Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.intl.elsevierhealth.com/journals/dema

Microbiome of titanium and zirconia dental implants abutments

Cássio do Nascimento[a,*], *Murillo Sucena Pita*[a], *Emerson de Souza Santos*[b], *Nadia Monesi*[b], *Vinícius Pedrazzi*[a], *Rubens Ferreira de Albuquerque Junior*[a,c], *Ricardo Faria Ribeiro*[a]

[a] *Faculty of Dentistry of Ribeirão Preto, Department of Dental Materials and Prosthodontics, Molecular Diagnosis Laboratory, Av. Café s/n°, Monte Alegre, Ribeirão Preto, SP 14040-904, Brazil*

[b] *Faculty of Pharmaceutical Sciences of Ribeirão Preto, Department of Clinical Toxicological and Bromatologic Analysis, University of São Paulo, Av. Café s/n°, Monte Alegre, Ribeirão Preto, SP 14040-903, Brazil*

[c] *Faculty of Dentistry, McGill University, Strathcona Anatomy & Dent, 3640, University Street, Montreal, QC H3A 2B2, Canada*

ARTICLE INFO

Article history:
Received 27 April 2015
Received in revised form 8 July 2015
Accepted 29 October 2015

Keywords:
Bacteria
Biofilm
Clinical outcomes
Dental implants
Molecular genetics

ABSTRACT

Objectives. This study employed culture-independent molecular techniques to extend the characterization of the microbial diversity of biofilm associated with either titanium or zirconia implant-abutments, including not-yet-cultivated bacteria species, and to identify and quantify species recovered from peri-implantar/periodontal sulci, supragingival biofilm and the internal parts of implants. Probing depth, clinical attachment level, bleeding on probing, and marginal bone level were also evaluated over time and correlated with biofilm formation.

Methods. Twenty healthy participants were analyzed. DNA-Checkerboard and 16S-rDNA-Pyrosequencing were used to quantify and determine species identity.

Results. 161 bacterial taxa representing 12 different phylotypes were found, of which 25% were non-cultivable. Species common to all sites belonged to genera *Fusobacterium*, *Prevotella*, *Actinomyces*, *Porphyromonas*, *Veillonella* and *Streptococcus*. While some species were subject-specific and detected in most sites, other species were site-specific. Moderate to higher levels of unclassified species were found colonizing titanium-related sites. Pathogenic and non-pathogenic species were detected colonizing oral sites in both materials. Titanium-related sites presented the highest total microbial count and higher counts of pathogenic species.

Conclusions. Our results revealed differences regarding microbial diversity and microorganisms counts in oral biofilm associated with titanium or zirconia. The obtained data suggests a possible relation between microbiological findings and clinical outcomes.

Significance. Next-generation methods of detection have provided new insights on complex microbiota colonizing different sites of oral cavity. The present study demonstrates relevant differences in the communities and microbial counts colonizing different tested substrates with consequent significant differences in the clinical-outcomes, suggesting a probably different mechanism for specific bacterial adhesion.

* *Corresponding author at*: Faculdade de Odontologia de Ribeirão Preto, Universidade de São Paulo Av. do Café s/n CEP: 14040-904 Ribeirão Preto-SP Brazil. Tel.: +55 16 3315 4095; fax: +55 16 3315 0547.
E-mail address: cassionasc@forp.usp.br (C. do Nascimento).

http://dx.doi.org/10.1016/j.dental.2015.10.014
0109-5641/

1. Introduction

In spite of the high success rates of long-term implant-supported restorations, recent studies have reported the presence of relevant microbial adhesion on the implant components [1,2]. Microbial colonization of dental implant assemblies is a consequence of the exposure of the components to the oral cavity [3,4] and one of the most important causes of early and late implant failure is related to the inflammatory process of the surrounding host bone and soft tissues that occurs in response to microbial contamination [5,6].

