The Peri-Implant Sulcus Compared with Internal Implant and Suprastructure Components: A Microbiological Analysis

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ABSTRACT

Purpose: A recent *in vivo* study has shown considerable contamination of internal implant and suprastructure components with great biodiversity, indicating bacterial leakage along the implant-abutment interface, abutment-prosthesis interface, and restorative margins. The goal of the present study was to compare microbiologically the peri-implant sulcus to these internal components on implants with no clinical signs of peri-implantitis and in function for many years. Checkerboard DNA-DNA hybridization was used to identify and quantify 40 species.

Material and Methods: Fifty-eight turned titanium Brånemark implants in eight systemically healthy patients (seven women, one man) under regular supportive care were examined. All implants had been placed in the maxilla and loaded with a screw-retained full-arch bridge for an average of 9.6 years. Gingival fluid samples were collected from the deepest sulcus per implant for microbiological analysis. As all fixed restorations were removed, the cotton pellet enclosed in the intra-coronal compartment and the abutment screw were retrieved and microbiologically evaluated.

Results: The pellet enclosed in the suprastructure was very similar to the peri-implant sulcus in terms of bacterial detection frequencies and levels for practically all the species included in the panel. Yet, there was virtually no microbial link between these compartments. When comparing the abutment screw to the peri-implant sulcus, the majority of the species were less frequently found, and in lower numbers at the former. However, a relevant link in counts for a lot of bacteria was described between these compartments. Even though all implants in the present study showed no clinical signs of peri-implantitis, the high prevalence of numerous species associated with pathology was striking.

Conclusions: Intra-coronal compartments of screw-retained fixed restorations were heavily contaminated. The restorative margin may have been the principal pathway for bacterial leakage. Contamination of abutment screws most likely occurred from the peri-implant sulcus via the implant-abutment interface and abutment-prosthesis interface.

KEY WORDS: abutment-prosthesis interface, contamination, dental implant, implant-abutment interface, leakage

INTRODUCTION

Essentially, two to three parts can be identified in all implant reconstructions. The implant includes the endosseous part and connects a suprastructure or prosthetic part, which ultimately restores function and aesthetics. Even though both are intimately attached at one-piece implants, a microgap seems inevitable at the interface. A second and deeper microgap is typically formed when two-piece implants are used in rehabilitation. Indeed, these include a removable transmucosal component or abutment connected to the implant on one hand and to the prosthesis on the other hand. As such, an implant-abutment

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interface and abutment-prosthesis interface are created. The magnitude of the former depends upon the manufacturer and seems limited to less than 50 μ for commonly used implant systems.[1,2] Larger gaps may be expected at the abutment-prosthesis interface,[1] which is not surprising as the prosthetic part is not prefabricated and thus, possibly less fitting. In addition, both microgaps may be further widened under loading conditionsv.[3] Because most of the oral bacteria are about 10 μ large, microbial pathways are created along the interfaces toward internal implant and suprastructure components and vice versa. A number of in vitro studies[2,4–10] and some *in vivo* studies[11–13] have confirmed this phenomenon, which is considered the most plausible explanation for the development of an inflammatory cell infiltrate along the interfaces.[14] These bacterial flows may also explain more initial bone loss around two-piece implants.[14,15] Indeed, their implant-abutment interface is located much closer to the alveolar crest than the microgap at one-piece implant types. In this regard, more inflammation and bone loss may be anticipated as more implants become submerged.[16]

Recently, the microbiota at internal implant and suprastructure components has been thoroughly described.[13] This cross-sectional *in vivo* study included data on 58 implants with no clinical signs of peri-implantitis supporting screw-retained full-arch bridges for an average of 9.6 years. The purpose of the present microbiological study was to compare the peri-implant sulcus to the internal components using checkerboard DNA-DNA hybridization. To our knowledge, this is the first investigation to explore a microbial link between the peri-implant sulcus and internal implant and suprastructure compartments.

