

Microbiological Aspects of Human Mandibular Subperiosteal Dental Implants

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Clinical, microbiological, and biochemical features of human mandibular subperiosteal dental implants exhibiting peri-implantitis were compared with those experiencing long-term peri-implant health. After evaluation of clinical parameters, submucosal plaque samples were obtained from perimucosal implant abutment posts exhibiting probing depths ≥ 5 mm and bleeding on probing in subjects with peri-implantitis ($n = 3$) and from posts with peri-implant health in subjects with long-term subperiosteal implant health ($n = 8$). The microbial specimens were transported in VMGA III and plated onto enriched Brucella blood agar and Hammond's selective medium with anaerobic incubation, and onto selective TSBV with 5% CO₂ incubation. Total anaerobic viable counts and selected bacterial species were identified using established phenotypic methods and criteria. In vitro resistance to doxycycline (2 $\mu\text{g}/\text{mL}$), amoxicillin (2 $\mu\text{g}/\text{mL}$), or metronidazole (4 $\mu\text{g}/\text{mL}$) was recorded per subject when bacterial pathogen growth was noted on antibiotic-supplemented isolation plates. Interleukin (IL)-1 β levels were measured with an enzyme-linked immunosorbent assay in peri-implant crevicular fluid samples from 5 study subjects. Significantly higher Plaque Index scores, higher total anaerobic viable counts, more red complex species, and lower proportions of gram-positive facultative viridans streptococci and *Actinomyces* species were detected on peri-implantitis-affected subperiosteal implants as compared with subperiosteal implants with long-term peri-implant health. No in vitro resistance to the 3 test antibiotic breakpoint concentrations studied was found, except a *Fusobacterium nucleatum* strain resistant to doxycycline at 2 $\mu\text{g}/\text{mL}$ from 1 peri-implantitis subject. Subperiosteal implants with peri-implantitis tended to yield higher peri-implant crevicular fluid IL-1 β levels. The level of peri-implant supramucosal plaque control and the composition of the peri-implant submucosal microbiome may be important determinants of the long-term clinical status of mandibular subperiosteal dental implants.

Key Words: subperiosteal, dental implant, microbial culture, Porphyromonas gingivalis, peri-implantitis, peri-implant health

INTRODUCTION

Subperiosteal dental implants represent a treatment option available for management of severely atrophic edentulous mandibles.^{1,2} Originating from Sweden in the early 1940s³ and introduced and refined in the United States in the 1950s,^{4,5} subperiosteal implants appear to be rarely used today in oral implantology practices as compared with an estimated 5000 annual patient placements in the United States during the late 1980s.⁶

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Complete mandibular subperiosteal implants are non-endosseous, epostal implants² that have load-bearing feet placed beneath the periosteum onto cortical bone surfaces, including areas of the external oblique ridge, lateral part of the ascending ramus, genial tubercle, mylohyoid ridge, and symphysis.⁵ They are custom cast to fit the existing topography of alveolar bone surfaces using a biocompatible surgical grade 60% cobalt–20% chromium–5% molybdenum alloy, or sometimes titanium.⁷ Bilateral anterior and posterior permucosal abutment posts extend into the oral cavity and often connect to an intraoral mesostructure (Brookdale) bar used to support prosthetic restorations.⁵ Subperiosteal implants for severely atrophic edentulous mandibles may offer potential advantages of minimizing ridge augmentation treatment needs, reducing the length of treatment time required to restore lost prosthetic function, and possibly providing a lower financial burden for some patients, in comparison to some alternative treatment plans involving endosseous root form dental implant systems.

Published long-term clinical evaluations of complete bilateral mandibular subperiosteal implants reveal 10- to 20-year survival rates ranging from 50% to 96%,^{8–15} with some successfully in function for at least 36 years.¹³ Spreading infections on permucosal abutment posts, clinically similar to peri-implantitis on endosseous implant systems, are prominent among complications reported with subperiosteal implants and are a leading reason underlying clinical decisions to resect out involved portions of the tissue-embedded implant metal substructure or fully remove the implant.^{16–20} Reasons as to why some human subperiosteal dental implants suffer marked spreading submucosal infections leading to implant failure and loss and others experience long-term peri-implant health and clinical stability have yet to be conclusively established. In this regard, no research attention has been given to the composition of bacterial plaque biofilms that colonize subperiosteal implants with peri-implantitis complications as well as subperiosteal implants demonstrating peri-implant clinical health.

