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Microflora around teeth and dental implants

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ABSTRACT

Background: When an implant is exposed to oral cavity, its surface gets colonized by micro-organisms. The aim of this study is to comparatively assess the microbiological parameters in sulci around the teeth and the crowns supported by dental implants.

Materials and Methods: In this prospective, cross-sectional study, 34 partially edentulous patients aged between 40 and 50 years with total 50 anterior maxillary single implants with cemented crowns (depth of sulci <4 mm) and 34 similar teeth in the same jaw of the same patients were included. Excluded were the patients with compromised systemic and periodontal health and smoking habits. None of the patients had used any antimicrobial mouthwashes during at least two weeks before the study. All of the implants (ITI) were at least 6 months in place covered by definitive prostheses. Samples of gingival sulci were taken around teeth with paper cone and transported to Stuart transport medium. Samples were cultured and examined by a dark field microscope and eight laboratory tests were performed to determine the micro-organisms The data were evaluated statistically using Chi-square test (α=0.05).

Results: Six anerobic bacteria found in teeth and implants sulci were Gram-positive cocci, Gram-negative cocci, Prevotella, *Porphyromonas gingivalis*, *Bacteroid Fragilis* and *Fusobacterium*. Gram-positive cocci and Gram-negative cocci had maximum and minimum percentage frequency in the two groups, respectively. There were no significant differences between the two groups (*P* value >0.05).

Conclusion: The present study indicated that microflora in implant sulci is similar to the tooth sulci, when the depth of sulci is normal (<4 mm). As a result, implants' susceptibility to inflammation is the same as teeth.

Key Words: Dental implants, microflora, tooth

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INTRODUCTION

Use of dental implants is a widespread treatment modality to restore missing teeth and edentulous cases;[1-3] however, a successful implant treatment depends on the lack of inflammation in peri-implant tissues.[4-6] Being exposed to the oral cavity, implant's surface gets colonized by micro-organisms.[7] A recent *in vivo* study indicated that bacterial colonization occurred within 30 min after implant placement.[8] Another research showed that, following exposure of dental implants to the oral cavity, streptococci were predominant after 4 h and anerobic bacteria increase after 48 h.[9]

We should point out that some characteristics of dental implant – for example roughness – play an important role in bacterial biofilm formation, and biofilms on dental implant surface are the main source of pathogens for peri-implantitis.[10] Moreover, subsequent to the accumulation of plaque on implant surface, dense inflammatory infiltration occurs in connective tissue, which weakens the attachment of overlying epithelium.[11]

Apical progression of the plaque is associated with clinical and radiographic manifestations of tissue

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injury. The soft tissue density of inflammatory infiltration and bone resorption is higher around implants compared to teeth. Also, peri-implant lesions involve the supra-crestal connective tissue and damage to bone marrow.[11]

Opportunistic periodontal pathogens like *Actinobacillus, Actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Peptostreptococcus microbes* and *Fusobacterium nucleatum* are responsible for peri-implantitis in partially edentulous patients.

These micro-organisms are also common among peri-implantitis and periodontal diseases.[12]

Meijndert *et al*. assessed the prevalence of seven periodontal marker pathogens, before implant placement and 1 year after loading. They concluded that in almost half of periodontal healthy individuals, the subgingival biofilm harbors periodontal pathogens above threshold values. Keller *et al*. compared clinical and microbiological features in the peri-implant area of implants carrying either screw retained or cemented suprastructures and investigated the relationship between the peri-implant microflora, the microbiota on the inner surface of removable suprastructures, and the periodontal microflora within the same subject. They found that the microbial leakage through the gap between the suprastructure and the abutment plays an important role in the bacterial colonization of the internal part of screw retained crowns and bridges. The study furthermore confirmed the impact of the dental microflora on the microbial colonization of implants.

