Archives of Oral Biology 83 (2017) 145–152

Contents lists available at ScienceDirect

Archives of Oral Biology

journal homepage: www.elsevier.com/locate/archoralbio

Research paper

Microbiome of *peri*-implantitis affected and healthy dental sites in patients with a history of chronic periodontitis

Danae Apatzidou[a], David F. Lappin[b], Graham Hamilton[c], Christos A. Papadopoulos[a], Antonis Konstantinidis[a], Marcello P. Riggio[b,*]

[a] Department of Preventive Dentistry, Periodontology and Implant Biology, School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece
[b] Oral Sciences Research Group, Dental School, University of Glasgow, Glasgow, UK
[c] Glasgow Polyomics, Wolfson Wohl Cancer Research Centre, University of Glasgow, Glasgow, UK

ARTICLE INFO

Keywords:
Peri-implantitis
Periodontal disease
Bacteria
Microbiome
16S rRNA gene sequencing

ABSTRACT

Objective: To determine the composition of the microbiome of *peri*-implantitis sites and corresponding dental sites in subjects with a history of chronic periodontitis.
Design: Clinical and radiographic examination assessed the periodontal/peri-implant disease status. Plaque samples were collected from one diseased implant with *peri*-implantitis, functional for at least two years and healthy sites in ten non-smokers who had received periodontal treatment prior to implant placement. Following DNA extraction, the bacteria present in each sample were determined by high-throughput sequencing of V3-V4 region of the 16S rRNA gene using the Illumina MiSeq platform. OTUs were picked using QIIME. Differences between dental and implant sites were determined using linear discriminant analysis, effect size and diversity analyses were conducted using PAST v3.02.
Results: The microbiomes of healthy samples were more diverse than those found in disease, although disease was associated with a higher abundance of taxa relative to health. The genera *Actinobacillus* and *Streptococcus* were most closely associated with health, whereas *Prevotella* and *Porphyromonas* were most discriminative for disease. *Synergistetes* were highly associated with *peri*-implantitis.
Conclusion: In patients with a history of periodontitis, putative periodontal pathogens prevailed in the microbiome of diseased implants. Diseased implants and corresponding healthy sites appear to have distinct microbiological ecosystems.

1. Introduction

Previous studies have indicated that teeth may serve as reservoirs of bacteria for the colonisation of implants since periodontal sulci appear to harbour a similar microbiota to that residing in implant crevices (Apse, Ellen, Overall, & Zarb, 1989; Leonhardt, Adolfsson, Lekholm, Wikstr & m, & Dahlén, 1993; Mombelli, van Oosten, Schurch, & Land, 1987; Quirynen & Listgarten 1990). The *peri*-implantitis-related biofilm was shown to be similar to that of periodontitis, comprising high levels of putative periodontal pathogens (Botero, González, Mercado, Olave, & Contreras, 2005; Canullo et al., 2016; Hultin et al., 2002; Shibli et al., 2008). In the absence of *peri*-implantitis few differences in the prevalence of bacterial species were found between dental and implant sites (Salvi, Fürst, Lang, & Persson, 2008). Culture techniques have suggested that the microflora present in the oral cavity before implantation determines the composition of the newly establishing microflora on implants (Mombelli, Marxer, Gaberthüel, Grunder, & Lang, 1995), implying that patients with a history of periodontal disease may be at greater risk for *peri*-implantitis (Mombelli & Décaillet, 2011).

However, *peri*-implant infections may be related to microorganisms not typically found in chronic periodontitis, harbouring high numbers of peptostreptococci or staphylococci (Mombelli & Décaillet, 2011). Although the qualitative composition of the microbial flora of *peri*-implantitis-associated biofilms is in agreement with periodontitis, cutting-edge diagnostic techniques have demonstrated dissimilarities in the microbial diversity between the subgingival and submucosal biofilms (Belibasakis, Charalampakis, Bostanci, & Stadlinger, 2015; Faveri, Figueiredo, Shibli, Pérez-Chaparro, & Feres, 2015). The percentage of pathogenic bacteria (red and orange groups) in infected *peri*-implant tissues reaches 40% while an increased diversity of species was present in the more advanced stages of disease (Al-Radha, Pal, Pettemerides, & Jenkinson, 2012). High-throughput sequencing analysis demonstrated that although in some cases certain periodontal

* Corresponding author at: Glasgow Dental Hospital and School, 378 Sauchiehall Street, Glasgow G2 3JZ, UK.
E-mail address: marcello.riggio@glasgow.ac.uk (M.P. Riggio).

http://dx.doi.org/10.1016/j.archoralbio.2017.07.007
Received 11 May 2017; Received in revised form 5 July 2017; Accepted 9 July 2017

pathogens may be in common between teeth and implants, the majority of the species remain distinct between the two ecosystems, suggesting that the composition of a microenvironment is not fully determined by mere geographic proximity (Dabdoub, Tsigarida, & Kumar, 2013). Interestingly, lower diversity in *peri*-implant than subgingival biofilms was observed in both health and disease, indicating that the *peri*-implantitis microbiome is less complex than that of periodontitis and represents a distinct ecosystem (Dabdoub et al., 2013; Kumar, Mason, Brooker, & O'Brien, 2012). In contrast, bacterial 16S rRNA gene sequencing analysis demonstrated a more complex microbiota in *peri*-implantitis sites than in corresponding periodontitis sites and periodontally healthy teeth (Koyanagi, Sakamoto, Takeuchi, Ohkuma, & Izumi, 2010). Similarly, at implants and teeth with clinical signs of mucositis and gingivitis, respectively a diverse bacterial composition was identified, suggesting that transmission of the complete bacterial microbiota from teeth to implants is highly unlikely (Heuer et al., 2011). Microbiome analysis has shown that although teeth and implants share a common ecological niche, they exhibit differences in their microbial communities (Charalampakis & Belibasakis, 2015).