Bi-directional bacterial microleakage through the implant-abutment interface has been reported in both *in vitro* studies and clinical investigations [3,7]. Microbial colonization of the peri-implant sulci and prostheses occurs in approximately 60% of the rehabilitated patients [8] and therefore it constitutes one of the major concerns related to the long term success of the oral rehabilitation with implant-supported restorations [2,5,6]. Oral microbiota represents a potential risk when associated to two-part dental implant systems. The gaps and cavities inherent to implant-abutment assemblies may act as traps, harboring bacterial species that can lead to inflammatory reactions in the peri-implant soft tissues with consequent bone resorption and implant failure [9]. Chrcanonivc et al. [10] performed a meta-analysis of several studies reported in the literature on the dental implant failure rates. The meta-analysis enrolled a total of 91 studies from 1989 until 2014. A total of 52 357 dental implants were inserted, with 2224 failures (4–36%). Periodontitis/peri-implantitis was shown to be a prevalent risk factor in implant failures.

Structural and topographical variations due to the different materials and manufacturing processes employed to fabricate implant components may influence the microbial adhesion and can lead to significant differences in the formed microbiome [1,11–13]. Nevertheless, studies about the microbial adhesion on the different abutment materials and the impact of the complex oral biofilm on the clinical parameters of implant restorations are still not conclusive. More recently, pyrosequencing of PCR-amplified 16S rDNA has provided a more comprehensive way to characterize the oral microbiome. In this context, we employed both DNA Checkerboard hybridization and pyrosequencing of PCR-amplified 16S rDNA to evaluate the microbial diversity of the biofilm associated either with titanium or zirconia implant-abutments. The microbiological findings were correlated with a set of clinical outcomes (probing depth, clinical attachment level, bleeding on probing and marginal bone level) at 3 different time points up to 6 months after functional loading. The null hypothesis tested in this study is that there is no significant difference in the microbial profile from titanium or zirconia abutments.

2. Material and methods

2.1. Participants

Participants were recruited among partially edentate individuals refereed to the prosthodontics clinic of the University of São Paulo (Ribeirão Preto, Brazil). Potential participants were invited to participate in the study if they: (a) were at least 18 years old; (b) were indicative of a cemented-retained single-unit implant-supported restoration in the anterior maxilla; (c) were indicative of a cemented-retained single-unit implant-supported restoration in the posterior maxilla/mandible; (d) possessed the contra-lateral and antagonists teeth; (e) have had no treatment or professional cleaning in the previous 3 months. Additional exclusion criteria were pregnancy, lactation, periodontal or antibiotic therapy in the previous 3 months, and any systemic condition which could influence the course of periodontal status. The study was approved by the local ethics committee (CAAE 0066.0.138.000-10) and all the experiments were undertaken with the informed and written consent of each subject according to ethical principles.

2.2. Experimental design

Twenty healthy individuals, 17 women and 3 men (mean-age 45.5 years), fulfilled the study's criteria. The clinical parameter probing depth was selected as a primary variable in determining the progression of peri-implant disease and also for determining the sample size (N). To compare two independent groups (Titanium and Zirconia) with repeated measures performed on the three proposed times (baseline, 3 months, and 6 months), and considering a standard deviation of 1.26 among individuals and 0.89 in the intra-individual analysis (values estimated from the deviations observed in the applied literature), the sample size provided a statistical power (power of study) equal to 84% for the factor "group" and 96% for the factor "time" and "group × time" interaction, with a significance level of 5%, and magnitude of effect (effect size) of 0.79 for the factor "group", and 1.12 for the "time" factor and "group x time" interaction. Participants were enrolled into 2 groups of 10 participants each, according to the investigated abutments and manufacturers' recommendations: the first group (8 women and 2 men, mean-age 47 years) comprehended individuals who received a two-part dental implant with a morse taper connection (Ankylos C/X, Dentsply, USA) in the anterior area of maxilla and were rehabilitated using esthetic pre-machined zirconia abutments (Ankylos Cercon Balance, Dentsply, USA) and the second group (9 women and 1 men, mean-age 48 years) comprehended individuals who received a two-part dental implant with a morse taper connection (Ankylos C/X, Dentsply, USA) in the posterior area of maxilla/mandible and were rehabilitated using pre-machined titanium abutments (Ankylos Regular Abutment C/X, Dentsply, USA). In both groups, implants were placed at the level of the alveolar bone crest followed by the insertion of transmucosal healing abutments. The healing abutments were different lengths so that the occlusal surface ended 1.0 mm above the gingival marginal level. After 75 days (following manufacturer' instructions), all the participants were rehabilitated with a total ceramic (in the anterior area) or a metalloceramic (in the posterior area) single unit cemented-retained restoration. All surgical and prosthetic procedures were performed by the same clinician.