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Biomarkers and Bacteria Around Implants and Natural Teeth in the Same Individuals

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Aim: This cross-sectional study assessed cytokine levels in peri-implant crevicular fluid (PICF)/ gingival crevicular fluid (GCF) and a selection of subgingival/submucosal plaque bacteria from clinically healthy or diseased sites in the same individuals.

Material and Methods: Samples from 97 implants/teeth (58 implants: 19 healthy, 20 mucositis, 19 peri-implantitis; 39 natural teeth: 19 healthy, 12 gingivitis, 8 periodontitis) in 15 systemically healthy patients were investigated by immunoassay, real-time PCR. Samples were obtained first and then probing depth, clinical attachment level, bleeding on probing, plaque index scores, keratinized tissue width were recorded. Data were analyzed by Wilcoxon, Mann-Whitney and permutation tests on dependent, independent, mixed dependent and independent samples and Spearman correlation.

Results: Interleukin-1beta levels were significantly higher in PICF samples of healthy implants than in GCF samples of healthy teeth ($p=0.003$), soluble activator of nuclear factor Kappa-B (sRANKL) concentrations were significantly higher in gingivitis than mucositis group ($p=0.004$). The biomarker levels were similar in peri-implantitis and periodontitis groups ($p>0.05$). *Actinomyces naeslundi* and *Streptococcus oralis* levels were significantly higher in healthy implant group than healthy teeth ($p<0.05$). *Prevotella intermedia* and *Treponema denticola* levels were lower in mucositis group than in gingivitis group ($p<0.05$). *Prevotella oralis* and *S. oralis* levels were significantly higher in the periodontitis group ($p<0.05$) and *T. denticola* levels were significantly higher in the peri-implantitis group ($p<0.05$).

Conclusion: There were many similarities but crucially some differences in biomarker levels (IL-1β and sRANKL) and bacterial species between peri-implant and periodontal sites in the same individuals suggesting similar pathogenic mechanisms.

MESH KEY WORDS

biomarkers; cytokines; dental implant; peri-implantitis; periodontitis.

Inflammatory reactions around dental implants may affect only the peri-implant mucosa leading mucositis or also the peri-implant hard tissues leading to peri-implantitis.[1,2] Peri-implant mucositis is characterized by bleeding on probing without bone loss, whereas peri-implantitis is defined as loss of supporting bone around osseointegrated implants that is often associated with pocket formation, suppuration and mobility of the implant.[3]

The prevalence of peri-implant mucositis varies between 36.3-64.6% and peri-implantitis between 8.9-47.1%.[4] Peri-implant diseases may disturb esthetics as well as function of the implants and progressive bone loss may cause implant failure.[5] Therefore, early detection and treatment of peri-implant diseases is of utmost importance. Various clinical measures including probing depth (PD), bleeding on probing (BOP), and plaque index (PI) are used to assess peri-implant health.[6] Inflammatory process of peri-implant tissues has been examined in peri-implant crevicular fluid (PICF) samples.[7] PICF is a valuable biofluid that is easily obtained by non-

invasive methods for examining biochemical mediators with potential for early detection of peri-implant inflammatory diseases. Recent studies have demonstrated that various biomarkers in PICF are selectively upregulated at sites of inflammation and tissue breakdown compared with healthy sites.[8,9]

Major differences between gingivitis and periodontitis sites are presence of a dysbiotic microbiota and a susceptible host.[10,11] Dysbiosis can either be the cause or the consequence of disease. Homeostasis is a condition of equilibrium or stability in a known system.[12] Recently, it has been proposed that periodontitis fundamentally represents disruption of host-microbial homeostasis by dysbiosis of the periodontal microbiota.[10] Periodontal pathogens and their virulence factors stimulate the release of immunological biomarkers such as tumor necrosis factor alpha (TNF-α), interleukin-1beta (IL-1β), IL-6, IL-17, receptor activator of nuclear factor kappa-B ligand (RANKL)[13]. Socransky et al.[14] stated that mainly anaerobic gram-negative bacterial species such as *Tannerella forsythia* (*T. forsythia*), *Treponema denticola* (*T. denticola*), and *Porphyromonas gingivalis* (*P. gingivalis*) are associated with periodontal destruction. So far, the levels of key biologic mediators in gingival crevicular fluid (GCF) and PICF samples have been comparatively investigated in few studies.[15,16] It was hypothesized that possible differences in cytokine content and/or bacterial species between healthy and diseased sites may reveal similar factors acting in pathogenesis of periodontitis and peri-implantitis. Therefore, the aim of the present study was to comparatively assess the levels of IL-1β, IL-17A, IL-17F, IL-17E, sRANKL, and osteoprotegerin (OPG) in PICF/GCF samples and presence of *A. actinomycetemcomitans, Actinomyces naeslundi, Fusobacterium nucleatum, P. gingivalis, Prevotella intermedia, Streptococcus mutans, T. denticola T. forsythia* and *Veillonella dispar* in subgingival/submucosal plaque samples from healthy and diseased teeth and implants in the same individuals.