MATERIAL AND METHODS

Study Sample

The data in the present study were collected from implant reconstructions that had been used to document bacterial contamination of internal implant and suprastructure components in an earlier report.[13] Fifty-eight turned titanium implants (Brånemark, Nobel Biocare, Gothenburg, Sweden) in eight systemically healthy subjects under regular supportive care were examined. All implants had been placed in the maxilla and loaded with a screw-retained full-arch bridge for an average of 9.6 years. Two patients were fully edentulous, whereas six still had natural teeth in the mandible. These teeth showed healthy periodontal conditions. Table 1 summarizes demographic details. All patients were thoroughly informed and signed a consent form. The ethical committee of the University Hospital in Ghent approved the study protocol.

TABLE 1 Patient Characteristics

Number of patients	8
Age	
Mean (SD)	58.3 (9.7)
Sex	
F	7
M	1
Smoker	1
Number of implants, mean (SD)	7.9 (0.4)
Years in function, mean (SD)	9.6 (3.1)
Plaque index (%), mean (SD)	32.6 (35.3)
Bleeding on probing (%), mean (SD)	31.2 (36.2)
Probing pocket depth (mm), mean (SD)	3.1 (0.9)
Radiographic bone loss (mm): mean (SD)	2.6 (1.4)

F, female; M, male; SD, standard deviation.

Clinical Examination

Prior to bridge removal, an experienced clinician examined all patients. The presence/absence of plaque and probing pocket depths were measured at all implants at six sites (mesial, central, distal; buccally as well as orally) using a manual periodontal probe (CP 15 UNC, Hu-Friedy Mfg. Co. Inc., Chicago, IL, USA). At the same sites, data on the presence/absence of bleeding on probing (BoP) were collected. Radiographs were taken using the long-cone parallel technique to evaluate the position of the crestal bone level in relation to the implant-abutment interface.

Microbiological Examination

After clinical examination, subgingival microbial samples were obtained from the deepest pocket of each implant. Supragingival plaque was first removed with sterile cotton pellets, and a sterile paper point (Mynol Plus, Ada Products, Milwaukee, WI, USA) was then inserted into the pocket until resistance was felt. After leaving it *in situ* for 20 s, each paper point was placed in a separate sterile and dry Eppendorf tube. Thereafter, the internal implant and suprastructure compartments were sampled. These are illustrated in Figure 1. First, the coronal seal of the screw access holes was removed. The

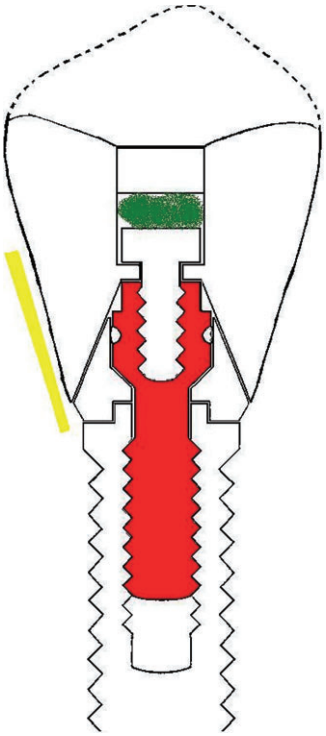

Figure 1 Schematic view of an implant and suprastructure showing the different sampled locations: green, cotton pellet at the intra-coronal compartment; red, abutment screw; yellow, paper point in the peri-implant sulcus.

