Evaluation of Microbial Contamination in the Inner Surface of Titanium Implants Before Healing Abutment Connection: A Prospective Clinical Trial

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Purpose: The objective of this investigation was to assess the microbiologic contamination in the inner surface of titanium implants prior to prosthetic abutment placement. Materials and Methods: The study population consisted of partially edentulous individuals who had previously received at least one internal hexagon titanium dental implant. A bacterial sample of the inner surface of the individual dental implant was taken after surgical reopening for healing abutment placement. The samples were allocated in order to evaluate three distinctive variables as follows: (1) location (mandible vs maxilla), (2) early exposure of implants to the oral cavity (cover screw) throughout the healing stage (exposed vs not exposed), and (3) existence or lack of keratinized mucosa (KM). The microorganism species detected were examined by checkerboard DNA-DNA hybridization. Results: A total of 32 partially edentulous patients with 78 implants placed in both the maxilla and mandible were enrolled: 8 men and 24 women, ranging in age from 27 to 64 years (mean age: 47.7 years). Bacteria were detected in 20 patients, distributed in 41 implants. Spontaneous early implant exposure and absence of KM did not increase bacterial contamination in the inner surface of implants. A significant increase in the detection of 22 bacterial species was found in the mandible when compared with the maxilla. Conclusion: Microbial biofilm accumulation in the implant’s internal surface might happen before healing abutment placement. Exposure of implants to the oral cavity and absence of KM were not directly related to a greater microbial biofilm count. The results suggested that submerged healing does not protect implants against bacterial colonization. Int J Oral Maxillofac Implants 2018;33:853–862. doi: 10.11607/jomi.5817

Keywords: checkerboard DNA-DNA hybridization, dental implants, microbiology, peri-implantitis

According to clinical, randomized, and longitudinal studies as well as recent systematic reviews, dental implants have become a routine treatment modality over the years due to their high predictability, effectiveness, and success rate.1–4 Among other reasons, the long-term survival of titanium dental implants is strictly related to the peri-implant tissue health.5–7 Recent evidence demonstrated, however, that implant failure does happen and falls broadly into two stages: early failure (before implant osseointegration) and late failure (after the osseointegration period).8 The long-term clinical effectiveness of dental implants is influenced by peri-implantitis (late failure), an inflammatory reaction resulting in peri-implant pocket and resorption of the supporting bone around the implant, which can lead to implant loss. It is mainly caused by a range of bacteria species including Tannerella forsythia, Porphyromonas gingivalis, Aggregatibacter actinomycetemcomitans, Treponema denticola, Fusobacterium nucleatum, and Bacterioides spp, affecting more than 14% of implants after 5 years in function.9–12

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Since peri-implantitis plays a pivotal role in the long-standing clinical success of dental implants, it is of critical importance to appreciate the different mechanisms of contamination by several microorganisms, from the primary interaction of the implant to the oral atmosphere. Despite the fact that peri-implant bone resorption as a result of periodontal pathogenic microorganism colonization has been satisfactorily identified, it is well recognized that such formation can appear immediately after surgical implant placement, when microorganisms could colonize on the occlusal aspects and internal surfaces of implants and cover screws. Interestingly, analysis of the microbiologic profile at failing dental implants has found comparable microbial species to those recognized at healthy locations, but with larger amounts of gram-negative anaerobic microorganisms.

Another factor to be considered when analyzing bacterial contamination of dental implants is the type of implant connection. It is already known that the connection design of the implants plays a crucial role in the quantity of microbial leakage and the consequent peri-implant inflammation. The microgap between the implant and healing cap or abutment connection has been connected to peri-implant bone resorption, especially when an external hexagon connection is used. A previous study has identified several bacterial species located on the apical side of abutment screws and inner surfaces with an external hexagon connection. However, according to previous in vitro and in vivo studies, internal connection implants showed decreased bacterial leakage when compared with external connection implants. These data suggest that the amount of bacterial leakage depends on the tight fit among the components, torque, and forces applied.