MATERIALS AND METHODS

Study Population

Fifteen systemically healthy patients were recruited in this cross-sectional study at the Department of Periodontology, School of Dentistry, Ege University, İzmir, Turkey between January 2014 and September 2014. Participants were sequentially recruited among patients referred for periodontal/peri-implant disease treatment. Healthy and diseased sampling sites were matched both for the implants and natural teeth in the same individuals. The inclusion criteria were; being systemically healthy, having implant-supported prosthesis in function for at least 12 months with at least one implant diagnosed with peri-implantitis based on: presence of at least one site with probing depth (PD) > 4 mm with bleeding on probing (BOP) and/or suppuration and radiographic evidence of bone loss ≥ 2 mm.[4] Moreover, the participants were required to have clinical diagnosis of periodontitis based on: presence of PD > 4 mm, clinical attachment level (CAL) ≥ 3 mm, and BOP. Peri-implant mucositis was diagnosed on the presence of BOP without loss of supporting bone.[3,4] Diagnosis of gingivitis was assigned, when there is BOP, PD <4 mm and no clinical sign of periodontitis was evident.[17] Un-inflamed implant and tooth sites with PD< 3mm and CAL< 3mm were considered to be healthy.

The exclusion criteria were presence of any known systemic disease, history of aggressive periodontitis, antibiotic and/or anti-inflammatory drug usage within the last 6 months before the start of the study, and presence of less than 16 teeth. This study was approved by the Institutional

Review Board, School of Medicine, Ege University (protocol no. 13-11.1/9). The study protocol was explained and written informed consent was received from each participant before enrolment in the study.

Sampling sites were selected according to the health status of the peri-implant/periodontal tissues in the same individuals. Teeth with the most severe periodontal tissue destruction comparable with the peri-implantitis sites; and the most severely inflamed sites were included in the periodontitis/gingivitis groups. The investigated dental implants were of two brands[**††] with conical connection design.

Clinical Periodontal Measurements

PD, CAL, BOP, plaque index (PI) scores and keratinized tissue width (Kr TW) were recorded. The clinical periodontal and peri-implant examinations were performed by two periodontists, who were trained and calibrated initially and also at the 6th month of the study (ÖG, PG). The initial intra-examiner kappa values were 0.96 (PD) and 0.86 (CAL) for PG and 0.93 (PD) and 0.79 (CAL) for ÖG. The interexaminer values were 0.92 (PD) and 0.75 (CAL). BOP was deemed positive if it occurred within 15 sec after probing. Presence of visible plaque was recorded dichotomously by visual examination.[18] A periodontal probe[‖] was used for measurement of PD and CAL at four sites (mesio-buccal, mid-buccal, disto-buccal, mid-lingual) of each tooth/implant present, except the third molars. Bite-wing radiographs were taken from all participants to determine the presence of bone loss around the dental implants.

Biofluid and Plaque Sampling

All samples were obtained before clinical periodontal recordings. GCF/PICF samples were obtained by the standard filter paper strips,[¶] from the buccal aspects of the teeth/implants. Filter paper strips were placed in the gingival/peri-implant sulcus and left in place for 30 sec. Care was taken to avoid mechanical trauma, and strips visually contaminated with blood were discarded. Sample volumes were estimated with a calibrated instrument.[#]

Before subgingival/submucosal plaque sampling, supragingival/supramucosal plaque was removed carefully by sterile curettes.[‡‡] The site was then isolated using cotton rolls and gently dried with an air syringe to avoid contamination with saliva. Another sterile Gracey curette was inserted to the bottom of the sulcus/pocket and removed applying a slight force towards the root surface. The tip of the curette was then inserted in the microcentrifuge tube containing 0.5 mL of distilled water and shaken until the plaque was removed from the curette. All samples were immediately frozen and stored at -40°C until the laboratory analyses.

Oral prophylaxis or nonsurgical periodontal treatment was not initiated before sample collection and then individuals were enrolled a periodontal treatment program as required.

Measurement of sRANKL, OPG, IL-1β, IL-17A, IL-17F, IL-17E and Albumin in GCF/PICF Samples

Commercial enzyme-linked immunosorbent assay (ELISA) kits were purchased for the measurement of OPG,[††] sRANKL,[‡‡] IL-17A,[§§] IL-17E,[‖‖] IL-17F,[¶¶] IL-1β[##] and assays were carried out according to the manufacturers' recommendations. The optical densities were read at 450 nm with a background subtraction at 570 nm and the samples were compared with the standards. The minimum detection limits for the assays were; OPG, 7.56 pg/mL; sRANKL, 7.56

pg/mL; IL-1β, 0.39 pg/mL; IL-17A, IL-17F, 0.79 pg/mL; IL-17E and IL-17A/F, 1.59 pg/mL. Albumin levels were determined by ELISA as previously described.[19] The biochemical data were expressed as total amounts per sampling time (pg/30sec) and also as concentrations (pg/μL).

Preparation and Assessment of Genomic DNA

A Gram positive DNA isolation kit[***] was used to prepare genomic DNA from the pelleted microbes present in the GCF sample bacteria and from known quantities of laboratory strains of the target microorganisms.[20] The amount of DNA and the purity was checked at 260 nm and 280 nm (260/280 nm ratio =1.8-2.0=good DNA purity) using a spectrophotometer.[†††] The DNA in standards and plaque samples was measured by fluorimetric analysis.[‡‡‡] The DNA content of the target species used to fix the copy number of its bacterial genome to use as standards for each real-time Quantitative Polymerase Chain Reaction (RT-QPCR) assay.

