The aim of this study was to evaluate the microbiota and surface of failed titanium dental implants from 4 manufacturers. Twelve mobile dental implants were retrieved from 10 smokers after 3 to 10 years of functional loading. Before implant removal, microbial samples were taken and evaluated using polymerase chain reaction. After implant removal, analyses of the failed implant surfaces were performed using scanning electron microscopy and energy-dispersive spectrometer x-ray. Periodontal pathogens such as Aggregactibacter actinomycetemcomitans, Campylobacter rectus, Eikenella corrodens, Fusobacterium nucleatum, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythia, and Treponema denticola were detected in all implants in different proportions. Surface analysis showed varying degrees of surface roughness between the samples and the presence of proteinaceous material, appearing mainly as dark stains. Foreign carbon, oxygen, sodium, calcium, aluminum, and silicon elements were also found. Although no material-related causes of implant failure were detected, several periodontal pathogens were identified independently of the surface topography or manufacturer.

Key Words: dental implants, energy dispersive spectroscopy, periodontal pathogens/microbiology, failed implants, scanning electron microscopy, tobacco smoking

INTRODUCTION

Dental implant failures have been reported in the current literature. These failures can be classified on the basis of both chronic (early or late) and etiologic aspects. Late implant failures are commonly associated with the occurrence of peri-implantitis, a destructive inflammatory process affecting the soft and hard tissues around osseointegrated implants. If left untreated, peri-implantitis can lead to the formation of a peri-implant pocket and loss of supporting bone.

In addition, the prevalence and severity of periodontal diseases are greater in smokers than non-smokers as characterized by an increased level of alveolar bone loss, deeper periodontal pockets, and
greater clinical attachment loss.4–6 Smoking may also influence the intraoral microbial composition by favoring the colonization of some microorganisms.7,8 In a similar manner, peri-implant tissues can suffer the same deleterious effects from smoking and thereby compromise long-term implant success.9,10 Several studies have described the similarity between the microbiota found in periodontal and peri-implant diseases.11–14 Microbiological studies of dental implants with clinically healthy marginal peri-implant tissues in humans15,16 and animals17–20 have demonstrated a scattered submucosal microbiota dominated by facultative Gram-positive cocci and rods. In contrast, diseased dental implants have been associated with periodontal pathogens such as *Tannerella forsythia*, *Aggregactibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Campylobacter rectus*.17–20 However, little data are available in the literature on the composition of microbiota in patients with implants who smoke.

Although the process of bone-to-implant contact has been ascribed to the biocompatibility of the surface oxide layer,21–23 several investigations have demonstrated that the titanium oxide layer is covered by a carbon (C)-dominated contamination layer and traces of nitrogen (N), calcium (Ca), phosphorus (P), chlorine (Cl), sulfur (S), sodium (Na), and silicon (Si).21–25 It has been hypothesized that contaminants released from tainted implant surfaces may enhance and perpetuate the inflammatory response, alter the healing process, and possibly even provoke the dissolution of titanium (Ti).26–28

The objective of this study was to analyze the microbiota, determine possible surface alterations, and identify any contaminating elements in the oxide layer of failed dental implants retrieved from smokers after moderate to long-term use.

**MATERIAL AND METHODS**

**Subjects and implants**

Twelve dental implants with different surface topographies were retrieved from 10 smokers who did not show any other medical or dental contraindication for implant placement (Table 1). Immediately after dental implant placement, no complications or infection were noted in any of the subjects.

