University of Groningen

Peri-implant infections

de Waal, Yvonne Catharina Maria

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2015

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
de Waal, Y. C. M. (2015). *Peri-implant infections*. [Thesis fully internal (DIV), University of Groningen]. University of Groningen.

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 23-05-2024

CHANGES IN ORAL MICROFLORA AFTER FULL-MOUTH TOOTH EXTRACTION: A PROSPECTIVE COHORT STUDY

Y.C.M. de Waal
E.G. Winkel
G.C. Raangs
M.L. van der Vusse
J.W.A. Rossen
A.J. van Winkelhoff

Edited version of: Journal of Clinical Periodontology 2014; 41: 981-989

ABSTRACT

Aim

The aim of the study was to evaluate the effect of full-mouth tooth extraction on the oral microflora, with emphasis on the presence and load of *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.

Material and methods

Adult patients (n = 30), with moderate to advanced periodontitis and scheduled for full-mouth tooth extraction, were consecutively selected. Prior to and 1 and 3 months after full-mouth tooth extraction saliva, tongue, buccal and gingival mucosa and subgingival plaque/prosthesis samples were obtained. Aerobic and anaerobic culture techniques and quantitative real-time polymerase chain reaction (qPCR) were employed for the detection of oral pathogens.

Results

Full-mouth tooth extraction resulted in reduction below detection level of *A. actinomycetemcomitans* and *P. gingivalis* in 15 of 16 and 8 of 16 previously positive patients using culture techniques and qPCR, respectively. Those patients remaining qPCR positive showed a significant reduction in load of these bacteria.

Conclusion

Full-mouth tooth extraction significantly changes the oral microflora. These changes include reduction of *A. actinomycetemcomitans* and *P. gingivalis*, frequently to levels below detection threshold. In some patients *A. actinomycetemcomitans* and *P. gingivalis* can persist in the edentulous oral cavity up to three months after full-mouth tooth extraction.

CLINICAL RELEVANCE

Scientific rationale for study

Consensus is lacking as to whether certain putative periodontal pathogens remain in the mouth after full-mouth tooth extraction. This might be of potential interest in decision making prior to placement of dental implants.

Principal finding

Significant changes in oral microflora occur as a result of full-mouth tooth extraction, including reduction of putative periodontal pathogens, frequently to levels below detection limit.

Practical implications

Although significantly reduced in numbers, putative periodontal pathogens can persist in the edentulous oral cavity up to three months after full-mouth tooth extraction. It remains unknown if low levels of these residual periodontal pathogens comprise a risk for future peri-implant infectious complications.

INTRODUCTION

During lifetime, major changes occur in the composition of the oral microbiome. Shortly after birth, the oral cavity is exposed to micro-organisms via contaminated animate and inanimate objects (Jenkinson & Lamont 2006). Whereas most of the present bacterial species are transient, some species may find a suitable habitat for attachment and growth (Könönen et al. 1991). The eruption of primary teeth around the age of 6 months greatly influences the oral environment, which is accompanied by a significant increase in gram-negative anaerobic species and the creation of a multiform gram-negative anaerobic microflora (Könönen et al. 1994). During the subsequent conversion from primary to mixed dentition relatively little changes occur in the subgingival microflora (Kamma et al. 2000b, Kamma et al. 2000a). After the onset of puberty however, extensive changes occur, ultimately leading to a subgingival microbial profile comparable to young adults (Tsuruda et al. 1995, Kumar 2013). This shift results from increase in abundance of certain species, rather than acquisition of new species (Kumar 2013). Eventually, a stable climax community is established, in which micro-organisms have achieved an equilibrium with the habitat provided by the host (Socransky & Haffajee 2005). Only alterations that will structurally impact the community or its habitat, such as age-related changes (*e.g.* reduced salivary flow rate, compromised immune system, hormonal changes), long-lasting behavioral changes (*e.g.* carbohydrate consumption, smoking, oral hygiene measures) or dental treatment, may shift the equilibrium and can result in the establishment of a new climax community (Socransky & Haffajee 2005).
Tooth extraction can be considered as one of those factors that may permanently alter the oral habitat and thus is expected to influence the composition of the oral microbial community. Danser et al. (1994) suggested that complete elimination of the subgingival environment by full-mouth tooth extraction initiates the elimination or prevalence