Real-Time Quantitative Polymerase Chain Reaction

A RT-QPCR assay using TaqMan chemistry[§§§] was used for the detection and quantification of bacterial cell copy numbers in 1 μg of plaque DNA. The primers and probes selected for the following bacteria were as published in the sources shown: *P. gingivalis, A. actinomycetemcomitans, P. intermedia, T. forsythia, F. nucleatum.*[21-24] *T. denticola,*[25] *A. naeslundi, S. oralis*, and *V. dispar*[26] were purchased.[‖‖‖] On a thermal cycler[¶¶¶] the TaqMAN assay PCR cycling parameters used were 10 min at 95°C, and 40 cycles of 30 sec at 95°C and 1 min at 60°C. All primer sets were validated by running four serial 1/10 dilutions of the standard DNA and calculating the efficiency of the reaction (E) where E= (10 -1/slope)-1. All reaction efficiencies calculated were acceptable (between 91-104%). All primer sets failed to amplify the DNA purified from different microbial standards.

Statistical Analysis

Previous studies on sRANKL and OPG in GCF[27] indicated that effect sizes (delta/sigma) > 2 were acceptable and that n > 6 would be sufficient to achieve 90% statistical power in a parametric test. With regard to IL-17 cytokines in GCF[28] n=15 may be sufficient to give 90% statistical power with an effect size >1.25. Although the intention was to make direct comparisons in the same individuals, there were repeat observations from some patients and missing observations from some others (Table 1). All samples were assayed separately, but in those individuals where more than one implant fell into the same category, these values were averaged. First, a Q-Q plot analysis indicated that the data were not normally distributed; therefore, distribution free statistical tests were used. Second, for the analysis of mixed dependent and independent data sets the approach described by Dubnicka et al.[29] was used. In brief, where there was direct concordance in sample numbers between disease categories, the Wilcoxon test was used for dependent sample comparisons in the same individuals. Where all samples were entirely independent between disease categories as between gingivitis and periodontitis, the Mann-Whitney-U test was used to compare the differences between the parameters recorded. Where there was a mixture of dependent and independent samples, a Monte Carlo method of 1000 random data combinations was used in a Permutation test (based on the Wilcoxon test). A Bonferroni correction was used to adjust the P value. Spearman correlations were performed to evaluate the relationship between clinical and biochemical data. All tests were

performed at a significance level of $\alpha = 0.05$. Statistical calculations were performed using the SPSS version 22.0[###] and R (R Project for Statistical Computing) statistical software packages.

RESULTS

GCF/PICF and subgingival/submucosal plaque samples were obtained from a total of 97 implants/teeth (58 implants, 39 natural teeth) in 15 patients. The clinical variables are presented in Table 1. The clinical parameters were similar in the gingivitis and mucositis groups and in periodontitis and peri-implantitis groups (p>0.05). In the healthy implant group PD, CAL, BOP, and PI data were significantly higher than the healthy tooth group (p<0.0001; p=0.01; p=0.009 and p=0.002, respectively).

Biomarkers

The biomarker data are outlined in Figure 1 and Figure 2. OPG total amounts and concentrations and albumin adjusted concentrations (data not shown) were significantly higher (p=0.042; p=0.010; and p=0.027, respectively) and IL-1β, sRANKL levels and sRANKL/OPG ratio were significantly lower in the healthy implant sites than those in the mucositis and peri-implantitis sites (p=0.001 and p=0.001, respectively). sRANKL/OPG ratios were significantly lower at the healthy implant sites than those at the mucositis and peri-implantitis sites (p=0.035 and p=0.008, respectively) (Fig. 1).

OPG concentrations were significantly higher (p=0.031 and p=0.022, respectively); and IL-1β levels, sRANKL levels and sRANKL/OPG ratios were significantly lower at the healthy teeth sites than those at the gingivitis and periodontitis sites (p=0.002 and p=0.018, respectively). IL-1β concentrations were higher at the gingivitis sites than those in the healthy group (p=0.003). Comparisons of natural teeth and implants with similar clinical health state revealed significantly higher IL-1β levels in the healthy implant group than those in the natural teeth (p=0.003), significantly higher sRANKL concentrations at gingivitis than in the mucositis sites (p=0.004) (Fig. 1).

The concentrations of IL-17A, IL-17-F, and IL-17E were similar at healthy and diseased sites (Fig. 2). There was no-significant difference in the concentrations of these cytokines in GCF with the exception of the IL-17E concentration, which was significantly lower at the gingivitis sites than at healthy tooth sites (p<0.05). The comparisons of peri-implantitis and periodontitis samples revealed similar levels and concentrations of the IL-17 family cytokines (Fig. 2).