**Microbiological Sampling and analysis**

Before implant retrieval, subgingival microbial biofilm samples were obtained with paper points from the peri-implant site with deepest pocket depth. Supragingival debridement at the peri-implant site was initially performed using a sterile plastic curette and dry gauze after isolation from saliva using cotton tips/wool and suction. Two sterile paper points were subsequently inserted into each peri-implant pocket, as far apical as possible, for 30 seconds. The paper points were removed and placed into different labeled microtubes containing 3 mL sterilized Milli-Q water. All samples were collected by the same operator and coded by an assistant for blind identification.
The polymerase chain reaction (PCR) amplification of the conserved region of 16SrRNA gene was tested for periodontal pathogens, including *A. actinomycetemcomitans* (forward primer 5'-GCTAATACGCGTAGAGTCGG-3' and reverse 5'-ATTTCACCTCACTTAAGGT-3'), *C. rectus* (forward primer 5'-TTTCGGAGGCGTAACACTCTTACCTGTAAG-3', and reverse 5'-TTTTGTGGGAGATAACCGG-3'), *E. corrodens* (forward primer 5'-TCAACATCTCTGTATCCTGCGT-3', and reverse 5'-ACTGTTAGCACAATACGGATG-3'), *P. intermedia* (forward primer 5'-TTTGTTGGGGAGTAAAGCGG-3', and reverse 5'-TCAACATCTCTGTATCCTGCGT-3'), *P. gingivalis* (forward primer 5'-AGGCAGCTTGCCATACTGCC-3' and reverse 5'-ACTGTTAGCACAATACGGATG-3'), *T. forsythia* (forward primer 5'-GCGTAGCAGCTGTC-3' and reverse 5'-TCAAAAGACCATTCTCTCTCTCTTA-3'), and *T. denticola* (forward primer 5'-AACTGTTAGCACAATACGGATG-3', and reverse 5'-ACTGTTAGCACAATACGGATG-3'). All these PCR primers were obtained commercially (Gibco BRL, São Paulo, SP, Brazil). Between 30 and 100 ng of genomic DNA was added to the PCR mixture, which contained 1 μmol/L of the primers, 2.5 U of Taq polymerase in 1× buffer and 0.2 mmol/L of dCTP, dGTP, dATP, and dTTP in a total volume of 50 μL. Amplification was performed for 30 cycles of 30 seconds at 95°C, 30 seconds at 55°C, and 30 seconds at 72°C in a thermocycler (Pekin Elmer, Gene Amp PCR System, Norwalk, CT). Positive and negative controls were included with each set. The negative control included all the PCR reagents except for the sample DNA. The positive control contained all the PCR reagents, together with positive controls for the target periodontal pathogens. Twenty microliters of each PCR reaction mixture was electroforesed in 1.0% agarose gel in TBE buffer, and the amplicom were visualized under 302 nm ultraviolet light on ethidium bromide-stained gels.

**Dental implant retrieval and processing**

All the retrieved dental implants were mobile and surrounded by a radiolucent line on radiograph (Figure 1). The dental implants were retrieved under local anesthesia by gently unscrewing them with stainless steel forceps, which were carefully positioned on the abutment to avoid any possible contamination of the dental implant surface. The dental implants were rinsed with saline solution, immersed in 4% formalin, and stored in a sterile plastic vial. The failed dental implants were inspected for macroscopic soft tissue remnants, which were removed using titanium tweezers.

**Scanning electron microscopy and energy dispersive spectroscopy**

The failed dental implants were dehydrated in a graded series of alcohol, mounted on a metallic stub using double-sided tape, and introduced into the vacuum chamber of a scanning electron microscope (SEM) (JEOL JSM-T330A, JEOL Ltd, Tokyo, Japan). They
were micrographed with different magnifications at an electron beam voltage of 20 kV and at $10^{-6}$ beam current, as previously reported. Thereafter, the implant surfaces were observed by means SEM and submitted to an element analysis. The regions of interest and the element detection were done simultaneously by verification of electron beam-induced x-ray radiation. An energy-dispersive spectrometer x-ray (EDX) equipped with a Si(Li) detector (EDS, Noran Instruments, Inc, Middleton, WI) was coupled to the JEOL JSM-T330A SEM. The spectral resolution of the detector was 138 eV at 5.7 kV (MnKα1). The microprobe used to acquire the spectra was set at 20kV high tension, 250 pA probe current, and a working distance of 80mm.

**Data analysis**

Fisher’s exact test was used to calculate the different detected proportions of target bacteria around failed implants ($P < .05$). The EDX analysis showing the element detected was present only as descriptive data.

**Results**

**Microbiological evaluation**

Figure 2 shows the prevalence of all target periodontal pathogens. *A actinomycetemcomitans* was detected in 16.67% of the implants. *P gingivalis* was most frequently detected in peri-implant pockets ($P = .030$) and was detected in 66.60% of samples. *P intermedia* and *T forsythia* were detected in 33.30% of peri-implant samples. *E corrodens*, *T denticola*, and *C rectus* were detected in 41.66% of the failed implants.