Thus, the objective of the present study was to compare clinical, microbiological, and biochemical features of human mandibular subperiosteal dental

implants exhibiting peri-implantitis to those experiencing long-term peri-implant health and clinical stability.

MATERIALS AND METHODS

Subjects

A total of 11 systemically healthy adults (2 men, 9 women; aged 64–83 years; mean [\pm SD] 74.0 \pm 6.7 years), with atrophic edentulous mandibles treated with complete bilateral mandibular subperiosteal dental implants, prosthetically restored with removable overdentures, and occluding with tissue-borne maxillary complete dentures, were included in the present study. All of the implant frames were cast using a cobalt-chromium-molybdenum alloy (Vitallium, presently Dentsply Austenal, York, Penn). Three study subjects, with subperiosteal implants in function for a mean (\pm SD) of 11.7 \pm 1.5 years (range, 10–13 years), presented with peri-implantitis lesions, characterized by marked gingival tissue inflammation, suppuration, and increasing probing depths, at 1 or more permucosal implant abutment posts (Figure 1). For 8 study subjects, with subperiosteal implants present for a mean (\pm SD) of 12.4 \pm 4.9 years (range, 9–22 years), long-term peri-implant health and clinical stability characterized their mandibular subperiosteal dental implants (Figure 2). None of the study subjects had received systemic or locally delivered antibiotics within the prior 6 months.

Clinical evaluations

Clinical parameters were scored at affected permucosal implant abutment posts in subjects with peri-implantitis lesions and at all permucosal implant abutment posts in subjects with long-term subperiosteal implant health. The Gingival Index was used to score peri-implant mucosal tissue inflammation, and the Plaque Index was used to quantitate supramucosal plaque, both on a 0 to 3 scale.^{21,22} Suppuration was dichotomously scored as present or absent. Peri-implant probing depths were measured to the nearest millimeter using a calibrated periodontal probe with color-coded Williams markings, with the presence or absence of bleeding on probing within 30 seconds additionally recorded.

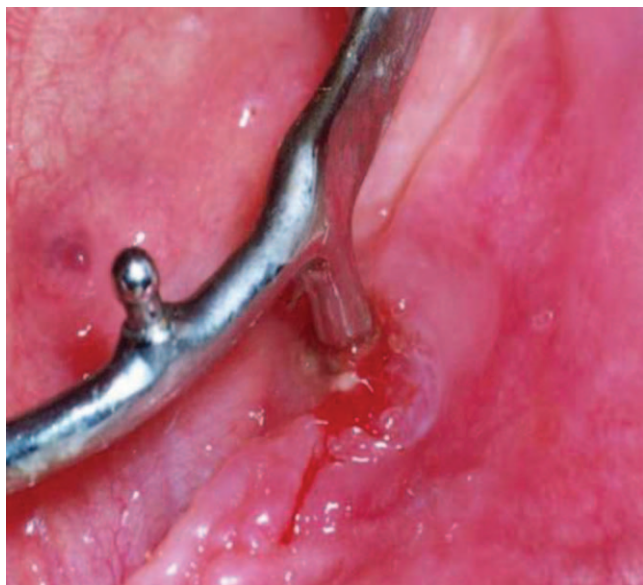


FIGURE 1. Subperiosteal implant permucosal abutment post with gingival inflammation, bleeding on probing, suppuration, and a 7-mm probing depth.

Microbial sampling and transport

Submucosal plaque biofilm specimens were obtained from permucosal implant abutment posts exhibiting probing depths ≥ 5 mm and bleeding on probing in subjects with peri-implantitis, and from permucosal implant abutment posts in subjects with long-term subperiosteal implant health. After isolation with cotton rolls and removal of saliva and

supramucosal deposits, 1 to 2 sterile, absorbent paper points (Johnson & Johnson, East Windsor, NJ) were advanced into each selected permucosal site for approximately 10 seconds. Upon removal, all paper points per study subject were pooled into a glass vial containing 6 to 8 small glass beads and 2.0 mL of anaerobically prepared and stored VMGA III transport medium,²³ which possesses a high preservation capability for oral microorganisms during postsampling transit to the laboratory.^{23,24} The submucosal samples were then transported within 24 hours to the Oral Microbiology Testing Service (OMTS) Laboratory at Temple University School of Dentistry, which is licensed for high-complexity bacteriological analysis by the Pennsylvania Department of Health.