Since oral cavity is a main source of bacteria responsible for oral biofilm-related diseases such as periodontal and peri-implant diseases[13] and bacterial infection is an important cause of peri-implant bone loss, the aim of this study was to comparatively assess the microflora in periodontal and peri-implant tissues to put a small step forward in understanding the etiology of complications encountered following implant surgeries.

MATERIALS AND METHODS

Design and overview

This cross-sectional study intended to comparatively assess the microbiologic content of the gingival sulci of teeth and implant-supported crowns with shallow (<4 mm) pockets. This study was conducted during a 16-month period from January 2007 to May 2008 in Dental Implants Research Clinic. Sulcular samples were cultured and examined at Isfahan Medical School microbiology laboratory and examined microscopically to determine the microflora.

ITI implants 6 months after placement were included. A periodontist examined all implants and confirmed absence of any acute or chronic clinical signs of exudates, inflammation, swelling, gingival recession or periodontal diseases for the included implants. None of the patients had used any antimicrobial mouthwashes during at least two weeks before the study. Prosthetic reconstruction was already incorporated for all patients at the time of the study. Selected patients were partially edentulous and their pocket depths were assessed by the same periodontist.

Patients with only implants or only natural dentition, systemically compromised patients, smokers and also patients with poor oral hygiene or improper cooperation were excluded from the study.

Experimental set

Thirty-four partially edentulous patients aged between 40 and 50 years with total 50 anterior maxillary single implants with cemented crowns (depth of sulci <4 mm) and 34 same teeth in the same jaw of the same patients were included. Sulci were first examined by a periodontal probe and gingival bleeding and other clinical signs were recorded. The gingiva around the teeth and the implants were then air dried and isolated. Sulcular samples were taken using a paper cone placed into the sulcus for 15 s [Figure 1]. Samples were kept in Stuart transport medium (STM) and transferred to the microbiology laboratory within 3 h.

Laboratory procedures

Paper cones were taken out of the transport tubes

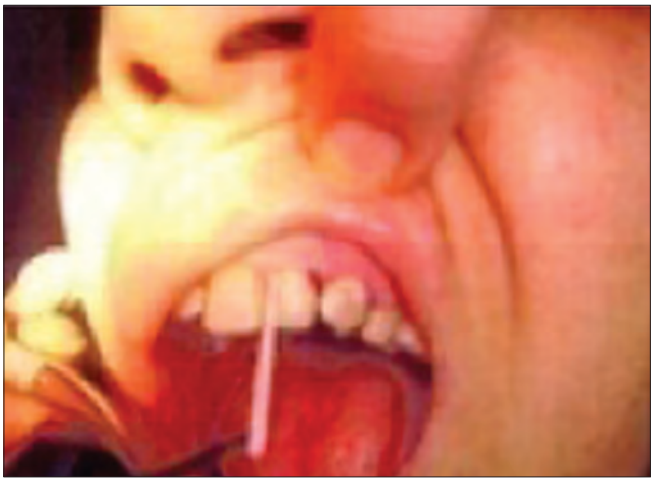

Figure 1: Sampling by paper cone

using sterile forcipes and immediately transferred to anerobic culture mediums (42 plates; each containing two samples). Two types of culture media were used in this study; non-specific and specific. All anerobic micro-organisms grow in non-specific cultures including a base of Columbia agar, Brucella agar or BHI (Brain Heart Infusion) agar; however, only certain anerobic micro-organisms can grow in specific culture [Figures 2 and 3] which includes a base of Columbia, Brucella and BHI with Vancomycin and Kanamycin added next.

Laboratory tests

After bacterial culturing, the following nine laboratory tests were performed: (a) Gram test; for bacterial morphological assessment [Figures 4-6]. (b) Catalase test; to detect anerobes which can not synthesize catalase enzyme. (c) DNAase test; to find DNAase producing bacteria. (d) Triple sugar iron (TSI) test; to detect enterobacteriaceae species. (e) Urease test; for detection of bacteria which are capable of urea-breakdown. (f) (Sulfide-Indole-Motility) medium motility test; to determine the capability of H2S production, and bacterial motility. (g) Gelatinase test. (h) Bile-esculin test to detect streptococcus; and (i) Lipase and Lesetinase. The data was evaluated statistically using Chi-square test (α=0.05).