Microbiological Parameters

The microbiological data are presented in Figure 3. The copy numbers of *A. naeslundii, P. gingivalis, F. nucleatum* and *T. forsythia* levels were significantly higher at the mucositis than the healthy implant sites (p<0.05). Numbers of *F.nucleatum, P. gingivalis, P. intermedia* and *T. forsythia* were significantly higher at the peri-implantitis sites than at the healthy implant sites (p<0.05). *F. nucleatum, P. intermedia* and *T. denticola* levels were significantly higher at gingivitis sites than at healthy teeth (p<0.05). *S. oralis, T. denticola, F. nucleatum* and *T.* forsythia levels were significantly greater at periodontitis sites than at healthy tooth sites (p<0.05). Periodontitis sites revealed significantly higher *P. gingivalis, P. oralis* and *S. oralis* levels than the gingivitis sites (p<0.05). *A. naeslundii* levels were significantly lower in the healthy implants than in the healthy teeth (p<0.05). *P. intermedia*, *T. denticola* levels were lower at the mucositis sites than at the gingivitis sites (p<0.05). *P. oralis, S. oralis* and *T. denticola*

levels were significantly higher in the periodontitis sites than in the peri-implantitis sites ($p<0.05$).

Correlations

At the implant sites, the amount of IL-1β and sRANKL/OPG ratio correlated positively with PD, (rho=0.497 and rho=0.293, respectively), and IL-1β concentration with PD (rho=0.228). There were significant negative correlations between OPG concentration and PD (rho=-0.387) and between the amounts of OPG at implant sites with PD (rho= -0.277). There was also a significant positive correlation between the IL-17A/IL-17E ratio and CAL (rho=0.323) at implant sites. PICF volume correlated significantly with IL-1β (rho=0.659), sRANKL (rho=0.533), and the RANKL/OPG ratio (rho=0.332).

At tooth sites, significant positive correlations were found between the amount of IL-1β and PD (rho=0.511), the amount of sRANKL, sRANKL/OPG and PD (rho=0.375 and rho=0.397), IL-1β concentrations and CAL (rho=0.223). Significant negative correlations consisted of OPG concentration with PD (rho= -0.423), OPG levels with PD (rho= -0.348). GCF volume correlated with IL-1β (rho=0.742); sRANKL (rho=0.513); OPG (rho=- 372) and the RANKL/OPG ratio (rho=0.478). There were numerous significant correlations between the microbiological and clinical data (Table 2). Significant positive correlations were found at both tooth and implant sites between the PD and the presence of *F. nucleatum, P. gingivalis, T. denticola* and *T. forsythia* with the addition of *A. actinomycetemcomitans* and *P. oralis* at the tooth sampling sites. In contrast, *S. oralis* showed a negative correlation with PD at tooth sites. At implant sites, *P. intermedia* and *A. naeslundii* showed weak positive correlations with CAL while *A. actinomycetemcomitans* had a weak negative correlation with CAL (Table 2).

P. gingivalis, F. nucleatum, and *P. intermedia T. denticola, T. forsythia* and *V. dispar* levels correlated positively with the amount of IL-1β. *P. gingivalis, P. intermedia, T. denticola* and *T. forsythia* with the amount of sRANKL and the sRANKL/OPG ratio (Table 3). Significant negative correlations were found between *A. actinomycetemcomitans* and the amount of sRANKL, sRANKL concentration and sRANKL/OPG ratio and *P. gingivalis* with OPG (Table 3).

DISCUSSION

To the best of our knowledge, this is the first cross-sectional study evaluating the biomarkers and microbiological data in implants versus natural teeth matched with regard to the clinical health status in the same individuals. Implants and teeth from the same individuals have been studied before but little information on their disease status was provided.[30] The present study was undertaken to investigate levels of IL-1β, IL-17, sRANKL, and OPG in GCF/PICF and presence and counts of *A. actinomycetemcomitans, A. naeslundi, F. nucleatum, P. gingivalis, P. intermedia, S. mutans, T. denticola, T. forsythia* and *V. dispar* around teeth and implants with different health status in the same individuals.

Like other cross-sectional studies[8,30,31,32] a major limitation is in relating the cause and effect of the disease and an accurate relationship between measurable parameters, however, strength of this type of investigation is the ability to survey a large number of parameters for potential associations. Another limitation of the present study is the rather low number of sampling sites available for periodontitis category to match with the peri-implantitis sites in the same

individuals. The approach of matching the different disease categories in the same individuals severely limited the number of patients eligible for the study. The major challenge this posed was that an exact match of healthy sites with the different disease categories was not possible; however, attempts were made to deal with this issue by carrying out the permutation analysis. An alternative approach could be combining the diseased groups at implants and teeth to compare with the corresponding healthy implants and teeth respectively, but this would have still resulted in a degree of mismatch and would have made the comparisons with the inflamed implant and tooth sites difficult because of the different level of disease at these locations.

Implants with conical implant-abutment connection had negligible bacterial contamination due to the decreased microgap and microleakage under *in vitro* conditions. Microgap and microleakage act as a reservoir of bacteria and this may contribute to the establishment of peri-implantitis.[33] For standardization purposes, only implants with a conical connection design were included in this study.