Microbial culture and incubation

At the OMTS Laboratory, the specimen vials were warmed to 35°C to liquefy the VMGA III transport medium, and sampled microorganisms were mechanically dispersed from the paper points with a Vortex mixer at the maximal setting for 45 seconds. Serial 10-fold dilutions of the dispersed bacteria were prepared in Möller's VMG I anaerobic dispersion solution, composed of prereduced, anaerobically sterilized, 0.25% tryptose–0.25% thiotone E peptone–0.5% NaCl.²³ Appropriate dilution aliquots were plated onto nonselective enriched Brucella blood agar (EBBA) primary isolation plates,²⁵ com-

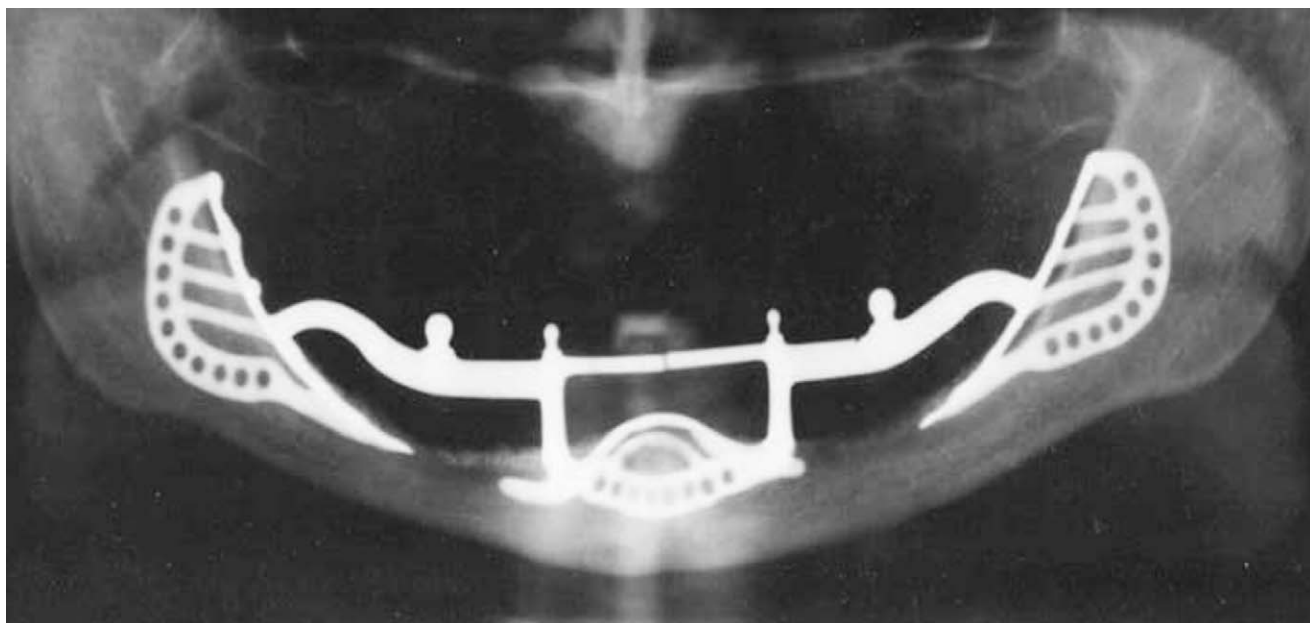


FIGURE 2. Example of subperiosteal implant with long-term peri-implant health and clinical stability over 9 years.

posed of 4.3% Brucella agar supplemented with 0.3% bacto-agar, 5% defibrinated sheep blood, 0.2% hemolyzed sheep red blood cells, 0.0005% hemin, and 0.00005% menadione, onto Hammond's selective *Campylobacter* medium²⁶ and onto selective trypticase soy-bacitracin-vancomycin (TSBV) agar.²⁷ EBBA and Hammond's selective medium plates were incubated at 35°C for 7 days in a Coy anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich) containing 85% N₂-10% H₂-5% CO₂, and TSBV plates were incubated at 35°C for 3 days in 5% CO₂-95% air.