RESULTS

Six groups of bacteria were found around the implants and the teeth, all of which were anerobic. Percentage frequency of these bacteria in the teeth sulci and the implants sulci is illustrated in Table 1 and Figures 7 and 8.

Gram-positive anerobic micro-organisms including *Fusobacterium* and *Bacteroid Fragilis* were found in the sulci of teeth and implants, as well as prevotella (a black-pigmented organism) and *Porphyromonas gingivalis* which are also Gram-negative anerobes.

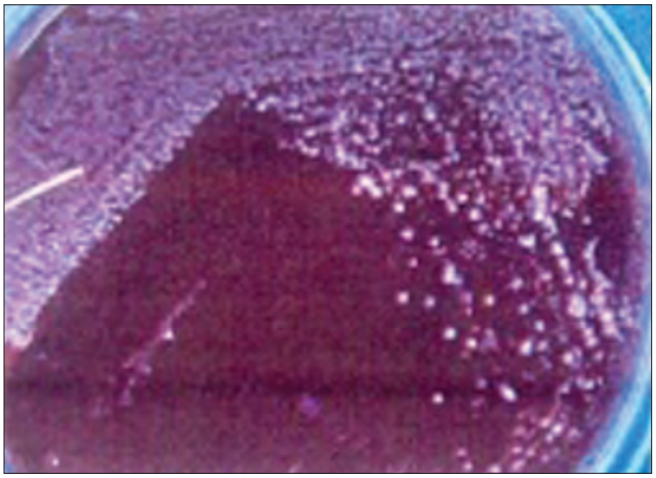

Figure 2: *Prevotella intermedia* in specific culture

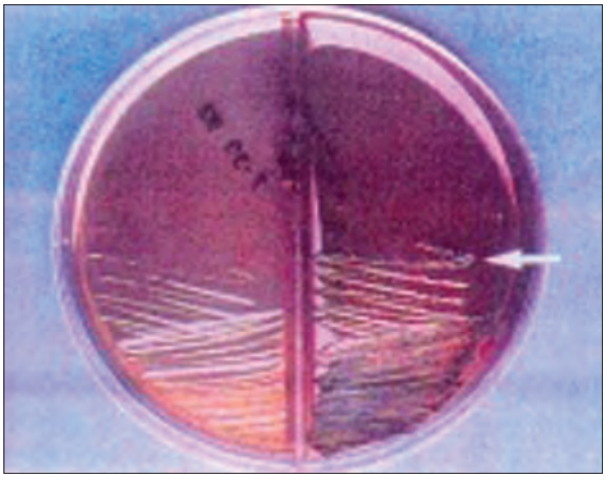

Figure 3: *Porphyromana Gingivalis* in specific culture

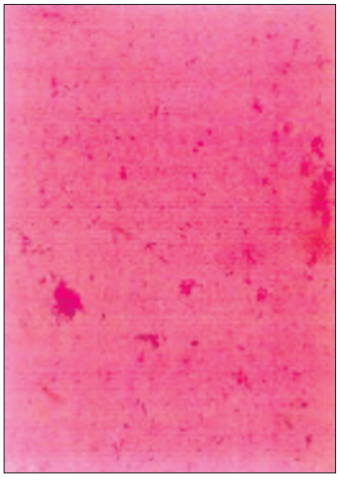

Figure 4: Gram staining: *Fusobacterium*

Figure 5: Gram staining: Gr⁻ Cocci

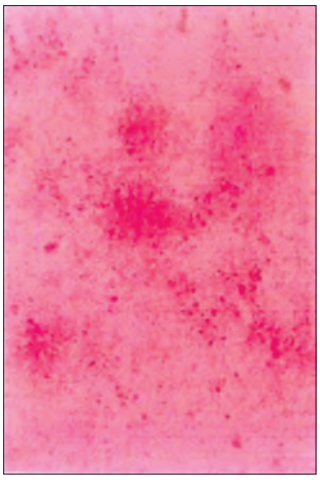

Figure 6: Gram staining: Gr⁺ Cocci

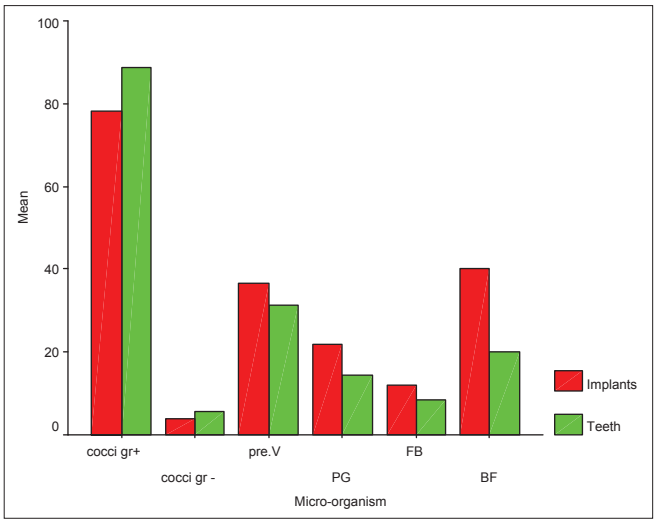

Figure 7: Frequency of different micro-organisms among teeth and sulci

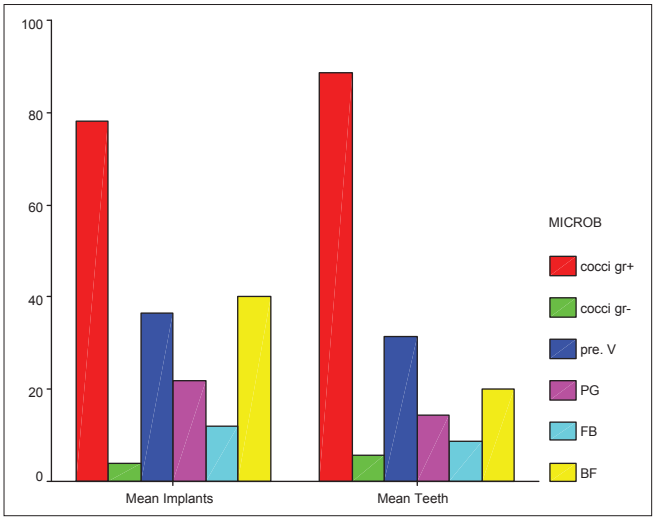

Figure 8: Frequency of different micro-organisms among teeth and implants

Table 1: Relative frequency of species according to the study groups

Bacteria	Location		
	Tooth sulcus (%)	Implant sulcus (%)	*P* value
G⁺ cocci	88.6	78	0.082
G⁻ cocci	5.7	4	0.537
Prevotella	31.4	36.6	0.471
Porphyromonas Gingivalis	14.3	22	0.371
Fusobacterium	8.6	12	0.557
Bacteroid Fragilis	20	40	0.064

Gram-positive cocci form bacteria were also observed in many samples.

The last cultured bacteria were Gram-negative cocci which were seen only in three samples, two in peri-implant sulci and one in tooth sulcus. Overall, no significant differences were observed between these two groups.

DISCUSSION

Shape, type and design of the implants are the determining factors in the development of peri-implantitis.[14] Sardin assessed the adherence effect of streptococcus on the alloy used in the fabrication of implants. He concluded that the alloy used might be a contributing factor in the attachment of micro-organisms and the development of infection around the implants.[15] The present study included ITI implants. The alloy used in the fabrication of these implants might have served as a contributing factor in the attachment of Gram-positive anerobes.