cotton pellet from the intra-coronal compartment was harvested and individually placed in a sterile and dry Eppendorf tube. Thereafter, the bridge was unscrewed and the abutment screw was retrieved and sonicated in a sterile Eppendorf tube filled with 0.15 ml DNA-free and sterile water for 10 seconds. Special care was given not to contaminate the pellets and the screws while removing them. All samples were sent to the microbiology laboratory at the University of Bern, Switzerland for analysis using the checkerboard DNA-DNA hybridization technique. This assay included a panel of 40 bacterial species, which are listed in Table 2. Details of the procedures have been described elsewhere.[17–20] Briefly, the samples were individually placed in Eppendorf tubes containing 0.15 ml Tris-HCL and ethylenediaminetetraacetate (10 mM Tris-HCL, 1 mM EDTA, pH 7.6). Within 30 min, 0.1 ml 5 M NaOH was added to each tube. Bacterial DNA was extracted, concentrated on nylon membranes (Roche Diagnostics GmbH, Mannheim, Germany), and fixed by cross-linking using ultraviolet light (Stratalinker 1,800, Stratagene, La Jolla, CA, USA). The membranes with fixed DNA were placed in a Miniblotter 45 (Immunetics, Cambridge, MA, USA). A 30×45 "checkerboard" pattern was produced as described by Socransky and colleagues[21] and Katsoulis and colleagues.[18] Chemiluminescent signals were detected using the Storm Fluor-Imager (Storm 840, Amersham Biosciences, Piscataway NJ, USA). In order to receive a full detailed account of the identified bacteria, the digitized information was analyzed by a software program (ImageQuant, Amersham Pharmacia, Piscataway, NJ, USA), allowing comparison of signals against standard lanes of known bacterial amounts. Signals were converted to absolute counts by comparison with these standards and studied as the proportion of sites defined as having $\geq 1 \times 10^4$ bacterial cells. The outcome variables for all 40 species were detection frequency and bacterial level.

Statistical Analysis

The implant was the unit of analysis in all calculations. For bacterial levels, means and standard deviations were calculated per compartment (sulcus, pellet, abutment screw) and per bacterium. Correlations between clinical parameters and sulcular bacterial levels were evaluated using the Spearman's correlation coefficient for each of the 40 species included in the panel. Differences in bacterial detection frequencies between the three compartments were analyzed using the McNemar test. Disparities in bacterial levels between the three compartments were analyzed using the Wilcoxon signed rank test. Correlations between the different compartments in terms of bacterial levels were evaluated using the Spearman's correlation coefficient. The level of significance was set at 0.01 for each comparison.

RESULTS

Clinical Findings

Tables 1 and 3 present clinical and radiographic findings on the subject, and implant level, respectively. These are indicative of peri-implant health at the time of examination. Radiographic evaluation by an experienced clinician confirmed the absence of pathologic peri-implant bone loss.

Microbiological Findings

Correlation between Clinical Parameters and Sulcular Bacterial Levels. Plaque and BoP scores showed a significant correlation with sulcular levels of 30 ($r \geq 0.36$; $p \leq .01$), respectively 28 ($r \geq 0.35$; $p \leq .01$) out of the 40 species. Only for four species (*Streptococcus oralis,*

TABLE 2 Bacterial Species and Subspecies Included in the DNA-DNA Checkerboard Kit