Microbial identification

Test putative pathogens examined for in this study included *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia/nigrescens*, *Parvimonas micra*, *Fusobacterium nucleatum*, *Campylobacter rectus*, *Streptococcus constellatus*, *Staphylococcus aureus*, *Enterococcus faecalis*, gram-negative enteric rods/pseudomonads, and *Candida* species. Other organisms of less putative pathogenic potential examined for in this study were *Capnocytophaga* species, *Actinomyces* species, and viridans group streptococci (encompassing mitis and salivarius subgroup species and excluding anginosus subgroup organisms).

Total anaerobic viable counts, *P. gingivalis*, *T. forsythia*, *P. intermedia/nigrescens*, *P. micra*, *S. aureus*, *E. faecalis*, *Capnocytophaga* species, *Actinomyces* species, and viridans group streptococci were identified on EBBA plates using a ring-light magnifying loupe and presumptive phenotypic methods and criteria previously described.²⁸⁻³⁰ *F. nucleatum* was similarly identified on EBBA plates as long-wave ultraviolet light autofluorescent chartreuse positive,³¹ gray, iridescent colonies of gram-negative, filamentous, spindle-shaped, nonmotile rods. *S. constellatus* on EBBA plates was defined as gram-positive, lactose MUG-test negative,³² nonmotile, facultative cocci demonstrating small white, opaque, circular, beta-hemolytic, surface colonies with irregular edges and positive for α -D-glucosidase enzyme activity, with or without β -D-fucosidase, β -D-glucosidase-positive reactions, as determined with the Fluo-Card Milleri test kit (Key Scientific Products Co, Stamford, Tex).³³ *C. rectus* was quantitated on Hammond's medium³⁴ and *A. actinomycetemcomitans*, gram-negative enteric

rods/pseudomonads, and *Candida* species on TSBV agar.^{25,27} Proportional subject recovery of the various test microorganisms was calculated as the percentage recovery of the test species colony-forming units (CFU) among total submucosal anaerobic viable CFU counts as determined on nonselective EBBA plates.

In vitro antibiotic resistance testing

Aliquots of submucosal sample dilutions from the study subjects were additionally inoculated onto EBBA primary isolation plates supplemented with either doxycycline at 2 μ g/mL, amoxicillin at 2 μ g/mL, or metronidazole at 4 μ g/mL (all antimicrobials obtained as pure powder from Sigma-Aldrich, St Louis, Mo) and incubated anaerobically for 7 days. In vitro resistance to the antibiotic breakpoint concentrations of doxycycline (2 μ g/mL), amoxicillin (2 μ g/mL), or metronidazole (4 μ g/mL) was recorded per subject when test putative pathogen growth was noted on the respective antibiotic-supplemented EBBA plates.^{25,30,35,36} *Bacteroides thetaiotaomicron* ATCC 29741, *Clostridium perfringens* ATCC 13124, and a multi-antibiotic-resistant clinical periodontal isolate of *F. nucleatum* were employed as positive and negative quality controls for all antibiotic resistance testing on drug-supplemented EBBA plates.

Crevicular fluid interleukin-1 β measurement

A subset of 5 study subjects were sampled for evaluation of their peri-implant crevicular fluid. After supramucosal plaque levels were scored and removed, but prior to other clinical evaluations and microbiological sampling, peri-implant crevicular fluid was collected from 1 affected permucosal implant abutment post in each of 2 study subjects with peri-implantitis and from 1 permucosal implant abutment post in each of 3 study subjects with long-term peri-implant health. After isolation with cotton rolls and gentle air drying, 1 absorbent Periotron filter paper strip (Harco Electronics, Winnipeg, Manitoba, Canada) was gently inserted from the facial surface of each selected implant abutment post into the submucosal area for 30 seconds, placed into a plastic microfuge tube, and stored at -20°C until laboratory testing. Interleukin (IL)-1 β levels were measured in the crevicular fluid samples using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Cistron

Biotechnology, Pine Brook, NJ), following the manufacturer's instructions. The results were read by a microplate reader, and IL-1 β concentrations were expressed as picograms (pg) per microgram (μ g) of sample extract.

