy implant and one with peri-implantitis (detailed methods in Supplemental File 1)

Sample processing:

Human: The peri-implant crevicular tissue was homogenized, total RNA isolated, rRNA depleted and mRNA enriched and stabilized. cDNA libraries were created from the stabilized mRNA.

Microbial: Microbial cells were lysed, total RNA isolated, rRNA depleted, mRNA enriched, polyadenylated and cDNA libraries synthesized.

Sequencing and analysis:

Microbial and human cDNA libraries were separately pooled and independently clustered on the Illumina HiSeq 4000 platform and sequenced using 150bp paired-end chemistry. Sequence data was aligned to the human transcriptome (GRCh38) and normalized transcript counts obtained. Transcripts were annotated using the Ensembl project. Protein ANalysis THrough Evolutionary Relationships (PANTHER,) classification system was used to infer the functions of these genes.

Microbialtranscripts were quality filtered and aligned against the Human Oral Microbiome Database (HOMD)[18] protein database (as of 2016-03-29). Aligned sequences were annotated to the KEGG database. Virulence factor analysis was performed by aligning microbial transcripts against the Virulence Factor Database. The phylogenetic profile of each sample was determined using Kraken v1.1[19].

Data analysis:

To reduce spurious associations that can occur from extreme values, transcripts that were uniquely present only in either health or peri-implantitis were filtered out. DESeq2 v1.18.0[20]was then used to perform differential expression analysis of the remaining microbial and mucosal transcripts. The default parameters for dispersion shrinkage were used to filter low count genes, flags genes with large outlier counts and exclude genes

with very high within-group variance from estimation of the dispersion prior and dispersion moderation. Size factor was normalized by dividing every gene count by the geometric mean of read counts across all samples and using the median of these ratios as a sample's size factor for normalization. *P*-values were computed for the Wald statistic and adjusted for multiple testing with the Benjamin-Hochberg procedure to control for false discovery rate. The COMPARTMENTS database was used to localize human proteins to subcellular locations (https://compartments.jensenlab.org). Spearman and Kendall's correlations were calculated between all significant and unique host and bacterial transcripts. Correlations with Spearman's rho and Kendal tau<|0.85| and p>0.01 were removed. Host-bacterial interaction networks were created using the Python library networkx v1.11[21] and visualized with Gephi v0.91 and the ForceAtlas2 layout algorithm[22]. All supplemental tables can be accessed at https://figshare.com/s/d81b730c01bc67e03ca3

RESULTS

Microbial metatranscriptomic and mucosal transcriptomic sequencing yielded226 million analyzable sequences.

Overview of the Human Transcriptome:

945 genes were differentially expressed between health and peri-implantitis; with 726 upregulated in disease and 219 downregulated ($p < 0.05$, FDR-adjusted Wald test, Figure 1a and Supplemental Table 1). Additionally, 7269 genes were uniquely identified in peri-implantitis and 1165 in health. These genes encoded 24 functional or metabolic pathways, with 35% of transcripts encoding metabolic processes, 11% coding for transcription and translation, 10% for cellular processes, 9% encoding immune system and cellular defense, and 7% related to protein modification.

In both health and peri-implantitis, the nucleus, cytoplasm and cytoplasmic organelles were sites with greatest numbers of transcriptionally active genes, followed by cell membrane, vesicle, ribosome, cytoskeleton and chromosome(Figure 1b). Among the differentially expressed genes, the ribosome was a predominant compartment in health but not in disease, while the nucleoplasm and extracellular exosomes were sites of greater transcriptional activity in disease. One-third of genes encoding immune-related functions localized to vesicles in health and to the cell membrane in disease. Similarly, >33% of the genes encoding zinc finger proteins (ZFPs) localized to cytoplasm in health, and to the nucleus in disease, with >25% of the localization in chromosomes. Proapoptotic genes predominantly localized to the cytoplasm, extracellular space, and vesicle in both health and disease; additionally, these genes localized to the nucleus in health. Genes encoding cellular communication and intercellular signaling localized mainly to cell membrane in peri-implantitis, however, in health, the genes additionally localized to several subcellular compartments including extracellular matrix, Golgi apparatus, microtubules, and cytoskeleton.

