Purpose: The All-on-4 treatment concept has been shown to be an effective clinical procedure; however, to date, no studies have analyzed the subgingival microbiota present in these restorations. The purpose of this study was to evaluate the microbial profile of the subgingival biofilm around dental implants placed in the All-on-4 protocol and compare the microbial profile around axial and tilted implants. Materials and Methods: Fourteen subjects treated by the All-on-4 concept were evaluated clinically and microbiologically. Subgingival biofilm was collected from each patient, and the amount of 40 species of bacteria was assessed using the checkerboard DNA-DNA hybridization technique. Results: The results for the indices of probing depth (PD), bleeding on probing, marginal bleeding, and visible plaque were 2.32 mm, 46%, 60%, and 57%, respectively. Tilted implants presented a significantly higher mean PD and Plaque Index compared with axial implants (P < .05). Fusobacterium nucleatum ssp vincentii, Veillonella parvula, and Fusobacterium nucleatum ssp polymorphum were found in higher levels; however, no difference in the microbial composition was observed between tilted and axial implants (P > .05). Tilted implants presented statistically higher mean levels for the orange complex in relation to the axial implants (P < .05). Conclusion: Despite the clinical success rate of the All-on-4 protocol, the subgingival biofilm of tilted implants presented a higher proportion for the orange complex pathogens in comparison to axial implants. These data could suggest that subjects with this modality of implant-supported restoration must be aware that they need a more rigorous maintenance protocol. Int J Oral Maxillofac Implants 2018;33:1339–1344. doi: 10.11607/jomi.6690

Keywords: All-on-4, dental implant, microbiota, prosthesis, subgingival biofilm

The treatment of atrophic arches using implant-supported restorations is a challenging procedure during daily practice. Several strategies have been applied to solve this problem; among them, the All-on-4 treatment concept (Nobel Biocare) has shown satisfactory clinical results. This technique was previously described and was based on a total of four dental implants: two dental implants placed straight medially and two tilted distally. Therefore, application of four implants, instead of six or more, could avoid advanced surgical techniques, and thus, reduce the involved expenses and the morbidity of the treatment.

Clinical studies have shown that the All-on-4 approach is predictable and generates an implant survival rate of approximately 98% in the mandible and maxilla, in a 5-year follow-up. Recently, a study followed for 7 years 111 edentulous patients treated with 532 implants placed by the All-on-4 concept, and the authors reported a survival rate of 94.5% for these implants. The authors also showed that the mean marginal bone loss was 1.27 mm and 1.34 mm for tilted
and axial implants, respectively. Furthermore, current literature shows that clinical parameters and marginal bone loss did not present a statistically significant difference between tilted and axial implants in the All-on-4 protocol,7 ratifying previous systematic reviews evaluating maxillary and mandibular full-arch implant-supported rehabilitations.6,9

Complementary, full-arch implant-supported rehabilitations presented high bacterial biofilm accumulation on the surface of these restorations and implants, since All-on-4 restorations may be more difficult to maintain than the standard ones. Abi Nader et al10 assessed the Plaque Index on the fitting surface of 20 All-on-4 restorations. The authors reported that the biofilm covered 28% of the restoration’s fitting surface. In addition, the restorations showed three times higher incidence of the presence of dental biofilm around the palate than the buccal area (52.5% and 17%, respectively). Consequently, it could be suggested that plaque accumulation may increase the risk of the development of peri-implant infection in these patients. Recently, Cavalli et al11 analyzed 196 dental implants used in 49 All-on-4 rehabilitations and reported a prevalence of 8.7% and 6.8% for mucositis and peri-implantitis, respectively. These findings indicate that the variations in the subgingival population around implants used in this treatment protocol should be understood. However, to date, there were no studies evaluating the subgingival biofilm around dental implants placed in All-on-4 restorations. Thus, the aim of this cross-section study was to describe the subgingival microbial profile of patients restored with at least one fixed complete-arch prosthesis sustained by two axial and two tilted dental implants. A secondary aim of this study was to assess and compare the microbiota around the axial and tilted implants.

**MATERIALS AND METHODS**

**Subject Population**

Subjects who received All-on-4 treatments at the Oral Implantology Clinic of Guarulhos University between September 2013 and April 2016 were selected for a retrospective analysis. Microbiologic sampling was performed between September 2015 and April 2016. The study protocol was approved by Guarulhos University’s Ethics Committee in Clinical Research (IRB: 61544116.7.0000.5506). Accordingly, the study protocol was explained to each subject, and the ones who agreed to participate in the study signed the informed consent.

**Inclusion Criteria**

The inclusion criteria were: totally edentulous subjects treated by the All-on-4 concept at maxillary or mandibular full-arch implant-supported rehabilitations in function for at least 2 years.

**Exclusion Criteria**

Subjects were excluded if they presented moderate to severe periodontal disease (ie, suppurative and/or periodontal pockets with probing depth [PD] > 4 mm and/or bleeding on probing [BOP] in more than 30% of the sites); had used any antibiotics or anti-inflammatory medication 6 months previously to the study; had been submitted to periodontal or peri-implant therapy within 6 months; presented a chronic medical disease; or were smokers, pregnant, or lactating.