**Surface analysis**

Different degrees of organic residues were detected on retrieved samples (Figure 3). These residues appeared mainly as dark areas of proteinaceous material. Machined implant surfaces were dominated by grooves and ridges along the machining direction and appeared essentially unchanged on the retrieved implants. The EDX analysis showed that all failed dental implant surfaces consisted of Ti oxide, with varying amounts of contaminants. The dominant element detected was C, followed by O, N, Na, Ca, and P (Table 2).

**Discussion**

The present study evaluated the surface composition and the microbiological features of retrieved implants from smokers. Microbiological analysis revealed a periopathogenic microbiota around failed implants. The detection of *P gingivalis, P intermedia*, and *F nucleatun* agree with previous studies. These microorganisms are commonly associated with progressive periodontal diseases and virulence factors that could be important to peri-implantitis progression and treatment.

*A actinomycetemcomitans* and *T forsythia* were detected in peri-implant sites in agreement with other reports. Previous studies have demonstrated that dental biofilm can be an important source of bacteria colonizing dental implant surfaces. However, the presence of putative periodontal pathogens around oral implants does not necessarily mean disease. These studies evaluated diseased implants in nonsmokers, suggesting that the microbial composition between smokers and nonsmokers might be similar. Earlier studies that evaluated periodontal diseases in smokers found similar results.

The EDX surface analysis showed the incorporation of several contaminants in failed dental implant surfaces. The inorganic contaminants present in failed implants, such as C, Ca, Na, and P, were probably relevant to the absorption mechanism of solvated ions that naturally occur in body fluids, as previously reported. In addition, the degree of contamination may determine the mechanical stability and osseointegration qualities of the Tiia surface. A large C signal, a smaller N signal, and traces of Cl, S, and Ca were detected in some samples, probably to adsorption during preparation procedures. These authors also reported that the Ca usually persisted throughout the oxide layer and may have been the result of surface segregation of minute Ca quantities in the commercially pure Ti stock.

The Si and P found are probably traces of the finishing step in the Ti preparation. The presence of Ca...
and Na probably come from body fluids. In contrast, the fact that Si is one of the major constituents of glass could explain the observed Si contamination; likely, it was mainly attributed to ion dissolution from the glass vials, as previous studies have suggested.\textsuperscript{21–23} In addition, sources of contamination by Si and C could also be the residues left by rubber gloves.\textsuperscript{40}

The contact time of dental implant with air before EDX analysis could be critical for the Ti levels, which seemed to be the result of absorption of C.\textsuperscript{41} Bioactivity is associated with the surface energy of a given biomaterial. Contamination of the biomaterial surface with hydrocarbons, other molecules, and foreign elements can reduce the surface energy and thereby also the potential bioacceptability of the surface.\textsuperscript{41,42}

Earlier studies\textsuperscript{24,25} evaluated failed implant surfaces using x-ray photoelectron spectroscopy and Auger electron spectroscopy (AES), which present a much higher surface sensitivity than the equipment used in our study. The use of EDX and differences among the aforementioned studies could explain the different elements detected in our sample data, as previously suggested.\textsuperscript{21,22}

Differences in implant surface topographies may influence bacterial adsorption.\textsuperscript{43} Physical and chemical factors can affect the attachment of biofilms to hard surfaces. The roughness of the surface can increase surface area and hence may increase the bacterial colonization. Roughness also provides protection from shear forces. Studies\textsuperscript{44,45} have shown that after initial bacterial colonization, supragingival plaque formation developed faster on roughened surfaces. The initial colonization of an intra-oral hard surface starts from surface irregularities, such as cracks, grooves, or abrasion defects and subsequently spreads out from these areas as a relatively even monolayer of cells. The roughness of different dental implant surfaces can facilitate initial bacterial adhesion and negatively
affected oral hygiene procedures. Although the present study did not correlate the presence of periodontal pathogens with implant surface contaminants, we may speculate that dental biofilm interacts with implant surface contaminants. The layer of proteinaceous film covering the dental implant surface can also facilitate the adsorption early colonizers.

**CONCLUSION**

Within the limits of the present study, the EDX analysis does not show any material that caused early or late dental implant failure. The microbiological analysis detected several periodontal pathogens around failed/mobile dental implants independently of the surface topography or manufacturer; however, these results should be considered with caution and further investigations must be conducted.

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**REFERENCES**


ANALYSIS OF FAILED IMPLANTS IN SMOKERS