Overview of the Microbial Metatranscriptome:

Functional mapping of the sequence data identified 968 genes grouped into 28 KEGG functional families. Functions contributing to increased biofilm thickness, namely, capsular biosynthesis and transport, alginate biosynthesis, exopolysaccharide synthesis as well as cell wall synthesis and transport demonstrated a 4–200-fold upregulation in peri-implantitis when compared to health ($p<0.05$, FDR-adjusted Wald test, Figure 2a and Supplemental Table 2). Heme transport, iron-mediated respiration, and gram-negative cell membrane components, namely, lipopolysaccharide, fimbriae and flagella, demonstrated similar upregulation in disease.

258 species belonging to 71 genera mapped to these transcripts (Figure 2b and supplemental table 3). Peri-implantitis demonstrated significantly higher organismal diversity when compared to health ($p = 0.036$, Mann-Whitney U of Simpson's Index). Additionally, peri-implantitis demonstrated lower variance in diversity than healthy ($p = 0.03$, Levene's test), indicating a narrower landscape of variation in disease-associated organisms.

Overview of host-bacterial interactions in health and peri-implantitis:

In health, the predominant microbial transcripts and 52% of the mucosal transcriptome mapped to metabolic activities, with metabolism of cofactors and vitamins, amino acid, carbohydrate and lipid metabolism, energy metabolism, transcription and translation, and genetic information processing representing 84% of the bacterial mRNA. also related to metabolic activity. 21% of mucosal transcripts encoded translation, transcription, folding, sorting and degradation, mRNA and mitochondrial biogenesis, membrane trafficking, ubiquitination and chaperone proteins. Cell growth and development (morphogenesis, regulation of cell development, cell adhesion and cell-cell signaling) accounted for 20% of the mucosal transcriptome.

The healthy peri-implant crevice demonstrated ten times more microbial-mucosal correlations than either inter-bacterial or intra-mucosal (247,686 microbial-mucosal correlations versus 26,682 intra-mucosal and 22,046 inter-bacterial correlations, Supplemental Table 4). In peri-implantitis, microbial-mucosal correlations were 3-fold fewer than health, 3-fold lower than intra-mucosal correlations and 5-fold lower than inter-microbial ones (intra-mucosal=265626, inter-microbial=41428 and microbial-mucosal=84212).

In health, 67% of the mucosal correlates encoded cellular development, integrity and gap junctions, and correlated positively with bacterial amino acid metabolism (predominantly phenylalanine, tyrosine and tryptophan biosynthesis) and negatively with bacterial adhesion proteins, elongation factor Tu and Lipid A. 18% of the mucosal transcripts related to immuno-inflammatory pathways and correlated negatively with glycan biosynthesis, biotin metabolism, methionyl-tRNA formyltransferase, bacterial proteases, and positively with aminoacyl-tRNA biosynthesis (particularly cysteinyl-tRNA synthetase).

In disease, five distinct hubs were evident. Each of these will be discussed in the following sections.

ZFPs:

In general, peri-implantitis showed higher numbers of ZFPs (632) when compared to healthy sites (248). In peri-implantitis, ZFPs mediating apoptotic response, TNF/NFkB activation, cell morphology and cytoskeleton organization were upregulated (Figure 1a and Supplemental Table 1), as were transcription factors related to DNA methylation (112/632 ZFPs). Inflammation and disease-specific ZFPs, housekeeping and physiologic cellular functions (nucleic acid binding transcription factors and transcriptional regulators) also demonstrated higher abundance in disease. These genes correlated positively with microbial transcripts encoding oxidative stress response, capsule and alginate biosynthesis, flagella, hemoglobin utilization and iron transport, immune evasion and immunomodulation, lipopolysaccharide synthesis and quorum sensing (Supplemental Table 4).