Pro-inflammatory cytokines induce alveolar bone resorption by increasing the expression of RANKL and reducing OPG in osteoblasts and stromal cells.[34] Mogi et al.[27] reported significantly greater ratio of RANKL/OPG in patients with periodontal disease. Vernal et al.[35] found higher levels of RANKL in untreated patients with chronic periodontitis than healthy controls. An increased RANKL/OPG ratio was reported in GCF samples obtained from periodontitis sites compared to gingivitis/healthy sites.[36] Rakic et al.[8] found higher sRANKL levels in peri-implantitis sites. However, Sarlati et al.[9] reported similar data in the study groups. In their study, 40 implants were categorized as clinically healthy, peri-implant mucositis and peri-implantitis according to the clinical and radiographic findings. Similar RANKL/OPG ratio was found in peri-implantitis sites and healthy controls,[8,37] while Duarte et al.[38] observed an increase in RANKL/OPG ratio in peri-implantitis. In the present study, the healthy implant group revealed significantly higher OPG total amounts and concentrations than the mucositis and peri-implantitis groups; together with significantly lower sRANKL levels and sRANKL/OPG ratios. The present finding of significantly higher OPG concentration in healthy teeth than gingivitis and periodontitis groups is consistent with the existing literature.[27,35] sRANKL concentrations were significantly higher in the present gingivitis than the mucositis group. A recent study reported significantly higher IL-1β levels in peri-implantitis sites compared to healthy implants.[39] Accordingly, we found significantly higher IL-1β levels in the peri-implantitis sites than in the healthy implants; also in the gingivitis, periodontitis than the healthy teeth sites. These findings provide further support for the previous studies suggesting that peri-implant and periodontal diseases have similar patterns in terms of host response and bone destruction. However, when natural teeth and implants are compared, IL-1β levels were significantly higher in the healthy implant group than the healthy teeth sites, and this in part contradicts the findings of a longitudinal study that showed elevated IL-1α levels at healthy tooth sites than at healthy implant sites[40] and loosely in agreement with the cross sectional study by Recker et al.[31] where IL-17A and TNF were higher at implant than at tooth sites. Furthermore, IL-1β and TNF levels were higher at implants than at teeth.[32] According to the present findings, sRANKL concentrations were significantly higher at the gingivitis than at mucositis sites. This may be explained by a rather more prominent innate immune response at peri-implant sites than natural teeth without bone resorption. However, in the current study IL-1β, sRANKL and OPG levels were similar in the peri-implantitis and periodontitis groups.

IL-17 family of cytokines consists of IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F. IL-17A synergizes with other cytokines, including IL-1β, TNF-α.[41] IL-17A increases RANKL expression and concomitantly decreases OPG expression in osteoblastic cells *in vitro* and *in vivo.*[42] IL-17F also plays a critical role in host defense upon bacterial and fungal infection by recruiting neutrophils and producing antimicrobial-peptides.[43] The presence of IL-17A/F heterodimers substantially influences the potency, specificity and the spectrum of the activity of cytokines.[44] In contrast with the other IL-17 family cytokines, IL-17E is an anti-inflammatory cytokine, which opposes the functions of IL-17A and IL-17F.[45] Evidence for this role of IL-17E in the oral environment is manifested in experiments with oral epithelial cells. In the current study, IL-17E concentrations were significantly lower at gingivitis sites than at healthy sites but no significant differences were observed for the other IL-17 cytokines.

Lester et al.[42] found increased IL-17A concentrations in gingival tissue supernatants at sites of severe attachment loss where a positive correlation was detected between the tissue level of this cytokine and CAL. Increased IL-17 levels in chronic periodontitis patients have been reported in other clinical studies.[28,35] Indeed, local tissue expression of the IL-17 probably contributes to higher amounts of IL-17 detected in GCF of periodontitis patients when compared with healthy individuals[28,35] and this finding may suggest an important role for IL-17 in the pathogenesis of chronic periodontitis. In the present study, GCF/PICF IL-17A, IL-17-F, IL-17E levels and IL-17A/IL-17E ratios were similar at all evaluated sites. It has to be conceded that the relatively low numbers of samples in different study groups may have a role in this finding, since studies with larger GCF sample numbers have reported clear differences in IL-17A and in IL-17A/IL-17E ratios.[28] On the other hand, the IL-17A/IL-17E ratio correlated positively with CAL at implant sites in the present study.

The biofilm plays a significant role in initiation and progression of peri-implant and periodontal diseases. An animal study[46] and a human cross-sectional study[47] have revealed that similar bacterial species are associated with periodontitis and peri-implantitis. Shibli et al.[48] evaluated the supragingival and subgingival microflora and concluded that higher mean counts of *P. gingivalis, T. denticola* and *T. forsythia* were observed in the peri-implantitis group. Canullo et al.[49] compared microbiological composition at healthy implants and implants with peri-implantitis and while orange complex species (*P. intermedia, P. micros, F. nucleatum*) were prevalent in both groups, their prevalence was higher in the peri-implantitis group. Implant sites were reported to be positive for *A. actinomycetemcomitans, P. gingivalis, P. intermedia, T. forsythia* and *T. denticola* during the 10-days period after surgery.[50] Red complex microorganisms *(P. gingivalis, T. forsythia, T. denticola*) were found to be more prevalent at peri-implantitis sites than at healthy implants.[39] Consistent with previous reports, significantly lower amounts of *A. naeslundii, P. gingivalis, F. nucleatum* and *T. forsythia* were detected in the healthy implant group than the mucositis group. *F. nucleatum, P. gingivalis, P. intermedia* and *T. forsythia* levels were significantly higher in the peri-implantitis group than the healthy implant group.