Species	Collection*
Aggregatibacter actinomycetemcomitans (a)	ATCC 29523
Aggregatibacter actinomycetemcomitans (Y4)	ATCC 43718
Actinomyces israelii	ATCC 12102
Actinomyces naeslundii (type I + II)	ATCC 43146
Actinomyces odontolyticus	ATCC 17929
Campylobacter gracilis	ATCC 33236
Campylobacter rectus	ATCC 33238
Campylobacter showae	ATCC 51146
Capnocytophaga gingivalis	ATCC 33612
Capnocytophaga ochraceae	ATCC 33596
Capnocytophaga sputigena	ATCC 33612
Eikenella corrodens	ATCC 23834
Eubacterium saburreum	ATCC 33271
Fusobacterium nucleatum nucleatum	ATCC 25586
Fusobacterium nucleatum polymorphum	ATCC 10953
Fusobacterium nucleatum naviforme	ATCC 49256
Fusobacterium periodonticum	ATCC 33693
Lactobacillus acidophilus	ATCC 11975
Leptotrichia buccalis	ATCC 14201
Parvimonas micra	ATCC 19696
Neisseria mucosa	ATCC 33270
Prevotella intermedia	ATCC 25611
Prevotella melaninogenica	ATCC 25845
Prevotella nigrescens	ATCC 33563
Porphyromonas gingivalis	ATCC 33277
Propionibacterium acnes (type I + II)	ATCC11827/28
Selenomonas noxia	ATCC 43541
Staphylococcus aureus	ATCC 25923
Streptococcus anginosus	ATCC 33397
Streptococcus constellatus	ATCC 27823 (M32b)
Streptococcus gordonii	ATCC 10558
Streptococcus intermedius	ATCC 27335
Streptococcus mitis	ATCC 49456
Streptococcus oralis	ATCC 35037
Streptococcus sanguinis	ATCC 10556
Streptococcus mutans	ATCC 25175
Tannerella forsythia	ATCC 43037 (338)
Treponema denticola	ATCC 35405
Treponema socranskii	D40DR2
Veillonella parvula	ATCC 10790

*ATCC, American Type Culture Collection.
*D, Sample from Forsyth Institute, Boston, MA.

Capnocytophaga gingivalis, Campylobacter showae, and *Fusobacterium nucleatum sp. polymorphum*), a significant link between PPD and sulcular levels was found ($r \geq 0.36$; $p \leq .01$).

Detection Frequency. The prevalence of the 40 included species was sorted by location and is presented in Table 4. In the peri-implant sulcus, high detection frequencies (27.3–100%) were found for practically all

TABLE 3 Implant Characteristics ($n = 58$)

Plaque index (%), mean (SD)	29.0 (34.7)
Bleeding on probing (%), mean (SD)	26.7 (30.9)
Probing pocket depth (mm), mean (SD)	3.2 (0.7)
Radiographic bone loss (mm), mean (SD)	2.4 (1.2)

SD, standard deviation.

bacteria included in the panel. For 25 species, the detection frequency was at least 75%. Also, at the intra-coronal compartment, high detection frequencies (27.3–86.4%) could be shown for a lot of species. At least 75% of the suprastructures were contaminated by *Streptococcus intermedius, C. showae, Fusobacterium nucleatum nucleatum, Fusobacterium periodonticum, Leptotrichia buccalis*, and *Prevotella melaninogenica*. At the abutment screw, detection frequencies ranged from 8.3 to 60.4%. More than half of the screws were contaminated by *C. showae, F. nucleatum sp. Nucleatum*, and *F. periodonticum*. When comparing the prevalence of the species between the sulcus and the intra-coronal compartment, only five species were significantly less frequently detected at the intra-coronal compartment. A comparison between the sulcus and the abutment screw revealed, however, a significantly higher detection frequency for 35 out of the 40 species in the sulcus. When comparing the internal compartments, there was a significantly higher detection frequency for 23 out of 40 species at the intra-coronal compartment.[13]

Bacterial Levels. The mean levels of the 40 included species were sorted by location and are presented in Table 5.

In the peri-implant sulcus, high bacterial levels above 10^5 genome equivalents were found for about half of the species. At the intra-coronal compartment, such high levels were found for the following 14 species: *Veilonella parvula, Aggregatibacter actinomycetemcomitans Y4, Capnocytophaga ochracea, Campylobacter gracilis, C. showae, F. nucleatum sp. nucleatum, F. nucleatum sp. polymorphum, Fusobacterium nucleatum sp. naviforme, F. periodonticum, L. buccalis, Parvimonas micra, P. melaninogenica, Tannerella forsythia,* and *Treponema socranskii*. At the abutment screw levels above 10^5 genome equivalents were only found for three species: *F. nucleatum sp. nucleatum, F. periodonticum*, and *L. buccalis*.