Apoptosis:

Fundamental regulatory apoptotic genes such as ADAM metallopeptidases were observed in health while pro-apoptotic genes such as Caspase 7, genes involved in MAP-kinase pathways, and tumor-inducing TNFAIP8 genes were upregulated in disease (Figure 1a and Supplemental Table 1). Apoptosis was an important transcriptional activity in peri-implantitis (1% of mRNA sequences). Apoptosis-related microbial-mucosal correlations were 25-fold greater in peri-implantitis than health. Mucosal transcripts positively correlated with flagella, siderophores, type-VI secretion systems, lipopolysaccharide and lipo-oligosaccharide biosynthesis, environmental signaling and resistance to phagocytosis in peri-implantitis (Supplemental Table 4). In health, microbial transcripts contributing to siderophore and other iron transport correlated with negative regulation of apoptosis, anatomic structure development and DNA repair in the mucosal transcriptome.

Membrane transport/signal transduction:

1909 genes related to membrane transport were observed in disease versus 332 in health, (Figure 1a and Supplemental Table 1). Healthy and diseased sites exhibited different pathways for signal transduction and transport functionalities. Solute carrier family-related genes, G-protein coupled receptors/kinases, rhomboid-5 homolog 2 genes, cyclin dependent kinases, endoplasmic reticulum-associated membrane and lipid-raft-related genes accounted for nearly 50% of membrane transport/signal transduction transcripts in disease, while in health, potassium voltage-gated channel subfamily genes, RAS Protein related pathways, and AP2 Kinases constituted 50% of signal transduction-related transcripts. Additionally, nearly 50% of gene sets encoding endoplasmic reticulum proteins were identified only in peri-implantitis. Strong positive correlations were observed between these over-expressed mucosal transcripts and bacterial genes encoding motility, heavy metal efflux pumps, Type-VI secretion systems, neutrophil activating protein, pyochelin and pyoverdine (Supplemental Table 4).

Immuno-Inflammatory System:

Peri-implantitis demonstrated 1.6–3.6x higher upregulation of immune response genes than health (Figure 1a and Supplemental Table 1). Pattern recognition receptors (PRRs) were identified in both health and peri-implantitis, however, genes encoding PRR functions

were site-specific. For example, mannan-binding lectin (MBL) associated serine proteases (MASP) were identified only in peri-implantitis, and sialic-acid-binding-Ig-like proteins only in health. Interestingly, we observed greater apoptotic gene expression in peri-implantitis (as described above) as well as significant positive correlations between these transcripts and microbial mRNA encoding macrophage inactivation (Supplemental Table 4). Additionally, transcripts related to inflammasome (NACHT, LRR AND PYD Domains), C-Reactive Proteins and T-cell receptor signaling pathways were identified only in peri-implantitis, as was interleukin-10, providing further evidence for site-specific immune-damage control. Transcripts of IL-17 and IL-1 were two-fold higher in peri-implantitis, while IL-4, IL-6, IL-8, IL-12 were unique to peri-implantitis. IL-1 and IL-12 contributed to 58% and 30% respectively of the abundance of Interleukin-associated transcripts in peri-implantitis. Tumor Necrosis Factor (TNF-a), complement system, and interferons were upregulated in peri-implantitis.

Cell-cell adhesion:

Fewer genes encoding cell junctions were expressed in health than peri-implantitis (Figure 1a and Supplemental Table 1). In peri-implantitis, a 3–5-fold downregulation of genes encoding tight junction proteins (claudin, occludin and junctional adhesion molecule) and a 14-fold downregulation of Cadherin-1 was noted, while transcripts for beta-catenin were not detectable. On the other hand, Cadherin-3 was identified in all sites with peri-implantitis but not in health. Integrins were 10-fold higher in peri-implantitis when compared to health, however, within this family, certain integrins were under-expressed, notably integrin alpha-1 and 3, which demonstrated at 5-fold lower expression. In health, 2,600 network edges were observed between these adhesion transcripts and microbial iron transport, lipopolysaccharide, stress response and biofilm formation (Supplemental Table 4). By contrast, in peri-implantitis, >12,400 edges were evident, with bacterial fibronectin anchoring a hub containing integrins and cadherins.