During surgical placement of titanium implants, the presence of microorganisms in the external area of the implant surface can be addressed by the biologic host protection. Nevertheless, microbiologic contamination in the internal area of the implant surface can stay for extended periods of time and possibly lead to contamination and infection. The microgap existing between the platform or shoulder of the implant and the healing abutment or cover screw can potentially favor microorganism settlement. This fact might hamper the osseointegration process during the healing phase. Nonetheless, there is limited understanding related to the bacterial colonization that can settle inside the implant surfaces, after their placement, during the phase of primary osseointegration of two-stage dental implants. In this context, it is important to recognize bacterial species that are strictly involved in periodontal and peri-implant diseases in the microgap between the implant and the healing abutment or cover screw.

Experimental data that directly evaluate the microbiologic contamination in the inner area of titanium implants before prosthetic loading are scarce. Thus, the aims of this prospective study were to identify the presence, the frequency, and the composition of bacterial biofilm in the inner surface of titanium implants placed into human alveolar ridges prior to healing abutment placement by means of checkerboard DNA-DNA hybridization. Moreover, it was investigated whether the absence of keratinized mucosa (KM) and the exposure of the cover screw to the oral environment would increase bacterial contamination. The study hypothesis was that there would be bacterial colonization in the inner part of the implants before healing abutment placement. Also, it was hypothesized that there would be a significant increase in bacterial contamination in the inner part of the exposed implants with the absence of KM.

### MATERIALS AND METHODS

#### Study Population and Implants

This prospective clinical trial involved partially edentulous patients who were enrolled from both private practice and the postgraduate program in Implant Dentistry of the Brazilian Dental Association (ABO/RN/Brazil) from March to December 2014.

The inclusion criteria were as follows: patients with implant sites free from clinical infection, periodontally healthy/stable (ie, patients who did not present more than 30% of the sites with probing depth $\geq 5$ mm, bleeding on probing, and suppuration), caries-free, who had previously received at least one two-stage internal hexagon dental implant (Dentoflex) placed for at least 4 months prior to sampling. Moreover, patients were excluded if they presented with systemic diseases such as uncontrolled diabetes mellitus, usage of medications such as antibiotics and anti-inflammatory drugs within 4 months prior to sample collection, and patients who were pregnant. Moreover, smokers and recreational drug users were also excluded from this study.

The working protocol was accepted by the Ethics Committee of the Federal University of Rio Grande do Norte (Protocol #151/06), and all participants signed a written informed consent to undergo all procedures prior to the initial treatment.

#### Sample Characteristics

Before the stage-two surgery for healing abutment placement, demographic (based on the participant files) and clinical data were obtained from each patient, such as age, sex, history of smoking, implant insertion site, length and width of the implant, exposure or not of the cover screw, and width of KM measured at the buccal aspect.
Implants were assessed clinically, by means of visual inspection of the soft tissue around the implant and by resonance frequency analysis to confirm primary osseointegration. Radiographic evaluation, by means of digital standardized periapical radiography, was also performed to verify if there was evidence of any pathologic radiolucent lesion. Data collection was performed by the same experienced examiner throughout the study.

All samples were distributed to evaluate three distinctive variables: (1) location (mandible vs maxilla), (2) early implant exposure (cover screw) during the healing phase of osseointegration (exposed vs not exposed), and (3) existence of KM (present vs absent). The classification proposed previously by Tal was employed to define if early implant exposure to the oral environment might impact inner contamination. Therefore, this clinical trial was distributed into two groups: (1) implants that have revealed a certain degree of exposure to the oral cavity at stage-two surgery (exposure classes 1 to 4), or (2) implants totally covered by soft tissue (class 0).

Furthermore, the present study evaluated whether the existence or lack of KM around implants could modify bacterial composition in the inner surface of two-stage titanium implants. For this, KM was measured with the aid of a periodontal probe on the buccal and palatal sides of the implant, immediately before the stage-two surgery, and implants were allocated between two groups, according to the existence or lack of KM (Figs 1a and 1b).