When comparing bacterial levels between the sulcus and intra-coronal compartment, only those of *T. forsythia* and *Streptococcus mutans* were significantly lower at the latter. A significant correlation between the sulcus and intra-coronal compartment was found for only three species: *A. actinomycetemcomitans Y4* ($r = 0.46$; $p \leq .01$), *F. nucleatum sp. polymorphum* ($r = 0.54$; $p \leq .01$), and *Aggregatibacter actinomycetemcomitans a29523* ($r = 0.48$; $p \leq .01$). Thirty-six out of 40 species were detected in significantly lower counts at the abutment screw when compared with the sulcus. However, a significant correlation between the sulcus and abutment screw was found for a lot of species (16/40; $r \geq 0.38$; $p \leq .01$). When comparing internal compartments, significantly higher levels were found for 7 out of 40 species at the intra-coronal compartment. There only was a significant correlation for the levels of *F. nucleatum sp. polymorphum* ($r = 0.41$; $p \leq .01$) between the internal compartments.[13]

DISCUSSION

A number of in vitro studies have been published documenting bacterial leakage along the interface at implant-abutment assemblies. Essentially, these indicated a bidirectional flux of fluids and bacteria via the microgap,[4] which occurred within days following inoculation,[5,6] at all implant types and implant-abutment configurations.[2] The phenomenon was reduced when the closing torque of the abutment screw was increased.[7] In addition, more bacterial penetration was found along the transversal screw of screw-retained assemblies when compared with the microgap separating the implant from the abutment.[6] Even though these are interesting findings from an exploratory point of view, they should be interpreted with caution as inoculation was usually done by only one test microorganism.[2,4,6,10] Bacterial leakage may be underestimated, especially when relatively large species such as *F. nucleatum* are selected for this purpose.[10] Furthermore, the implant-abutment assemblies had usually not been loaded prior to examination with the exception of one study.[8] Again, this may underestimate bacterial leakage. These drawbacks clearly indicate that *in vivo* studies are compulsory to scrutinize the phenomenon.

The presence and quantity of a large number of species at the internal implant and suprastructure components of the reconstructions included in the present study have been earlier described.[13] Detailed information was previously lacking on this topic given the very few *in vivo* studies that had been published describing

TABLE 4 Percentile Detection Frequency for Each of the 40 Species Sorted by Location

	Sulcus	Pellet	Screw
Fusobacterium periodonticum	100	81.8	54.2*‡
Fusobacterium nucleatum sp. nucleatum	100	75	52.1†‡
Campylobacter showae	100	75	60.4†‡
Leptotrichia buccalis	98.1	86.4	47.9*‡
Streptococcus mutans	96.3	65.9	29.2*‡
Streptococcus intermedius	96.3	77.3	47.9‡
Eubacterium saburreum	96.3	65.9	35.4*†‡
Campylobacter gracilis	96.3	70.5	29.2*‡
Streptococcus gordonii	94.4	68.2	37.5*‡
Prevotella melaninogenica	94.4	75	47.9*‡
Campylobacter rectus	94.4	63.6	29.2*‡
Streptococcus oralis	92.6	59.1	33.3†‡
Parvimonas micra	92.6	68.2	45.8‡
Aggregatibacter actinomycetemcomitans (Y4)	92.6	68.2	27.1*‡
Streptococcus mitis	90.7	63.6	33.3*‡
Fusobacterium nucleatum sp. naviforme	90.7	63.6	33.3*‡
Staphylococcus aureus	87.3	68.2	39.6*‡
Treponema denticola	87	59.1	31.3*‡
Streptococcus anginosus	87	59.1	29.2*‡
Treponema socranskii	85.2	54.5	27.1‡
Streptococcus constellatus	85.2	59.1	22.9*‡
Capnocytophaga gingivalis	85.2	56.8	20.8*‡
Streptococcus sanguinis	83.3	56.8	29.2*‡
Aggregatibacter actinomycetemcomitans (a)	83.3	59.1	27.1*‡
Lactobacillus acidophilus	80	54.5	25‡
Tanerella forsythia	74.1	36.4	29.2†‡
Capnocytophaga ochracea	70.4	50	25‡
Veillonella parvula	66.7	54.5	31.3‡
Fusobacterium nucleatum sp. polymorphum	63	52.3	33.3‡
Porphyromonas gingivalis	61.1	38.6	14.6‡
Actinomyces odontolyticum	58.2	45.5	12.5*‡
Selenomonas noxia	55.6	36.4	22.9‡
Capnocytophaga sputigena	55.6	27.3	22.9‡
Prevotella intermedia	51.9	34.1	18.8‡
Prevotella nigrescens	50	34.1	18.8
Eikenella corrodens	46.3	38.6	8.3*‡
Actinomyces neaslundii I & II	43.6	52.3	18.8*
Propionibacterium acnes	40.7	45.5	22.9*
Neisseria mucosa	33.3	31.8	10.4
Actinomyces israelii	27.3	40.9	12.5*