**Clinical Parameter Evaluation**

The clinical parameters evaluated were supragingival visible plaque, gingival bleeding, BOP, and suppurative. All these indices were dichotomically assessed (0/1). PD was measured in millimeters, at four sites per implant (mesial, buccal, distal, and lingual), using a North Carolina periodontal probe (Hu-Friedy). A single trained and calibrated examiner achieved all clinical measurements.

**Microbiologic Sampling**

Samples of four subgingival sites per implant (two axial and two tilted implants) were collected in each subject. The subgingival biofilm was collected with sterilized mini-Gracey curettes (#11-12) (Hu-Friedy), after the clinical parameters had been recorded and the supragingival plaque had been removed. The subgingival biofilm collected was immediately placed in separate Eppendorf tubes added with 0.15 mL of Tris-EDTA Buffer (TE) (10 mM Tris-HCl, 1 mM Ethylenediaminetetraacetic acid [EDTA], pH 7.6). After the addition of the sample, each tube received 100 µL of 0.5 M NaOH, and the sample was dispersed by a vortex mixer. The checkerboard DNA–DNA hybridization technique was used to determine the levels of 40 bacterial species, as previously described.12,13

**Statistical Analysis**

Each individual clinical parameter was computed per implant and then per patient. In addition, mean counts (× 103) of individual bacterial species and the percentage of the total DNA probe were determined initially in each site, then per patient and averaged across patients in the axial and tilted implant groups. The proportions for the species were distributed into the six complexes and the “other” group, as defined by Socransky et al.14 Differences between axial and tilted implants for the clinical and microbiologic parameters were sought using the Wilcoxon signed rank test. Adjustments for multiple comparisons15 were
performed when the 40 bacterial species were evaluated simultaneously. The level of significance was set at 5%.

RESULTS

Demographic data of the subjects and the characteristics of the dental implants included in the study are presented in Table 1. Fourteen patients (7 men and 7 women), with a mean age of 61.8 ± 7.6 years, were included in the study. A total of 64 implants were included in this study. All implants presented the same internal tri-channel connections (4.3 mm, Derig) and the same micro unit abutment connection (4.1 mm, Derig). Abutment angulation was standardized into 0 degrees for axial implants and 25 degrees for tilted implants. The mean timing of implant loading was 2 years. Table 2 presents clinical parameters for the axial and tilted implants. The mean periodontal parameter scores for all implants were 2.92 ± 0.60 mm for PD, and the percentage of sites with gingival bleeding, BOP, and Plaque Index were 57%, 60%, and 46%, respectively. Tilted implants showed significantly higher mean PD and percentage of sites exhibiting plaque, gingival bleeding, and BOP compared with axial implants (P < .05).

Figure 1 presents mean levels ($\times 10^5$) and the mean proportion (%) of the 40 evaluated species in all implants. The species were grouped according to the microbial complexes. In general, all species were detected in all samples. *Fusobacterium nucleatum* ssp *vincentii*, *Fusobacterium nucleatum* ssp *polymorphum*, and *Veillonella parvula* were the three species found in higher levels, and *Actinomyces naeslundii*, *Neisseria mucosa*, and *Actinomyces oris* were the species found in the highest proportions when all samples were analyzed together. On the other hand, *Streptococcus constellatus*, *Propionibacterium acnes*, and *Aggregatibacter actinomycetemcomitans* were the species found in the lowest levels and proportions in the subgingival biofilm.

Figure 2 shows the proportions of the six microbial complexes and the “other” group defined by Socransky et al. In all samples. The orange complex (28.7%) and the *Actinomyces* group (24.8%) presented the highest mean proportion, while the pathogens from the red complex (5.2%) showed the lowest mean proportion.

No difference was observed between axial and tilted implants when the 40 bacterial species were analyzed individually ($P > .05$, data not shown). However, tilted implants showed a significantly higher mean proportion of orange complex ($P < .05$) in comparison with axial implants (Fig 3).

### Table 1  Demographic Characteristics of Subjects and Clinical Parameters of Dental Implants

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. or mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>61.8 ± 7.6</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>7/7</td>
</tr>
<tr>
<td>Implant loading (y)</td>
<td>2</td>
</tr>
<tr>
<td>Probing depth (mm)</td>
<td>2.92 ± 0.60</td>
</tr>
<tr>
<td>% of sites with:</td>
<td></td>
</tr>
<tr>
<td>Supragingival plaque</td>
<td>46.0 ± 24.0</td>
</tr>
<tr>
<td>Gingival bleeding</td>
<td>57.0 ± 10.0</td>
</tr>
<tr>
<td>Bleeding on probing</td>
<td>60.0 ± 15.0</td>
</tr>
<tr>
<td>Suppuration</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

DISCUSSION

This retrospective study investigated the subgingival microbial profile of dental implants placed in patients treated with full-arch, implant-supported, immediately loaded rehabilitations following the All-on-4 protocol. In addition, this study compared the microbial profile of axial and tilted implants. Several studies have shown that the All-on-4 approach is predictable and yields a high cumulative implant survival rate. Therefore, it is important and necessary to describe the subgingival microbial profile of implants used in this rehabilitation to understand the longevity of this treatment protocol as well as possible risk factors in order to build further strategies for compliance and maintenance programs.