**Sampling Procedure and Microbiologic Assessment**

During the stage-two procedure for healing cap placement, the implant site was cautiously isolated from blood and saliva using sterilized gauze to decrease contamination of oral microorganisms and fluids. Subsequently, the cover screw was removed, and two sterilized paper points (Dentsply) with 28-mm width and number 55, were concurrently introduced for 20 seconds into the inner side of each implant until the most apical position was reached. Afterward, individual samples were instantaneously inserted in individual Eppendorf tubes comprising 0.15 mL TE. Every 30 minutes of sampling, 0.10 mL of 0.5 M NaOH was added to each tube. Then, the tubes were stored at −20°C until further analysis. After sample collection, the healing abutments were placed for all implants. All procedures were accomplished by the same experienced examiner (G.A.B.L.) to standardize the sampling collection.

**Checkerboard DNA-DNA Hybridization**

Total counts of 40 bacterial species were determined utilizing the well-known technique of checkerboard DNA-DNA hybridization (Table 1), as described elsewhere. Succinctly, bacterial samples were simmered for 10 minutes and deactivated with 0.8 mL of 5M ammonium acetate (Sigma-Aldrich). Then, the DNA released was inserted into a special apparatus through individual lanes (Minislot-30, Immunetics), on a 15 × 15-cm nylon membrane completely charged (Boehringer Mannheim), and secured to the membrane by baking at 120°C for 20 minutes. Subsequently, the membrane was positioned in a second apparatus (Minibloter-45, Immunetics), and the probes were diluted to ~20 ng/mL in hybridization solution. Then, probes were positioned in separate channels, and hybridized for 12 hours at 42°C. Membranes were cleaned twice at high stringency for 20 minutes at 68°C in phosphate buffer, according to a previously published protocol.

**Detection and Enumeration of Taxa**

The protocol for the detection and enumeration of taxa was based on a previous study with slight...
Briefly, membranes were blocked for 60 minutes and were then incubated in blocking buffer. Hybrids were detected by revealing the membranes to an anti-digoxigenin antibody conjugated to alkaline phosphatase (Roche Diagnostics) for 30 minutes. Signals were detected by incubating the membrane in a chemiluminescent agent (CDP-Star, Roche Diagnostics) for 5 minutes at room temperature (RT) and exposed to autoradiographic films for 10 minutes. Signals were assessed visually for comparison with values for the test species. These were documented as: 0, not detected; 1, < 10^5 cells; 2, ~10^5 cells; 3, 10^5 to 10^6 cells; 4, ~10^6 cells; and 5, > 10^6 cells. The sensitivity of this technique was adjusted to permit detection of ~ 10^4 cells for each bacterial species. Failure to detect a signal was recorded as 0.

### Statistical Analysis

Statistical analysis was performed using the GraphPad Prism software (version 6.0, GraphPad Software). All data were expressed as the mean ± standard error of the mean (SEM). The mean proportion of each bacterial species on the internal surface of the implants was processed for each implant and averaged within groups. The prevalence was calculated by stipulating the amounts of dental implants colonized by distinct bacterial species and averaging these amounts across different groups. After testing for a normal distribution (D’Agostino and Pearson test), the differences were analyzed with the Mann-Whitney U test. Differences were considered significant at P < .05.

### Results

A total of 32 partially edentulous healthy individuals were enrolled in this clinical trial. There were 24 women and 8 men, and they ranged in age from 27 to 64 years (mean age: 47.7 years). All patients denied use of alcohol and cigarettes. A total of 78 two-stage screw-type implants were distributed among this clinical study in both the maxilla and mandible. All dental implants were of an internal hexagon connection design, and had been placed for at least 4 months prior to sampling.