*Statistically significant difference between pellet and screw.
†Statistically significant difference between sulcus and pellet.
‡Statistically significant difference between sulcus and screw.

limited microbiological data confined to abutment screws in a limited number of implants.[11,12] Van Aelst and colleagues[13] showed that a high proportion of the implants was contaminated by the species included in the panel in frequently high numbers, as well on the abutment screw as in the intra-coronal compartment. Bacterial contamination was higher at the latter. Furthermore, the bacterial composition at the internal

TABLE 5 Mean Levels of the 40 Species Sorted by Location ($\times 10^5$)

	Sulcus		Pellet		Screw	
	Mean	SD	Mean	SD	Mean	SD
Fusobacterium periodonticum	2.55	2.53	1.96	3.38	1.01	2.05*‡
Fusobacterium nucleatum sp. nucleatum	4.03	3.86	2.19	3.64	1.37	2.41‡
Campylobacter showae	1.43	1.54	1.14	2.96	0.55	1.08‡
Leptotrichia buccalis	2.87	2.15	2.77	6.84	1.26	2.32‡
Streptococcus mutans	1.41	0.89	0.83	1.67	0.48	0.94†‡
Streptococcus intermedius	0.79	0.81	0.61	1.83	0.22	0.46‡
Eubacterium saburreum	1.60	1.37	0.87	1.26	0.34	0.53‡
Campylobacter gracilis	1.34	1.06	1.34	3.68	0.45	0.99‡
Streptococcus gordonii	1.10	1.02	0.85	2.81	0.49	1.17‡
Prevotella melaninogenica	1.35	1.21	1.85	4.86	0.85	1.62‡
Campylobacter rectus	2.10	2.04	0.93	1.60	0.45	0.80‡
Streptococcus oralis	0.55	0.61	0.30	0.55	0.09	0.15‡
Parvimonas micra	2.34	2.77	1.14	2.09	0.48	0.81‡
Aggregatibacter actinomycetemcomitans (Y4)	2.00	1.71	1.61	3.70	0.56	1.09‡
Streptococcus mitis	0.82	0.84	0.39	0.57	0.31	0.56‡
Fusobacterium nucleatum sp. naviforme	3.19	6.10	2.42	6.97	0.28	0.51‡
Staphylococcus aureus	1.13	1.36	0.57	0.86	0.28	0.50‡
Treponema denticola	1.81	1.97	0.65	1.02	0.33	0.52‡
Streptococcus anginosus	0.93	1.08	0.49	0.86	0.22	0.38‡
Treponema socranskii	1.44	1.35	1.06	2.19	0.37	0.65‡
Streptococcus constellatus	0.86	0.81	0.45	0.72	0.17	0.37‡
Capnocytophaga gingivalis	1.12	0.99	0.52	0.80	0.21	0.46‡
Streptococcus sanguinis	0.65	0.73	0.40	0.84	0.09	0.15*‡
Aggregatibacter actinomycetemcomitans (a)	0.92	0.83	0.86	2.42	0.27	0.58‡
Lactobacillus acidophilus	0.72	0.83	0.49	0.8	0.09	0.18*‡
Tanerella forsythia	6.40	17.45	2.33	9.38	0.52	0.94†‡
Capnocytophaga ochracea	1.74	1.69	1.18	1.85	0.55	1.19‡
Veillonella parvula	1.37	2.89	1.52	2.42	0.25	0.45*‡
Fusobacterium nucleatum sp. polymorphum	2.74	3.88	1.99	5.09	0.77	1.30‡
Porphyromonas gingivalis	0.71	2.12	0.48	1.31	0.05	0.14‡
Actinomyces odontolyticum	0.49	0.69	0.36	0.46	0.09	0.27*‡
Selenomonas noxia	0.34	0.41	0.28	0.56	0.12	0.23‡
Capnocytophaga sputigena	0.77	0.86	0.33	0.69	0.35	0.73‡
Prevotella intermedia	0.46	0.61	0.29	0.62	0.11	0.24‡
Prevotella nigrescens	0.42	0.62	0.46	1.54	0.09	0.22
Eikenella corrodens	0.30	0.46	0.14	0.24	0.04	0.14‡
Actinomyces neaslundii I & II	0.21	0.43	0.25	0.37	0.02	0.05*‡
Propionibacterium acnes	0.12	0.17	0.23	0.65	0.1	0.23
Neisseria mucosa	0.58	1.04	0.38	0.69	0.32	1.08
Actinomyces israelii	0.14	0.28	0.17	0.24	0.05	0.15*