All laboratory procedures were performed on a blinded basis without knowledge of the clinical status of the study subjects or their inclusion in the present report. Approval for this analysis was provided by the Temple University Human Subjects Institutional Review Board.

Data analysis

Recovered test microorganisms were grouped for reporting purposes into submucosal bacterial clusters (ie, red complex, orange complex, and other species), as previously described.³⁷ Descriptive analysis was used to calculate mean subject probing depth, Plaque Index, and Gingival Index values; the proportion of test sites per subject positive for bleeding on probing and suppuration; the subject occurrence and proportional cultivable recovery of test micro-organisms; and the subject occurrence of in vitro test putative pathogen antibiotic resistance. The Student *t* test for differences in means and a *z* test for differences in proportions assessed the relationship of various clinical parameters, total anaerobic viable counts, and the subject occurrence of red complex microbial species between study subjects with peri-implantitis and those with long-term peri-implant health. A *P* value of $\leq .05$ was required for all tests of statistical significance.

RESULTS

Clinical findings

Table 1 shows that subperiosteal implants with peri-implantitis exhibited suppuration, bleeding on probing, and mean probing depths of 6.6 mm at affected permucosal implant abutment posts, and yielded significantly greater Plaque Index and Gingival Index values ($P < .001$) as compared with subperiosteal implants with long-term clinical health.

Microbiological findings

Total submucosal anaerobic viable EBBA counts averaged $2.9 \times 10^8 \pm 1.1 \times 10^8$ (SE) CFU/mL from subperiosteal implants exhibiting peri-implantitis,

which was significantly higher than what was recovered from subperiosteal implants with long-term clinical health (mean, $2.1 \times 10^7 \pm 7.3 \times 10^6$ [SE] CFU/mL; $P = .002$, *t* test).

Table 2 lists the distribution of test bacterial species recovered from the 11 study subjects with subperiosteal dental implants.

P gingivalis was recovered from 3 (100%) and *T forsythia* from 2 (66.7%) of the subperiosteal implant subjects with peri-implantitis, at mean submucosal proportions of 35.5% and 2.5%, respectively, in culture-positive subjects. These two red complex bacterial species occurred significantly more in subperiosteal peri-implantitis as compared with subperiosteal implant clinical health ($P < .05$), where they were not detected by culture.

Among orange complex species, both *P intermedia/nigrescens* at a mean proportional recovery of 5.4% and *P micra* at mean submucosal proportions of 22.6% were isolated from all 3 peri-implantitis subjects, along with *C rectus* in 2 subjects and *F. nucleatum* from 1 peri-implantitis subject. A lower occurrence of these species was found in subperiosteal implants with long-term clinical health, as only 1 subject yielded *P intermedia/nigrescens* and another trace proportions of *S constellatus* (Table 1).

Gram-positive facultative viridans streptococci and *Actinomyces* species, which were isolated from all study subjects, were present in significantly lower cultivable submucosal proportions from subperiosteal implants with peri-implantitis as compared with those with long-term clinical health (23.8% vs 62.2%, $P < .05$). *Capnocytophaga* species were additionally isolated from 2 subperiosteal implants with long-term clinical health (Table 1). One peri-implantitis and 1 clinically healthy subperiosteal implant revealed low submucosal proportions (0.001% and 0.2%, respectively) of *Candida* species. Cultivable submucosal *A actinomycetemcomitans*, *S aureus*, *E faecalis*, and gram-negative enteric rods/pseudomonads were not detected in any of the study subjects.

In vitro antibiotic resistance testing

No in vitro resistance to the 3 test antibiotic breakpoint concentrations studied was found among recovered putative bacterial pathogens on drug-supplemented EBBA primary isolation plates, except *F nucleatum* resistant to doxycycline at 2 μ g/mL in 1 peri-implantitis subject.