Using the checkerboard DNA-DNA hybridization method, 40 bacterial species were employed for the development of the whole genomic DNA Probes, according to Table 1. Bacterial strains were found in the implants of 20 subjects, totaling 62.5% of the sample. More than half of the implants placed (41 implants) demonstrated certain microorganism presence (52.6%). According to the findings presented in Table 2, a higher percentage of bacterial presence was detected in the mandible (77.42%) compared with the maxilla (36.17%). Furthermore, only 19 implants were exposed to the oral environment compared with 59 implants not exposed. However, only eight implants (42.11%) that were exposed to the oral cavity presented with bacterial contamination. Interestingly, 67 implants (86% of the total sample) presented with KM,

### Table 1  Bacterial Strains Employed for Development of Whole Genomic DNA Probes

<table>
<thead>
<tr>
<th>Species</th>
<th>Strain (ATCC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomyces naeslundii I</td>
<td>12104</td>
</tr>
<tr>
<td>Streptococcus constellatus</td>
<td>27823</td>
</tr>
<tr>
<td>Eubacterium nodatum</td>
<td>33099</td>
</tr>
<tr>
<td>Porphyromonas gingivalis</td>
<td>33277</td>
</tr>
<tr>
<td>Aggregatibacter actinomycetemcomitans a and b</td>
<td>43718 + 29523</td>
</tr>
<tr>
<td>Fusobacterium nucleatum sp. vincentii</td>
<td>49256</td>
</tr>
<tr>
<td>Campylobacter rectus</td>
<td>33238</td>
</tr>
<tr>
<td>Treponema socransii</td>
<td>D40DR2</td>
</tr>
<tr>
<td>Eubacterium saburreum</td>
<td>33271</td>
</tr>
<tr>
<td>Peptostreptococcus micros</td>
<td>33270</td>
</tr>
<tr>
<td>Veillonella parvula</td>
<td>10790</td>
</tr>
<tr>
<td>Actinomyces viscosus (naeslundi genospecies2)</td>
<td>43146</td>
</tr>
<tr>
<td>Streptococcus anginosus</td>
<td>33397</td>
</tr>
<tr>
<td>Streptococcus sanguinis</td>
<td>10556</td>
</tr>
<tr>
<td>Actinomyces gerencersemreae</td>
<td>23860</td>
</tr>
<tr>
<td>Streptococcus oralis</td>
<td>35037</td>
</tr>
<tr>
<td>Capnocytophaga ochracea</td>
<td>33596</td>
</tr>
<tr>
<td>Actinomyces israeli</td>
<td>12102</td>
</tr>
<tr>
<td>Streptococcus intermedius</td>
<td>27335</td>
</tr>
<tr>
<td>Treponema denticola</td>
<td>B1</td>
</tr>
<tr>
<td>Prevotella nigrescens</td>
<td>33563</td>
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<tr>
<td>Actinomyces odontolyticus I</td>
<td>17929</td>
</tr>
<tr>
<td>Fusobacterium nucleatum sp. polymorphum</td>
<td>10953</td>
</tr>
<tr>
<td>Campylobacter showae</td>
<td>51146</td>
</tr>
<tr>
<td>Fusobacterium periodonticicum</td>
<td>33693</td>
</tr>
<tr>
<td>Neisseria mucosa</td>
<td>19696</td>
</tr>
<tr>
<td>Fusobacterium nucleatum sp.</td>
<td>25586</td>
</tr>
<tr>
<td>Capnocytophaga gingivalis</td>
<td>33624</td>
</tr>
<tr>
<td>Streptococcus gordonii</td>
<td>10558</td>
</tr>
<tr>
<td>Tannenerella forsythia</td>
<td>43037</td>
</tr>
<tr>
<td>Selenomonas noxia</td>
<td>43541</td>
</tr>
<tr>
<td>Propionibacterium acnes I e II</td>
<td>11827 + 11828</td>
</tr>
<tr>
<td>Prevotella melaninogenic</td>
<td>25845</td>
</tr>
<tr>
<td>Streptococcus mitis</td>
<td>49456</td>
</tr>
<tr>
<td>Eikenella corrodenes</td>
<td>23834</td>
</tr>
<tr>
<td>Gemella morbillorum</td>
<td>27824</td>
</tr>
<tr>
<td>Capnocytophaga sputigena</td>
<td>33612</td>
</tr>
<tr>
<td>Leptotrichia buccalis</td>
<td>14201</td>
</tr>
<tr>
<td>Campylobacter gracilis</td>
<td>33236</td>
</tr>
<tr>
<td>Prevotella intermedia</td>
<td>25611</td>
</tr>
</tbody>
</table>

*American Type Culture Collection.*
but this fact did not decrease bacterial contamination in the internal implant surface.