Conclusion

Within the limitations of this cross sectional study, some interesting data were obtained that may be relevant to the differing levels of disease at implants and teeth. It might be concluded that peri-implant and periodontal sites in the same patients are similar in many aspects but some differences in biomarker levels were seen particularly in IL-1β, RANKL and OPG. Furthermore,

there were differences in the levels of a select number of bacterial species present at implants and teeth and at both healthy and diseased sites around implants and the dentition.

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Figure 1.

Box and whisker plots showing (A) the amount (B) concentration of IL-1β; (C) the amount, (D) concentration sRANKL; (E) the amount (F) concentration OPG and the (G) sRANKL:OPG ratio in PICF from Implant sites: clinically healthy, with mucositis, with peri-implantitis and in GCF taken from teeth: clinically healthy, gingivitis, and periodontitis.

**Statistically significant differences between implant sites and tooth sites with similar disease categories.*

†Statistically significant differences between the disease categories and the corresponding healthy implant or tooth sites.

†Statistically significant differences between mucositis and peri-implantitis or between gingivitis and periodontitis.

Figure 2.

Box and whisker plots showing (A) the amount, (B) concentration of IL-17A; (C) the amount, (D) concentration of IL-17F; (C) the amount, (D) concentration of IL-17E (IL-25) and (G) the IL-17A:IL-17E ratio in PICF from implant sites: clinically healthy (white), with mucositis (light grey), with peri-implantitis and in GCF from tooth sites: clinically healthy, gingivitis, and periodontitis.

**Statistically significant differences between the disease categories and the corresponding healthy implant or tooth sites ($p<0.05$).*

Figure 3.

The stacked bars represent, in alphabetical order top-bottom, (A) the cumulative copy numbers of microorganisms at implant and tooth sites and (B) the cumulative percentage of each organism at tooth and implant sites. Significant differences between the sample sites for each organism are indicated.

** significantly higher at the mucositis than healthy implant sites ($p<0.05$).*

† significantly higher at the peri-implantitis sites than at the healthy implant sites ($p<0.05$).

‡ significantly higher at the gingivitis sites than at the healthy teeth ($p<0.05$).

§ significantly higher at the periodontitis sites than at the healthy tooth sites ($p<0.05$).

‖ significantly higher at the periodontitis sites than at the gingivitis sites ($p<0.05$).

¶ significantly lower in the healthy implant samples than in the healthy tooth samples ($p<0.05$).

 # significantly lower at the mucositis sites than at the gingivitis sites ($p<0.05$).

*** significantly lower at the peri-implantitis sites than at the periodontitis sites ($p<0.05$)*

Table 1.

Clinical parameters recorded in the sampling sites and full-mouth values

	Implants			Teeth		
Clinical Parameter	Healthy	Mucositis	Peri-implantitis	Healthy	Gingivitis	Periodontitis
N	19 (15)	20 (15)	19 (15)	19 (15)	12 (10)	8 (6)
PICF/GCF volume (µL)	0.51 ± 0.48	0.7 ± 0.46	0.72 ± 0.34*	0.27 ± 0.26	0.53 ± 0.34	0.68 ± 0.38*
PD (mm)	2.09 ± 0.71	2.8 ± 0.77	5.47 ± 2.09†	1.86 ± .57	2.75 ± .51	4.67 ± 0.52†
CAL (mm)	2.3 ± 0.41	2.8 ± 0.77	6.79 ± 3.43*	1.29 ± 1.1	2.75 ± 2.41*	6.84 ± 2.24*
PI	0.05 ± 0.23	0.7 ± 0.47	0.89 ± 0.32*	0.33 ± 0.48	0.5 ± 0.45	1.0 ± 0.0*
KrTW (mm)	3.37 ± 1.26	2. ± 1.56	1.58 ± 1.54	4.88 ± 1.12	3.0 ± 0.8	3.67 ± 0.82
Full-mouth BOP	0.14 ± 0.10	0.63 ± 0.17†	0.67 ± 0.27†	0.3 ± 0.24	0.4 ± 0.21	0.58 ± 0.12
Full-mouth PI	0.14 ± 0.10	0.59 ± 0.13†	0.66 ± 0.21†	0.31 ± 0.25	0.4 ± 0.16	0.65 ± 0.05*
Full-mouth PD	2.42 ± 0.51	2.48 ± 0.3	2.76 ± .42	2.05 ± 0.15	2.0 ± 0.38	2.92 ± 0.2*
Full-mouth CAL	1.2 ± 0.92	2.33 ± 1.03	2.24 ± 1.43	1.8 ± 1.14	2.0 ± 0.48	1.75 ± 1.99
Time in function (months)	15.11 ± 3.43	21.0 ± 8.6	34.11± 14	0.0 ± 0.0	0.0 ± 0.0	12.0 ± 0.0

Significantly different from the healthy sites (*$P<0.05$; †$P<0.01$)

Table 2.