DISCUSSION

Since implant-level factors supersede patient-level factors in creating a high-risk environment[23], we used pairs of healthy and diseased implants from the same patient in order to control for variability in patient-level factors such as immune response and oral hygiene. We simultaneously characterized microbial as well as mucosal transcriptional events using combinatorial -omics and network analysis to elucidate events at cellular and subcellular levels, a method designed to provide greater mechanistic insight into disease etiology than simple biomarker identification. Before we discuss the implications of our findings, we wish to emphasize that the small sample size is a major limitation to the generalizability of our results to the larger population. However, our findings open numerous avenues for hypothesis generation.

We discovered that peri-implantitis represents abandonment of host-bacterial transactions that dictate health and instead is a move towards chronic programming of a non-healing wound. The first line of evidence comes from mRNA signals indicating an unmodulated apoptotic response. Apoptosis is a fundamental physiological cellular activity that plays an

important role in maintaining health, while dysregulation of this process has been implicated in various diseases[24] including periodontitis[25–27]. Apoptosis is a double-edged sword; one on hand, it is an innate immune defense mechanism that sacrifices infected cells to (a) eliminate pathogens at an early stage of infection and (b) encourage dendritic cells to induce a protective immune response by engulfing the apoptotic bodies. On the other hand, it can be hijacked by bacteria to exit host cells, acquire nutrients and evade host defenses[28]. In the healthy peri-implant sulcus, we observed that apoptosis-related gene expression was localized to the nucleus and cytoplasm, and that the predominant transcripts encoded DNA repair, bleb assembly, membrane trafficking and the endomembrane system. For example, ADAM metallopeptidases, which were upregulated in health, are type 1 transmembrane glycoproteins involved in cell-signaling, apoptotic resistance, and regulation functions[29]. Specific proteins of this family (for example, ADAM 28, ADAMTS1, ADAMTS4 etc.) effectively manipulate proliferation, apoptosis, and differentiation of human periodontal ligament stem cells[30]. These health-associated apoptosis-related transcripts correlated with microbial gene expression directed to metabolism, synthesis of cell wall and cell membrane components, and iron acquisition. Taken together, the data suggest that a health-associated microbiome influences routine cell turnover in the peri-implant crevice.

By contrast, microbially-driven dysregulation of the apoptotic process appears to predominate in peri-implantitis. For instance, Caspase 7 and MAPKinase, which are known to increase in response to bacterial stimuli and during progression of periodontal disease[31], were highly expressed in peri-implantitis, as was mannan binding lectin, which binds to senescent and apoptotic cells and plays a major role in ‘cell clearance’ by phagocytosis. We also observed a concomitant over-expression of genes encoding oxidative stress, ER stress, mitochondrial stress, DNA stress, inflammatory responses, and autophagy. Disruption of ER-related genes are reported to be ‘top-gene sets’ in severe chronic periodontitis[32, 33]. Various intra- and extra-cellular stimuli disrupt the functions of the ER and result in ER stress, leading to accumulation of unfolded proteins in the ER and eventual perturbation of cellular activities[34]. Bacteria orchestrate host cell death through three main pathways: the mitochondria-dependent pro-death pathway, NFκ-B–dependent pro-survival pathway, and inflammasome-dependent cell death pathway. In the present investigation, we discovered upregulation of ZFPs that regulate NFκ-B. Additionally, signals from the inflammasome pathway, T-cell receptor signaling, and acute phase proteins were identified only in sites with peri-implantitis, but not health. Thus, these observations support epithelial sensing of an infectious trigger and activation of the antimicrobial defense system of cell death.