This study also evaluated the frequency detection (Fig 2), the levels of colonization (Fig 3), and the level of detection of 40 bacteria species (Fig 4). The findings of the present study demonstrated that the prevalence of 22 bacterial species between the maxilla and mandible were statistically significantly different ($P < .05$) between both arches (Fig 4). Regarding the spontaneous early exposure of dental implants to the oral environment, 24% of the implants exhibited some level of exposure at the moment of sample collection. On the other hand, there were no differences between the groups exposed and not exposed to any of the bacterial species, and therefore, the prevalence of all microorganisms did not vary between them ($P > .05$).

All microorganism strains were more predominant in dental implants placed in areas with KM, but only a few bacterial species, such as Capnocytophaga sputigena, Leptotrichia buccalis, and Veillonella parvula demonstrated a significant difference ($P < .05$) between groups with or without KM.

Table 2 Proportional Distribution of Implants According to Groups/Variables

<table>
<thead>
<tr>
<th>Groups/variables</th>
<th>Bacteria detected</th>
<th>Bacteria not detected</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maxilla</td>
<td>36.17% (n = 17)</td>
<td>63.83% (n = 30)</td>
<td>100% (n = 47)</td>
</tr>
<tr>
<td>Mandible</td>
<td>77.42% (n = 24)</td>
<td>22.58% (n = 7)</td>
<td>100% (n = 31)</td>
</tr>
<tr>
<td>Exposed</td>
<td>42.11% (n = 8)</td>
<td>55.93% (n = 11)</td>
<td>100% (n = 19)</td>
</tr>
<tr>
<td>Not exposed</td>
<td>55.93% (n = 33)</td>
<td>44.07% (n = 26)</td>
<td>100% (n = 59)</td>
</tr>
<tr>
<td>KM present</td>
<td>58.21% (n = 39)</td>
<td>41.79% (n = 28)</td>
<td>100% (n = 67)</td>
</tr>
<tr>
<td>KM absent</td>
<td>18.18% (n = 2)</td>
<td>81.82% (n = 9)</td>
<td>100% (n = 11)</td>
</tr>
</tbody>
</table>

Fig 2 Frequency detection of 40 bacteria species.
Furthermore, additional statistical methods to estimate the effect of investigated risk factors and the bacterial loads were assessed utilizing correlation and linear regression analyses. The results of the evaluated factors were not statistically significant (data not shown).

**DISCUSSION**

This prospective clinical trial depicts an in vivo evaluation of microorganism contamination on the inner surface of titanium dental implants placed into human arches prior to the placement of either prosthetic abutments or healing cap. None of the 78 implants placed revealed any clinical symptoms of bleeding, suppuration, pain, or mobility signs at the moment of healing abutment placement (during the second surgical approach). The results of this study demonstrated that microbiologic contamination of the internal surface of the implants might happen before healing cap or prosthetic abutment placement. Exposure of implants to the oral cavity and absence of KM were not directly related to increased bacterial prevalence. Furthermore, a significant increase in the detection of 22 bacterial species, such as *L. buccalis*, *S. gordonii*, *C. gingivalis*, *T. denticola*, *T. forsythia*, *F. nucleatum*, and *A. actinomycetemcomitans*, was found in the mandible when compared with the implants placed in the maxilla. While selected studies have assessed supramucosal or submucosal bacterial biofilm composition of titanium implants, to the best of the present authors’ knowledge, this is the first prospective clinical trial to comprehensively outline the internal surface of titanium implants for a varied range of microorganism strains.