TABLE 1
Clinical peri-implant parameters in 11 adults with subperiosteal dental implants

	Subperiosteal Implants With Peri-implantitis (n = 3)	Subperiosteal Implants With Peri-implant Health (n = 8)	P Value
Mean \pm SE plaque index	2.3 \pm 0.1	0.2 \pm 0.1	<.001
Mean \pm SE gingival index	2.0 \pm 0	0.5 \pm 0.1	<.001
Sites with suppuration, %	100	0.0	<.05
Sites with bleeding on probing, %	100	0.0	<.05
Mean \pm SE probing depth, mm	6.6 \pm 0.4	1.6 \pm 0.1	<.001

Crevice fluid findings

All 5 subperiosteal implants sampled for crevice fluid ELISA analysis revealed measurable IL-1 β in their peri-implant crevice fluid. While no statistically significant clinical group differences were found with the relatively small number of crevice fluid samples studied, the 2 sampled subperiosteal implants with peri-implantitis tended to display higher average crevice fluid IL-1 β levels (mean [\pm SE], 70.2 \pm 15.9 pg/ μ g) as compared with the 3 sampled implants with long-term clinical health (mean [\pm SE], 22.8 \pm 8.3 pg/ μ g).

DISCUSSION

Subperiosteal dental implants for treatment of severely atrophic edentulous mandibles have received little or no study in regard to microbiological parameters related to their long-term clinical

outcomes. The present study provides the first data to date on cultivable bacterial populations associated with human subperiosteal implants afflicted with peri-implantitis, as well as subperiosteal implants with long-term peri-implant clinical health. Previously, the only microbiological data on subperiosteal implants were presented in a report by Becker et al,³⁸ in which *P gingivalis* and *P intermedia* were found with whole-genomic DNA probes in a single subject with a failing subperiosteal implant demonstrating mobility and peri-implant radiolucency.

Despite marked differences among varying dental implant systems in their design and surface characteristics, subperiosteal implants with peri-implantitis in the present study revealed submucosal bacterial profiles remarkably similar in composition to those on various types of endosseous dental implants affected by peri-implantitis, as well as natural teeth with periodontitis.³⁷⁻⁴⁰ Significantly

TABLE 2
Occurrence and proportional submucosal recovery of selected bacterial species in 11 adults with subperiosteal dental implants

	Subperiosteal Implants With Peri-implantitis (n = 3)		Subperiosteal Implants With Peri-implant Health (n = 8)	
	No. (%) of Culture-Positive Subjects	Mean \pm SE Recovery in Positive Subjects, %	No. (%) of Culture-Positive Subjects	Mean \pm SE Recovery in Positive Subjects, %
Red complex species				
<i>Porphyromonas gingivalis</i>	3 (100)	35.5 \pm 10.1	0	0
<i>Tannerella forsythia</i>	2 (66.7)	2.5 \pm 2.0	0	0
Orange complex species				
<i>Prevotella intermedia/nigrescens</i>	3 (100)	5.4 \pm 2.7	1 (12.5)	10.2
<i>Fusobacterium nucleatum</i>	1 (33.3)	0.1	0	0
<i>Parvimonas micra</i>	3 (100)	22.6 \pm 8.2	0	0
<i>Campylobacter rectus</i>	2 (66.7)	1.7 \pm 0.6	0	0
<i>Streptococcus constellatus</i>	0	0	1 (12.5)	0.5
Other species				
<i>Capnocytophaga</i> species	0	0	2 (25.0)	7.5 \pm 0.7
Viridans streptococci/ <i>Actinomyces</i> species	3 (100)	23.8 \pm 6.4	8 (100)	62.2 \pm 9.5

higher numbers of total anaerobic bacterial counts, and a significantly greater occurrence, with high submucosal proportions of the red complex microbial species *P gingivalis* and *T forsythia* were found on subperiosteal implants with inflammatory peri-implant tissue breakdown as compared with subperiosteal implants with long-term peri-implant health. In addition, all 3 peri-implantitis-affected subperiosteal implants were colonized by the orange complex putative pathogens *P intermedia/nigrescens* and *P micra* and 2 by *C rectus*. Peri-implantitis on many different types and brands of root-form dental implants and teeth with severe periodontitis also frequently harbor high numbers of these putative pathogenic microbial species.³⁷⁻⁴⁰

In comparison, subperiosteal implants with long-term peri-implant health in the present study revealed elevated submucosal proportions of gram-positive facultative viridans streptococci and *Actinomyces* species and a relative absence of putative bacterial pathogens, consistent with prior microbiological studies of other types of healthy dental implants and natural teeth.^{37,39,41,42}