Spearman correlations between the bacterial counts and clinical parameters

	Implants			**Teeth**		
Bacteria species	PD	CAL	PI	PD	CAL	PI
A. actinomycetem	-0.082	-0.222[*]	0.042	0.611[†]	0.111	0.172
A. naeslundi	-0.015	0.238[*]	0.229[*]	0.219	0.327	0.014
F. nucleatum	0.346[†]	-0.131	0.238[*]	0.673[†]	0.039	0.302
P. gingivalis	0.446[†]	0.079	0.419[†]	0.683[†]	0.134	0.337
P. intermedia	0.107	0.257[*]	0.175	0.080	0.017	0.057
P. oralis	-0.097	-0.066	-0.046	0.660[†]	0.235	0.359
S. mutans	0.039	-0.171	0.268[*]	-0.249	0.311	-0.202
S. oralis	-0.168	0.208	-0.007	-0.766[†]	-0.169	-0.446
T. denticola	0.261[*]	-0.011	0.146	0.373	0.192	0.201
T. forsythia	0.269[*]	-0.030	0.233[*]	0.595[†]	-0.100	0.302

Statistically significant correlations ([*]P<0.05; [†]P<0.01)

Table 3.

Spearman correlations between bacterial counts and cytokines

Bacterial species	sRANKL	OPG	IL-1β	sRANKL/OPG
A. actinomycetem	-0.228[*]	0.099	-0.067	-0.241[*]
A. naeslundi	0.025	-0.021	0.016	0.130
F. nucleatum	0.157	-0.104	0.337[†]	0.171
P. gingivalis	0.229[*]	-0.249[*]	0.384[†]	0.278[†]
P. intermedia	0.356[†]	-0.155	0.278[†]	0.214[*]
P. oralis	0.122	-0.060	0.103	0.045
S. mutans	0.046	-0.194	0.113	0.128
S. otalis	0.010	0.079	-0.146	-0.057
T. denticola	0.246[*]	-0.154	0.272[†]	0.224[*]
T. forsythia	0.239[*]	-0.168	0.261[†]	0.238[*]
V. dispar	0.366[†]	-0.385[†]	0.386[†]	0.284[†]

Statistically significant correlations ([*]P<0.05; [†]P<0.01)

[**] C1, MIS implant, Savion Isreal

[††] Conelog, Camlog implant, Basel, Switzerland

[‖] Williams, Hu-Friedy, Chicago, IL, USA

[¶] Periopaper®, Proflow Inc., NY, USA

[#] Periotron 8000®; Oroflow, NY, USA

[‡‡] Hu-Friedy, Chicago, IL, USA

[††] R&D systems, Abingdon, UK

[‡‡] Peprotech, London, UK

[§§] Peprotech, London, UK

[‖‖] Peprotech, London, UK

[¶¶] Peprotech, London, UK

[##] Thermo Fisher, Paisley, UK

[‡‡] Peprotech, London, UK

[***] Epicentre Masterpure, Cambio, Cambridge, UK

[†††] NANODROP 1000 Thermo Renfrew, UK

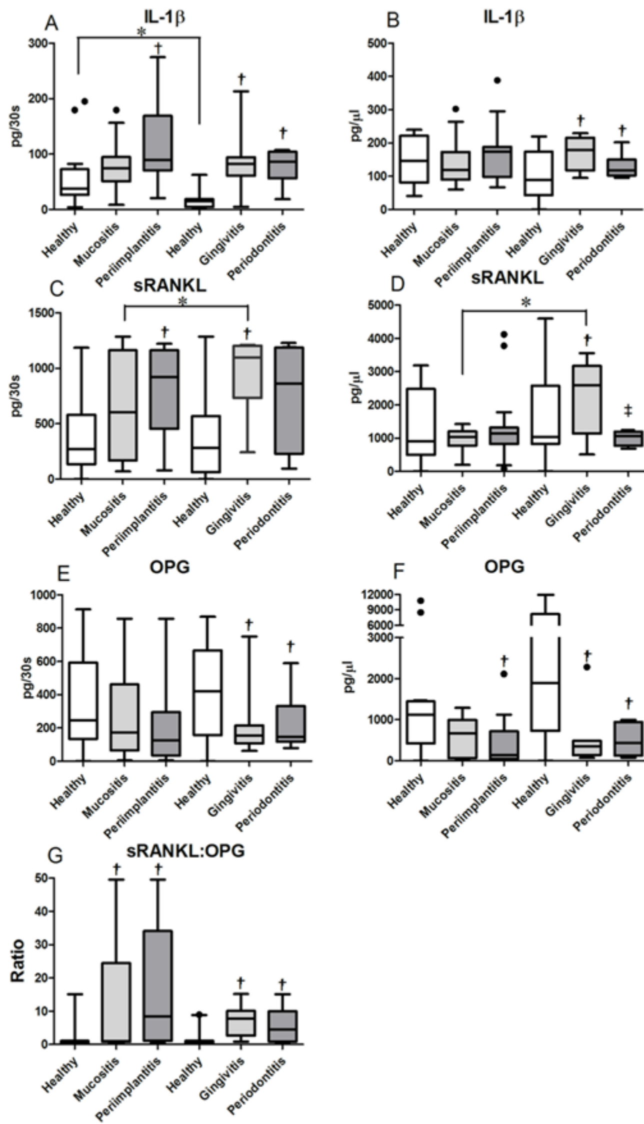
[‡‡‡] CYquant assay system, Invitrogen, Paisley, UK