A previous study evaluated by means of cell culture the microbial counts of seven particular periodontal pathogens in patients. Van Winkelhoff et al used comparable methods to collect the bacterial sample in the inner part of the implant with two paper points. In 9 of 20 individuals, bacterial samples revealed detectable numbers of microorganisms. The most frequently isolated bacterial species were *Bacteroides forsythus*, *Prevotella intermedia*, *Fusobacterium nucleatum*, and *Peptostreptococcus micros*. *Porphyromonas gingivalis*.

![Levels of colonization of the 40 bacteria species. Length of each bar indicates percentage of positive samples according to different levels of detection.](image-url)
and *Aggregatibacter actinomycetemcomitans* were not distinguished. These findings demonstrated that the source of these microorganisms could not be determined with assurance, but the authors suggested saliva as a possible cause of bacterial contamination.

The present study findings described in Fig 2 demonstrated that the highest commonly distinguished bacterial strains were as follows: *Capnocytophaga gingivalis*, *Capnocytophaga sputigena*, *Fusobacterium nucleatum* sp vicentii, *Leptotrichia buccalis*, *Streptococcus oralis*, *Treponema denticola*, and *Veillonella parvula*. According to Fig 3, none of the bacterial strains reached the level of 10^6 target bacterial cells, which closely resembled data found in a previous study. Hultin et al investigated the immune response and the microbiota of individuals with peri-implantitis. Several bacterial species were evaluated, but four were not found: *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces odontolyticus*, and *Actinomyces viscosus*. Accordingly, Renvert et al showed that *Fusobacterium nucleatum* sp and *Leptotrichia buccalis* are the most detectable bacterial species in the implant and teeth surfaces, 7 years after implant placement.

Máximo et al used the method of checkerboard DNA-DNA hybridization to investigate bacterial samples from 12 patients with mucositis and 13 individuals with peri-implantitis. According to this study, *Actinomyces*, *Fusobacterium* spp, *Porphyromonas gingivalis*, *Porphyromonas intermedia*, *S gordonii*, *S sanguinis*, *Tannerella forsythia*, and *Veillonella parvula* were found at increased levels in patients with peri-implantitis. Increased counts of *Actinomyces*, *P nigrescens*, *P gingivalis*, *Neisseria mucosa*, *Fusobacterium* spp, and *Capnocytophaga ochracea* were found in patients with mucositis. The present study data showed that *Actinomyces* were not present in the collected samples, because these microorganisms, alongside with *Streptococcus*, are recognized as initial colonizers in which they colonize the biofilm in the first 14 days, providing a perfect atmosphere for late bacterial species to thrive. From the time when samples were collected, ie, after 12 weeks of biofilm establishment, it is realistic to presume that *Actinomyces* are not existent in the samples. This finding parallels the observation made by Fürst et al, who evaluated early colonization on dental implants right after placement and throughout 12
weeks posthealing, and compared the microbial loads at implants and tooth sites. The authors demonstrated that 29 out of 40 bacterial species were frequently found after 4 months postsurgery, comprising *T. denticola*, *P. gingivalis*, and *T. forsythia*. These species are well-known gram-negative periodontal pathogenic microorganisms that cause periodontal disease and peri-implantitis. The authors also concluded that bacterial colonization might occur 30 minutes after dental implant placement.

The findings of the present investigation indicate that dental implants placed in the mandible were more susceptible to microbial contamination in their inner surface. More than 77% of the total bacterial species examined were highly present in the implants located in the mandible, comprising microorganisms commonly related to the initiation and development of mucositis and peri-implantitis, together with periodontitis, such as *C. gingivalis*, *C. rectus*, *S. oralis*, *T. denticola*, *T. forsythia*, and *V. parvula*. The authors have not been able to identify any studies correlating the composition of the microbiota according to the mandibular or maxillary arches. On the other hand, a previous investigation showed increased percentage of bone resorption and biofilm formation around submerged mandibular implants. Importantly, all the implants previously placed were complete cover of the implants by the soft tissue. In addition, during the implant placement, the inner part of the implants is not avoided of the manufacturer. Interestingly, the contamination probably happens in the moment of implant placement, because the exposure of the healing cap or cover screw to the oral cavity was not associated with increased microbial count. Nonetheless, a recent study revealed a positive correlation between incidence of healing abutment exposure and bone resorption and biofilm accumulation around submerged implants before stage-two surgery. In this retrospective clinical trial, more than 88% of submerged implants did not show exposure of cover screws to the oral environment (score 0). The authors concluded that spontaneous early exposure of cover screws is directly associated with crestal bone resorption. Accordingly, Van Assche et al suggested that crestal bone resorption is enhanced when the integrity of soft tissue is disturbed (ie, cover screw exposed to the oral cavity) during the postsurgical phase. These findings are in agreement with Tal et al, who encountered a positive correlation between healing abutment exposure levels and bone resorption over time. Nevertheless, further randomized controlled studies associating soft tissue perforations during healing time to microbial incidence are still required to support this hypothesis.