*Statistically significant difference between pellet and screw.
†Statistically significant difference between sulcus and pellet.
‡Statistically significant difference between sulcus and screw.

compartments showed high variation. Virulent microorganisms such as *Helicobacter pylori* and *Staphylococcus aureus* were identified in at least one-third of the samples. Anaerobic species associated with peri-implantitis were even more frequently found. The data illustrating these findings on the contamination of the internal compartments are also shown in Tables 4 and 5 of the present report, yet relating to 40 species.

The checkerboard DNA-DNA hybridization method has shown a high diagnostic sensitivity for the detection of microbiota around dental implants.[22] In addition, recent studies have been published using the same protocol and laboratory for the identification and quantification of microbiota in the peri-implant sulcus.[19,23] Gerber and colleagues[24] most frequently identified *A. actinomycetemcomitans*, which is in contrast with our results. On the other hand, the levels of *A. actinomycetemcomitans* between both studies were compatible. Renvert and colleagues[23] identified *Fusobacterium* species as the most prevalent bacteria in gingival fluid samples, which is in accordance with our findings.

Hitherto, no data have been published comparing the peri-implant sulcus to internal implant and suprastructure components for the presence and counts of bacteria. The purpose of the present study was to document this on implants with no clinical signs of peri-implantitis and in function for many years. Our results indicated that the microbiota under investigation showed little difference between the peri-implant sulcus and the intra-coronal compartment. Clearly, the cotton pellet was highly contaminated with a variety of species in frequently high numbers. One could speculate these were false-positive results due to contamination during the restorative treatment and/or retrieval of the cotton pellet for examination. Obviously, the former seems unlikely as it is generally accepted in microbiology that a hermetic closure of a compartment would result in death of all microorganisms, especially after several years.[11] In addition, contamination while collecting the samples was avoided as much as possible by applying cotton rolls at all times. As a result, microbial leakage along the abutment-prosthesis interface and the restorative margin of the composite restorative material filling up the screw access holes was considered the most probable explanation for the high contamination of the intra-coronal compartment. Interestingly, a significant association in bacterial levels between the peri-implant sulcus and the intra-coronal compartment was found for only three species out of 40, suggesting leakage had principally occurred via the restorative margin of composite restorative material and not via the abutment-prosthesis interface. This finding clearly questions the quality of the restorations filling up the screw access holes, which is supported by several reports in the endodontic literature. Indeed, an increased incidence of apical periodontitis has been associated with defective restorations suggesting bacterial leakage along the restorative margin.[25–27] In addition, a recent study has showed significantly higher bacterial detection frequencies in root-filled teeth with defective restoration when compared with root-filled teeth with intact restorations.[28] Clearly, it would have been interesting if we had scored the quality of the coronal restorations in the present study.