The results of the present study suggested that dental implants used in the All-on-4 concept presented a subgingival microbial profile compatible with healthy implants. Overall, the implants presented increased proportions of some beneficial bacterial species and strikingly reduced proportions of putative pathogens. However, the present study also found a trend that the bacterial biofilm found around axial implants presented a different microbial composition compared with tilted implants. Thus, the microbial profile of tilted implants was more similar to a subgingival profile of implants with mucositis instead of healthy implants. A higher mean level and proportion of *Fusobacterium* species as well as a statistically higher mean proportion of orange complex were observed in tilted implants compared with axial implants (Fig 3). In addition, tilted implants also showed a significantly higher mean proportion of red complex and a trend to show a higher mean proportion of red complex in comparison with axial implants. These bacteria species have pathogenic potential and have been implicated in severe forms of periodontal and peri-implant disease.
Fig 1  Mean counts (×10⁵) and mean proportion of 40 subgingival species in all samples from (a) axial and (b) tilted implants. The species were ordered according to the microbial complexes described by Socransky et al.¹⁴

Fig 2  Mean proportions of the microbial complexes in all samples from axial and tilted implants. The colors represent different microbial complexes (Socransky et al¹⁴) and Actinomyces species (blue) shown in Fig 1.

Fig 3  Mean proportions of the microbial complexes in axial (left) and tilted (right) implant samples. The colors represent different microbial complexes (Socransky et al¹⁴) and Actinomyces species (blue) shown in Fig 1. The significance of differences between groups was assessed using the Wilcoxon signed rank test.
analyzed the subgingival microbial profile of healthy implants and implants with peri-implantitis and found a mean proportion for the orange complex of 37.8% and 46%, respectively. Hultin et al\textsuperscript{23} also reported higher levels of \textit{Fusobacterium} species in peri-implantitis sites. The difficulty in maintaining good oral hygiene in these fixed, full-arch, implant-supported rehabilitations as well as higher mean PD, plaque, and gingival bleeding could explain the higher levels of orange complex in tilted implants compared with axial implants.

Complementarily, it is important to highlight that the present study found, in this short-term follow-up, an implant survival rate of 100%, and no implant presented peri-implantitis after 2 years of loading. These data corroborate with previous studies that showed good clinical outcomes with the All-on-4 treatment.\textsuperscript{1,2,4–7,16–21} Nevertheless, the results of the present study ratify the notion, which seems to be crucial, that subjects rehabilitated with this protocol, especially at tilted implants, need to receive a supportive program based on patient compliance and re-instruction in oral hygiene measures combined with professional implant cleaning.

The main weakness of this study was the lack of baseline microbiologic data and follow-up visits. Therefore, future research designs must be standardized, and prospective clinical trials must be conducted in order to help in better understanding the colonization around tilted and axial implants in the All-on-4 protocol. Another limitation of this study is the sample size. It is important to highlight that all patients selected in this retrospective study were treated by the same protocol, which included the same operator as well as the same implant brand and abutments, which reduces important bias in this type of analysis. However, a larger sample size could help in confirming these data.

CONCLUSIONS

Despite the clinical success rate of the All-on-4 protocol in a short-term follow-up, a high mean proportion of orange complex microbiota were established in the subgingival biofilm of tilted implants compared with axial implants. These data could suggest that subjects with this modality of implant-supported restoration must be aware that they need a more intense (or rigorous) maintenance protocol.

ACKNOWLEDGMENTS

The authors would like to thank Dr Magda Nagazawa for the clinical examinations and clinical organization facilities. The authors declare that there are no conflicts of interest for any author in the present paper.

![Table 2](image)

**Table 2. Clinical Parameters of Axial and Tilted Dental Implants Evaluated in This Study**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Axial implants</th>
<th>Tilted implants</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Probing depth</td>
<td>2.21 ± 0.84</td>
<td>3.14 ± 0.71</td>
<td>.0061</td>
</tr>
<tr>
<td>Supragingival plaque</td>
<td>34.0 ± 31.0</td>
<td>58.0 ± 21.0</td>
<td>.0262</td>
</tr>
<tr>
<td>Gingival bleeding</td>
<td>44.0 ± 37.9</td>
<td>69.0 ± 45.0</td>
<td>.0482</td>
</tr>
<tr>
<td>Bleeding on probing</td>
<td>51.0 ± 14.0</td>
<td>67.1 ± 15.0</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Suppuration</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
<td>&gt; .05</td>
</tr>
</tbody>
</table>

*Wilcoxon rank test.

REFERENCES


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