The current study aimed to determine the composition of the microbiome of *peri*-implantitis sites and periodontitis-free dental sites in the same individual using high-throughput sequencing of the bacterial 16S rRNA gene. A well-defined (for periodontal parameters) and homogenous (for absence of smoking, single implant system and low levels of remaining periodontal disease, if any) cohort of subjects participated in the current study. Microbial dissimilarities between diseased *peri*-implant tissues and corresponding healthy dental sites were determined. This aimed to address from a microbiological stand-point a clinical concern as to why *peri*-implantitis developed in individuals with otherwise controlled chronic periodontitis. In addition, in one individual with full-mouth implant restorations (subject #10) the microbiota of the *peri*-implantitis site was compared with that of healthy *peri*-implant tissues.

2. Materials & methods

2.1. Ethical approval

The clinical part of the study was conducted at the Postgraduate Clinic of the Department of Preventive Dentistry, Periodontology and Implant Biology (PDP & IB), Aristotle University of Thessaloniki, Greece (AUTh). The study was approved by the Ethical Committee of the School (275/14-12-2012) and all participants signed an informed consent.

2.2. Study population

Ten systemically healthy non-smokers who previously received implant therapy (loading of implants ≥ 2 years) and had at least one implant diagnosed with *peri*-implantitis were recruited at the Department of PDP & IB, AUTh. Subjects had a history of chronic periodontitis and underwent periodontal treatment prior to implant placement, but were not regular attenders for maintenance care. All the participants were of Caucasian origin and their demographic details and baseline information are shown in Table 1. Nine of the 10 subjects were partially edentulous and one subject was fully edentulous. Recruitment was made on the basis that each subject had at least one implant with *peri*-implantitis based on the following criteria: radiographic evidence of *peri*-implant bone loss of ≥ 2 mm in combination with probing pocket depth (PPD) ≥ 6 mm and simultaneous presence of BOP and/or suppuration in at least one implant surface after one year of loading. This study evaluated one type of implant in all participants (Biomet 3i, Impladend, Greece), however both screw- and cement-retained porcelain fused to metal restorations were included.

Exclusion criteria comprised smoking, history of systemic disease, pregnancy/lactation, untreated advance periodontitis, treatment of *peri*-implantitis and deep scaling at teeth within the previous 12 months, antibiotic intake in the past three months, implant placement and prosthetic loading within the previous 12 months.

Table 1
Demographic details and general information.

N = 10	Gender	Age (years)	Teeth present	Implants present	Loading years of implants
Case 1	F	61	25	5	6
Case 2	F	60	13	6	5
Case 3	M	73	20	4	5
Case 4	F	54	4	4	2
Case 5	F	52	10	1	5
Case 6	M	60	29	1	2
Case 7	F	40	25	1	3
Case 8	F	59	19	2	7
Case 9	M	62	4	8	10
Case 10	M	43	0	12	4
Overall[a]	6F/4M	56.4 (9.6)	14.9 (10.2)	4.4 (3.6)	4.9 (2.4)

All participants were of Caucasian origin and non-smokers. F, female; M, male.
[a] Mean (SD).

2.3. Clinical and radiographic examination

Full-mouth clinical recordings included bleeding on probing (BOP), Plaque Index (PI) (O'Leary, Drake, & Naylor, 1972) for presence/absence of plaque, PPD and clinical attachment levels (CAL) were determined using a manual periodontal probe (PCP-UNC 15; Hu-Friedy XP-23/QW, Chicago, IL, USA) to the nearest millimetre at six sites per tooth/implant and parallel to the long axis (mesio-, mid-, disto-, both buccally and lingually). CAL was determined as the distance between the cementoenamel junction of the tooth and the deepest aspect of the gingival sulcus, or as the distance between the prosthetic crown shoulders of the implant to the bottom of the *peri*-implant sulcus. PPD was recorded from the gingival margin of the tooth to the bottom of the gingival sulcus, or from the mucosal margin of the implant to the bottom of the *peri*-implant sulcus.

Intraoral digital radiography was utilised to assess the periodontal and *peri*-implant bone levels using the long-cone paralleling technique at a distance of 10 cm between the x-ray head and the digital sensor. The distance between the first bone to implant contact and implant shoulder was measured using the accompanying software (Kodak Dental Imaging Software, version 6.12).

The screening visit included initial periodontal/peri-implant clinical examination, intra-oral radiographic examination and fulfillment of inclusion/exclusion criteria. In case of suitability, a signed consent form was obtained from each subject who was subsequently recalled one week later for baseline full-mouth periodontal/peri-implant recordings and microbial plaque collection. All participants were scheduled to receive further treatment.

Patients were screened for eligibility and were assessed clinically by a single calibrated examiner, who also performed the radiographic assessment (CP). Probing pocket depth measurements were collected from one diseased implant (PPD ≥ 6 mm in at least one aspect) in five subjects who did not participate in the study. Measurements were repeated 24 h later and duplicate measurements were within 1 mm for > 90% of the time. Plaque samples and clinical data were coded, so that the statistical and laboratory analyses were performed in a blind manner.

2.4. Sample collection

Based on clinical and radiographic assessments, one diseased implant and four dental sites with periodontal health non-adjacent to the implant were selected for plaque collection in each subject. In one subject (case 10), who was fully edentulous, other implant sites in the