These mucosal apoptotic signals also demonstrated strong correlations with immune evasion, iron acquisition and cell invasion within the microbial metatranscriptome in peri-implantitis, suggesting that apoptosis is not purely a defense mechanism mounted by the peri-implant mucosa, but an active attempt by pathogens to manipulate the ecosystem for survival. Iron is indispensable for bacterial survival, especially anaerobic species, and they acquire this precious commodity using chelating proteins known as siderophores and hemophores. The host responds to iron chelation by upregulating apoptosis, mitophagy, hypoxia, and the production of inflammatory cytokines[35]. In support of this theory, while bacterial siderophores and hemophores demonstrated robust positive correlations with genes related to apoptosis in both health and disease, such correlations were ten times greater in

peri-implantitis when compared to health. A high degree of positive correlation was also observed between apoptosis and bacterial internalins and survival within macrophages. It has been proposed that internalins enable bacteria to gain entry into the cell at sites where the apoptotic cell is expelled from the epithelium[36]. Hence, it is possible that upregulated apoptosis promotes invasion of bacteria into the peri-implant epithelium, creating not only a haven from immune defenses, but also a reservoir of pathogens capable of reseeding the peri-implant crevice following therapy.

Further evidence for dysregulation of wound healing as a pathogenetic mechanism in peri-implantitis comes from gene expression patterns relating to barrier function, especially related to the cadherin/catenin complex. Cadherin 1 or E-cadherin as it was formerly known, is an essential adhesion molecule expressed by epithelial cells for barrier formation along with its complex partner, beta-catenin[37]. E-cadherins play a very important role in preventing uncontrolled cell division and proliferation, and recent studies have reported decreased epithelial gene expression in periodontitis[38]. In the present study, not only was Cadherin 1 downregulated, but Cadherin 3 was also only expressed in sites with peri-implantitis. The cooperation between Cadherin 1 and 3 has been reported to determine the invasive potential of cancers; while Cadherin 1 is anti-invasion, Cadherin 3 over-expression is associated with aggressive spread. Although it is impossible to extrapolate these findings from cancer research to microbially-driven chronic inflammatory diseases, it provides an interesting avenue of research and further studies are warranted.

Integrins and cadherins play central roles in maintaining adherens junctions as well as providing intracellular links to the actin cytoskeleton and regulating gene transcription[39]. Overproduction of integrins is a sentinel event in chronic wound programming, because it leads to premature senescent cellular (fibroblast, myofibroblast, endothelial, keratinocyte and macrophage) transformations and chronic inflammation[40]. The 10-fold higher production of integrin-related mRNA signals in peri-implantitis suggests that aberrant integrin signaling might be a potential event underlying the etiopathogenesis of peri-implantitis.

In the present study, ZFPs related to DNA methylation and NFkB were upregulated in peri-implantitis and showed positive correlations with many bacterial virulence factors. ZFPs make up 1% of the abundance of mammalian genomes and play roles in a number of housekeeping as well as pathologic cellular processes[41]. By binding to RNA and DNA molecules, ZFPs significantly influence transcriptional regulation, ultimately mediating downstream biological processes such as cellular development, differentiation, apoptosis, and inflammatory processes[42, 43]. The nuclear factor NFkB is a prototypical proinflammatory signaling pathway and has been demonstrated to play a role in a multitude of infectious diseases and cancer[44]. In chronic periodontitis, downstream NFkB regulation by ZF proteins has been shown to be increased[45]. Bacterially driven hypermethylation of genes that regulate inflammatory processes has been well described in the etiopathogenesis of periodontitis[46,47]and our study finds parallel events in peri-implantitis. In further support of this, several genes encoding both innate and adaptive immune responses were significantly over-expressed in peri-implantitis. Specifically, we report for the first time, NLRP2 (NOD-like receptor protein 2), NLRP8 and NLRP12-associated signals only in