[§§§] ABI, Invitrogen, Paisley, UK

[‖‖‖] Invitrogen, Paisley, UK

[¶¶¶] MRX3000 Agilent Edinburgh, UK

[###] IBM, Armonk, USA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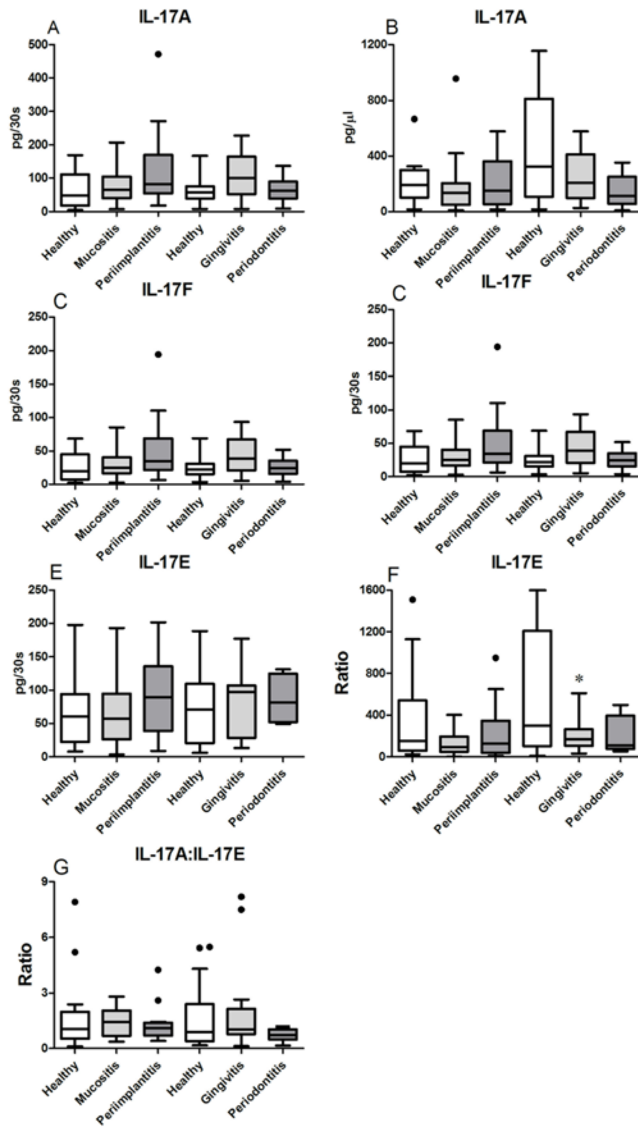
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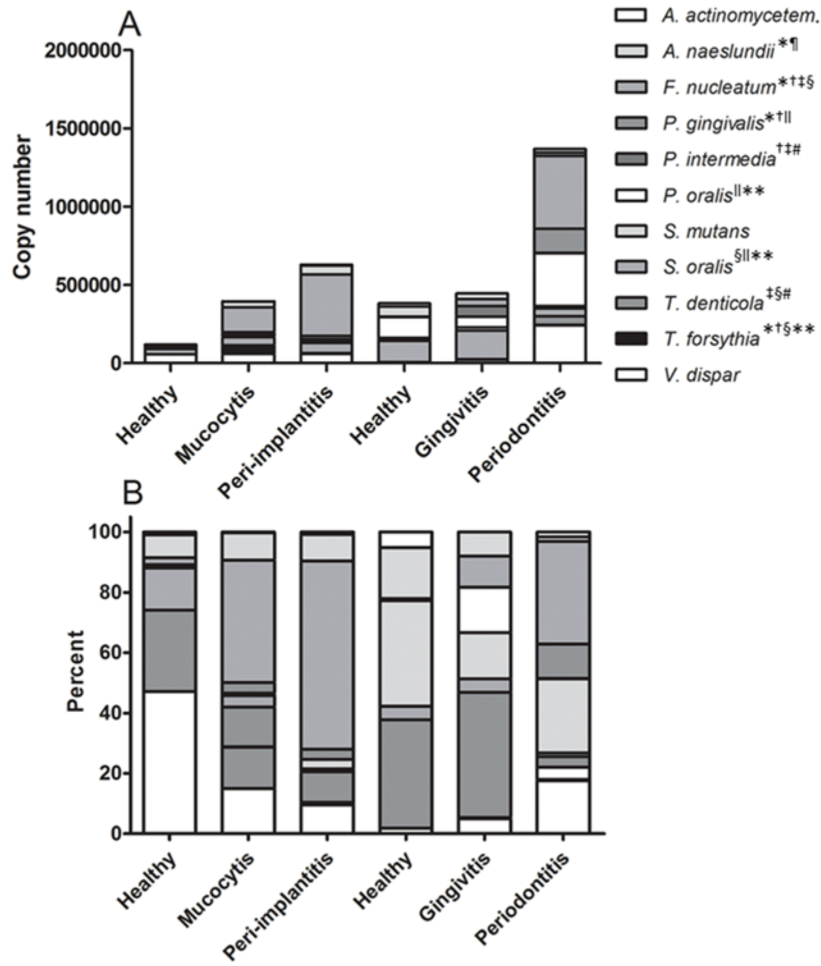
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F. nucleatum*†‡§
P. gingivalis*†||
P. intermedia†‡#
P. oralis||**
S. mutans
S. oralis§||**
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