Comparing one-stage and two-stage implants, Adell *et al*.,[16] did not find any Aa micro-organisms in the studied groups. PG bacteria was found in one stage implants. low levels of Prevotella Intermadia (PI) and high levels of *F. nucleatum* were found in the sulci of both implant groups.[16] All implants in the present study were one-stage. No Aa micro-organisms, B Forsythus and *F. nucleatum* were found in the study groups of our study while PG and PI were detected.

Koing[17] concluded that staphylococcus is one of the primary culprits for peri-implant bacterial infections. This micro-organism was not found in the present study which may confirm the periodontal and peri-implant soft-tissue health of the included cases.

Also in Mengel's[18] study, Aa was associated with acute periodontal diseases. Again, the absence of

this species in the present study may confirm that none of the included cases had acute periodontal problems. Moreover, Puchades-Roman[19] claimed that spirochetes are the dominant bacteria of the Astra and Branmark implants; however, in the present study they were not found. This could be attributed to the effect of surface conditions of the fixture to the microflora of the sulci.

In the study of Leonhardt *et al*.,[20] PG and Prevotella were among the bacteria detected in teeth and implants sulci, where PG was significantly of higher amounts in teeth sulci compared to implants' while Prevotella did not show a significant difference. However, in the present study, neither PG nor Prevotella were significantly different between the two groups. Nakazato *et al*.,[21] reported that anerobic Gram-positive cocci were the most prevalent bacteria in implant sulci. They did not isolate PG and spirochetes. In our study, also, Gram-positive cocci were the most obtained micro-organisms and no spirochetes were found, while PG was isolated in contrast to Nakazato study.

Rams *et al*,[22] Takanashi *et al*.,[23] Mengel *et al*.,[18] Rabel *et al*.,[24] the students and the professors of Geneva University, and Leonhardt *et al*.,[25] consistently isolated PG, Aa and PI from implant sulci. In the present study, the same micro-flora was detected except for Aa.

Leonhardt *et al*.,[25] did not find any difference between tooth and implant sulci with 4 mm pocket depth in terms of microflora. Similarly, no statistically significant differences were found between teeth and shallow implant sulci in terms of microflora in the present study.

In the study of Renvert *et al*.,[26] partial edentulousness was found to be related to presence of PG which seems to be in accordance to the findings of the present study since we found high counts of PG especially around implants.

Rams *et al*.,[27] stated that cocci were the most observed micro-organisms where implant sulci were of less than 5 mm. In the present study, however, anerobic Gram-positive cocci were the most obtained micro-organisms. Also, the amount of these micro-organisms in implant sulci was quite similar to tooth sulci in the present study, which supports the findings of Rams *et al*. Again, it should be noted that no statistically significant differences were found between study groups in terms of relative Gram-positive cocci counts.

Borgarello *et al*.,[28] reported the dominant micro-organisms around implants to be stomatococcus, *Prevotella intermedia*, peptostreptococcus, *Fusobacterium nucleatum* and a.a. *Prevotella intermedia* was only found in one case and stomatococcus was found only in three cases. Due to the lack of sufficient laboratory equipment, it was not possible to determine Prevotella and *Fusobacterium* in the present study; thus, we recommend to determine these two in the future studies. As all of the tests used in this study are not the newest methods to detect oral microflora, it is recommended to adopt the most recent methods for the determination of bacteria. Also, it is recommended to repeat the same study with different age ranges, different implant systems and designs and in different areas of the oral cavity to be able to distinguish the influence that each type of these criteria might have on the sulcular microflora, as adequate information about sulcular microflora may help in determining the prognosis of an implant treatment.

CONCLUSION

The results of this study show that as long as peri-implant pocket depth is in the normal range, the implant is not at stake in terms of periimplantitis; and clinicians must caution their patients to pay attention to their oral hygiene.

(This article is written based on the findings of the thesis: Evaluation of Microflora in Teeth and Implants; Project number: 386332).

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