The use of culture methodology for microbial recovery and identification is a limitation of the present study, with additional microbial species likely to be identified from subperiosteal implants with use of more sensitive culture-independent, molecular microbiology techniques.⁴³ Interestingly, almost no in vitro antibiotic resistance to therapeutic concentrations of doxycycline, amoxicillin, or metronidazole were detected among putative bacterial pathogens from subperiosteal peri-implantitis lesions, which is clinically relevant to the potential application of local and/or systemic antimicrobial therapies increasingly recommended as a part of peri-implantitis treatment.^{39,44} Relative to this, it is important to note that peri-implantitis-related pocketing around subperiosteal perimucosal abutment posts progresses laterally along implant metal substructure components positioned beneath gingival tissues,⁴⁵⁻⁴⁷ markedly compromising the access and effectiveness of conventional mechanical debridement procedures in limiting advancing submucosal pathogenic implant biofilms. However, it is not presently known whether adjunctive local and/or systemic antimicrobial therapy provides long-term benefits in such clinical situations.

A trend toward higher IL-1 β cytokine levels in

crevicular fluid was found on 2 subperiosteal implants with peri-implantitis as compared with 3 healthy subperiosteal implants, which is consistent with prior crevicular fluid studies on endosseous dental implants.⁴⁸⁻⁵⁰ No previous crevicular fluid data have been reported for subperiosteal dental implants. Although the pathogenesis of human peri-implantitis lesions remains to be conclusively delineated, a range of potential triggering factors in the etiology of peri-implantitis has been proposed,⁵¹ including presence of pathogenic bacterial species. In this regard, the findings of the present study underscore the potential importance of meticulous bacterial control in maintaining peri-implant health on subperiosteal implant surfaces. It is noteworthy that low Plaque Index scores, indicative of a high level of patient supramucosal plaque control, were found on subperiosteal implant abutment posts colonized by high submucosal proportions of gram-positive facultative viridans streptococci and *Actinomyces* species of relatively low pathogenic potential and exhibiting long-term peri-implant health. In contrast, significantly higher Plaque Index scores were associated with peri-implantitis-affected subperiosteal implant abutment posts yielding large submucosal populations of anaerobic microorganisms and a variety of putative pathogenic bacterial species, including *P gingivalis*, *T forsythia*, *P intermedia/nigrescens*, *P micra*, and *C rectus*. However, because of the cross-sectional design of the present study, any potential causal relationships between supramucosal plaque levels, as well as the profile of bacterial species detected in the peri-implant submucosal environment, with the onset of peri-implantitis on subperiosteal dental implants could not be ascertained.

Nevertheless, the level of peri-implant supramucosal plaque control and the composition of the peri-implant submucosal microbiota may be important determinants of the long-term clinical status of mandibular subperiosteal dental implants. Since a recent systematic review concluded that "there is no evidence for a single, universally superior treatment modality for the edentulous mandible,"⁵² renewed focus on use of subperiosteal dental implants for carefully selected patients with severely atrophic mandibles may be appropriate if their long-term clinical stability free of infectious complications can be enhanced. As a result, therapeutic

and prophylactic antimicrobial clinical strategies and procedures to improve patient plaque control and more effectively suppress or eliminate putative bacterial pathogens from perimucosal abutment posts may be of potential value in maintaining subperiosteal implant health and are in need of further research attention.

CONCLUSIONS

Significantly higher Plaque Index scores, higher total anaerobic viable counts, more red complex species, and lower proportions of gram-positive facultative viridans streptococci and *Actinomyces* species were detected on 3 peri-implantitis-affected subperiosteal implants as compared with 8 subperiosteal implants with long-term peri-implant health. The level of peri-implant supramucosal plaque control and the composition of the peri-implant submucosal microbiome may be important determinants of the long-term clinical status of mandibular subperiosteal dental implants.

ABBREVIATIONS

CFU: colony-forming units
 EBBA: enriched Brucella blood agar
 ELISA: enzyme-linked immunosorbent assay
 IL-1 β : interleukin-1 β
 OMTS: Oral Microbiology Testing Service
 TSVB: trypticase soy-bacitracin-vanomycin

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