sites with peri-implantitis. Inflammasomes are signaling platforms that play critical roles in the innate immune response. Minimally, these multimeric complexes contain a sensor, an adapter and an effector; and NLRP2, 8 and 12 function as sensors in these complexes[48]. Activation of these sensors leads to upregulation of IL-1b, IL-18 and Caspase-1, as well as gasdermin D (GSDMD), a chain of events that results in pyroptosis. In line with this, we identified parallelly upregulated gene expression relating to IL-1 and Caspase-1. Also significantly increased in peri-implantitis were MBLs, which not only play a role in apoptosis, but are pattern recognition molecules that play a pivotal role in defense against infectious organisms by activating the complement system.

A cell is markedly responsive to external stimuli and continuously adapts to these changes through signal transduction cascades[49]. In the present study, several G-protein coupled receptors/regulating kinases that play important roles in maintaining bone homeostasiswere significantly upregulated in disease, suggesting that the non-healing wound milieu extends beyond the peri-implant soft tissue environment and affects bone homeostasis[50].

CONCLUSION

We recognize the major limitations of this study are a very small sample size and a cross sectional study design, however, there is sufficient homogeneity in microbial and host gene expression for ustogain some insights into the complex host-bacterial interactions that underlie peri-implant health and disease. We find that the interaction between the peri-implant microbiome and the soft tissue of the peri-implant crevice might be critical to maintaining homeostatic barrier function and cell cycle. We also found evidenceto suggest that the crosstalk between a dysbiotic microbiome and the peri-implant soft tissue wall is suggestive of an environment resembling a chronic, non-healing wound. This has important clinical implications, since the longer a wound remains in the inflammatory phase the more cellular defects it accumulates, thereby decreasing responsiveness to treatment. These findings may explain the low success rate of current therapeutic modalities and the high rate of recurrence of this disease. We readily acknowledge that a cross-sectional study offers only a ‘snapshot’ view of a dynamic event, and that gene transcription does not directly correlate with protein levels. However, as a pilot investigation, this study serves to identify novel avenues for research on the etiopathogenesis of peri-implant diseases and candidate biomarkers of peri-implantitis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability Statement

No data is available for this research work.

Data availability: Sequence data used in the study can be made available upon request to the corresponding author.

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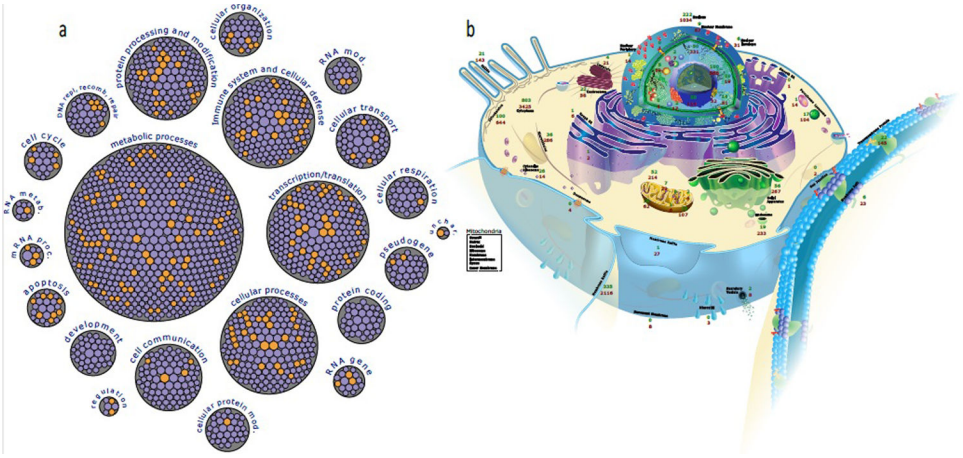

Figure 1: Characteristics of the mucosal transcriptome in peri-implant health and disease.