When comparing bacterial detection frequencies and levels between the peri-implant sulcus and the abutment screw, significant differences were found for a large majority of the bacteria. These species were less frequently detected at abutment screws and in lower numbers. The fact that a significant association in bacterial levels between the peri-implant sulcus and the abutment screw was still found for 16 species out of 40 does not contrast this observation. Indeed, as there was no immediate contact between the abutment screw and the intra-coronal compartment (Figure 1), the implant-abutment interface and abutment-prosthesis interface were the most obvious pathways for microbial transmission from the external environment (peri-implant sulcus) to the abutment screw and vice versa. Hence, an important link was expected.

Even though all implants in the present study showed no clinical signs of peri-implantitis, the high prevalence of numerous species associated with pathology was striking. In more than three out of four samples from the peri-implant sulcus, pathogenic species such as *A. actinomycetemcomitans, F. nucleatum, L. buccalis, P. micra, P. melaninogenica*, and *Treponema denticola* were found. At the intra-coronal compartment, their prevalence was well-above one in two samples, and at the abutment screw it was at least one in three. Under the assumption that these species are truly related to disease, the following implications should be taken into account. First, one could speculate it may only be a matter of time until this bacterial burden starts to initiate pathology around these implants that have been in a clinically healthy state for years. Second, when peri-implantitis

has developed, the presence of these species at the internal implant and suprastructure compartments forming a microbial reservoir could explain why nonsurgical treatment strategies for peri-implantitis show negligible efficacy.[24,29] This hypothesis is even consistent with a superior treatment outcome of surgical therapy for peri-implantitis[30] as the distance of the internal microbial reservoir to the peri-implant sulcus is drastically increased when resective surgery is performed. As such, the possible impact of this reservoir on the recolonization of the peri-implant sulcus by pathogens is reduced. Given these possible pathologic and therapeutic implications, it is obvious that the results of this study could become highly clinically relevant.

Besides pathogens that have been associated with peri-implantitis, other quite virulent species such as *S. aureus* were also frequently found as well in the peri-implant sulcus (about 90%) as in the implant (about 70%) and suprastructure compartments (about 40%). The presence of this species in the peri-implant sulcus has recently been described by Renvert and colleagues[23]; however, the significance of this finding is still unclear. Others studies have shown an association of serum IgG antibodies relative to *S. aureus* with early implant loss[31] and a link of *S. aureus* to aggressive periodontitis.[32]

In conclusion, in this cross-sectional *in vivo* study, the peri-implant sulcus was microbiologically compared with the internal implant and suprastructure components. The pellet enclosed in the suprastructure was very similar to the peri-implant sulcus in terms of bacterial detection frequencies and levels for practically all the species included in the panel. Yet, there was practically no microbial link between these compartments, suggesting important leakage along the restorative margin of the composite restorative material filling up the screw access holes. When comparing the abutment screw to the peri-implant sulcus, the majority of the species were less frequently found and in lower numbers at the former. However, a strong link in counts for a lot of bacteria was described between these compartments. Even though all implants in the present study showed no clinical signs of peri-implantitis, the high prevalence of numerous species associated with pathology was striking. Additional studies are needed to explore the relationship in terms of microbiota between the internal implant and suprastructure surfaces and the peri-implant crestal bone level. In addition, the dynamics of internal colonization needs to be thoroughly documented in longitudinal *in vivo* studies.

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