It has been well stated that KM around titanium implants plays an important role in peri-implant health. The findings of the present study showed that all microbial strains were more frequently found in dental implants placed in regions with KM; however, only 3 out of 40 target bacterial species displayed significant differences (*P < .05*) due to the KM existence. This outcome could potentially be because the majority of the implants were placed in areas with KM, representing more than 86% of the total sample. Several investigations linked the existence of KM with some clinical parameters related to the periodontal and peri-implant diseases, such as Plaque Index and Gingival Index, probing depth, crestal bone level, bleeding on probing, and mucosal recession. On the other hand, no studies comparing KM with peri-implant microbiota have been found in the scientific literature yet. Large-scale prospective studies should be performed to clarify whether there is an association between the existence or lack of peri-implant KM and the peri-implant bacteriologic profile.

The results of the present investigation proved the authors’ null hypothesis. It was evidently demonstrated that bacterial colonization occurs before healing abutment placement or prosthetic rehabilitation. On the other hand, the authors’ second hypothesis was rejected because implants exposed to the oral environment and the presence of KM did not interfere with the bacterial colonization in the inner part of the implants. Importantly, all the implants previously placed were within the same brand, presented with internal hexagon connection, and all the cover screws were precisely tightened according to the recommendations of the manufacturer. Interestingly, the contamination of the internal surface of the implants is not avoided even with the cover screw tightly fitted and with the complete cover of the implants by the soft tissue. In addition, during the implant placement, the inner part of the implants could be filled with saliva that could act as a carrier of the host commensal microbiota and keep this environment until stage-two surgery. Finally, the cover screws and the complete coverage of the
implant by soft tissue do not prevent the microbial contamination to the inner part of the implant.

To date, there are no data that could show that the presence of these microorganisms in the inner part of the dental implants could increase or even cause peri-implant infection. Recent in vitro data have shown that Morse taper connections improve the mechanical sealing on the implant-abutment junction, but none of the aforementioned studies depicted a sealed chamber in any connection (external, internal, or Morse taper).

Lastly, further clinical research should be performed in individuals with established periodontal disease, to investigate whether antimicrobial decontamination prior to dental implant surgery might eradicate or decrease this type of contamination. Additional studies are also essential to elucidate how the bacterial penetration occurs in the inner surface of dental implants, and what the implications of this contamination are for the long-standing success of implants.

CONCLUSIONS

The results of this study demonstrated that microbial contamination of the implant’s internal surface might happen before healing cap placement. Exposure of implants (cover screw) to the oral cavity and absence of KM was not directly connected with a higher microbial count. Furthermore, significant increases in the detection of 22 bacterial species, including *L. buccalis, S. gordonii, C. gingivalis, T. denticola, T. forsythia, F. nucleatum*, and *A. actinomycetemcomitans* were found in the mandible when compared with the implants placed in the maxilla. Taken together, the results of this study suggested that submerged healing does not protect implants against bacterial colonization in the conditions studied.

ACKNOWLEDGMENTS

The authors would like to acknowledge the statistical support provided by Kênio Lima, PhD, Department of Preventive Dentistry, Federal University of Rio Grande do Norte, Brazil. The authors reported no conflicts of interest related to this study.

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