Figure 1a is a hierarchical circle-packing graph of transcripts that have been grouped into nested higher order functions based on the Protein ANalysis THrough Evolutionary Relationships (PANTHER) database. Genes up-regulated in peri-implantitis are colored in purple, while those down-regulated in peri-implantitis are colored brown. Only genes with a 2-fold or greater change in transcription and $p<0.05$ (Fisher's exact test with Benjamini-Hochberg False Discovery Rate (FDR)) with are shown. The data supporting this figure can be found in Supplemental Table 1. Figure 1b is a representation of the subcellular localization of the transcripts in health and peri-implantitis, inferred from the COMPARTMENTS database. The number of transcripts (adjusted for sequencing depth) in health are in green font, and those in peri-implantitis are in red font.

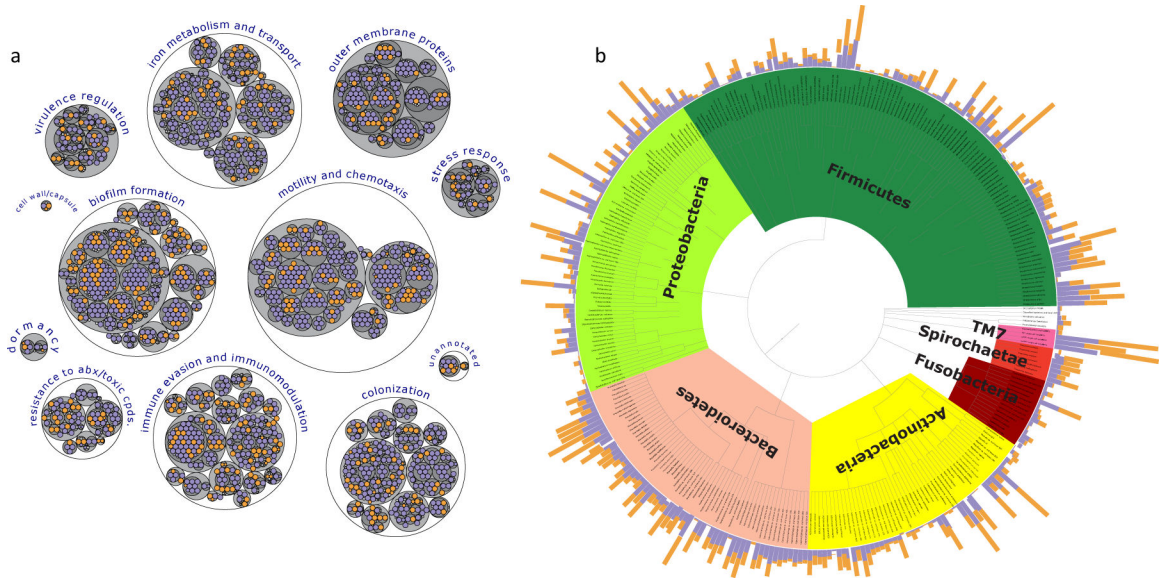

Figure 2: Characteristics of the microbial metatranscriptome in peri-implant health and disease.

Figure 2a is a hierarchical circle-packing graph of transcripts that have been grouped into nested higher order functions based on the Virulence Factor Database (VFDB) database. Genes up-regulated in peri-implantitis are colored in purple, while those down-regulated in peri-implantitis are colored brown. Only genes with a 2-fold or greater change in transcription and $p<0.05$ (DeSeq2 with Wald test for False Discovery Rate (FDR)) are shown. The data supporting this figure can be found in Supplemental Table 2. Figure 2b is a circular phylogenetic tree of 258 species-level OTUs (belonging to 71 genera) that mapped to the transcripts. The outermost circle represents the relative abundances of the OTUs, with the purple bars representing peri-implantitis and the orange bars representing peri-implant health. The inner circle shows the clades, which are colored by phylum. The data supporting this figure can be found in Supplemental Table 3.