below detection level of the periodontal pathogens *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. This spontaneous 'disappearance' of *A. actinomycetemcomitans* and *P. gingivalis* from the oral cavity has been disputed by Van Assche et al. (2009) and Quirynen & Van Assche (2011) who used quantitative real time polymerase chain reaction (qPCR) and checkerboard technology instead of anaerobic culture technique. They found that detection frequencies of periodontal pathogens in saliva and on the tongue remained unchanged after full-mouth tooth extraction, but significant reductions in counts of periodontal pathogens did occur. Unfortunately, no studies are available comparing culture technique *and* DNA-technique in the evaluation of the effect of full-mouth tooth extraction on the intra-oral microflora. Hence, the answer to the question whether some bacteria 'disappear' from the mouth after full-mouth tooth extraction remains inconclusive. This information can be of clinical importance because many fully edentulous patients eventually receive dental implants to support a dental prosthesis. Upon implant placement the newly introduced non-shedding hard surfaces and 'gingival-like' crevices quickly become colonized by micro-organisms present in the oral cavity (Quirynen & Van Assche 2011). After a prolonged period of implant function time peri-implant diseases may develop (Lindhe et al. 2008, De Waal et al. 2013). It has been shown that micro-organisms associated with periodontal disease, *i.e. A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythia* (Zambon 1996), may also be associated with peri-implant infection (Mombelli et al. 1987, Leonhardt et al. 1999, Shibli et al. 2008). Although the mere presence of these bacteria does not necessarily seem to jeopardize dental implant prognosis (De Waal et al. 2013, De Waal et al. 2014), it might improve the prognosis of dental implants when these bacteria are suppressed below detection level at the time of implant placement in susceptible individuals.

The objective of the present clinical study was to evaluate the effect of full-mouth tooth extraction on the oral microflora, with emphasis on the presence and numbers of *A. actinomycetemcomitans* and *P. gingivalis*, using both culture techniques and qPCR.

MATERIAL AND METHODS

Study design

The present study is a prospective cohort study evaluating changes in the oral microflora after full-mouth tooth extraction using both culture techniques and quantitative real-time PCR (qPCR). The study has been conducted in full accordance with the World Medical Association Declaration of Helsinki (version 2008) and was approved by the Institutional Review Board of the University Medical Center Groningen, the Netherlands (METc2012.097).

Participants

Adult patients with a hopeless residual dentition were consecutively recruited for participation in this study. Written informed consent was obtained from all participants before entering the study.

Inclusion criteria were:

- Presence of a hopeless residual dentition of at least 4 teeth for which extraction of all teeth was scheduled;
- Moderate or severe periodontitis (≥ 2 interproximal sites with clinical attachment loss ≥ 4 mm or ≥ 2 interproximal sites with probing pocket depth ≥ 5 mm (not on same tooth)) (Page & Eke 2007).

Exclusion criteria were:

- Medical and general contra-indications for the surgical procedures;
- A history of radiotherapy to the head and neck region;
- Chemotherapy during the last 6 months;
- Use of antibiotics during the last 3 months;
- Dental implants remaining in the oral cavity after full-mouth tooth extraction.

No exclusion was performed based on current oral hygiene measures and no advises were given for oral hygiene measures during the course of the study. Patients performed oral hygiene measures according to their own insights; 27% of the patients used a mouth rinse at some point during the study.

Outcomes

Prior to full-mouth tooth extraction (T_0) and four weeks (T_1) and three months (T_2) after full-mouth tooth extraction the following microbiological samples were collected:

- *Unstimulated saliva*: approximately 1.5 ml;
- *Tongue*: a sterile swab (Copan®, Copan Flock Technologies, Brescia, Italy) was rubbed over the dorsum of the tongue from the circumvallate papilla to the apex in a zigzag movement;
- *Buccal mucosa*: a sterile swab was rubbed over the left and right buccal mucosa five times each side covering approximately 2 cm^2 per side;
- *Gingiva*: a sterile swab was rubbed over the buccal gingival area of the upper jaw five times from first premolar region to first premolar region;
- *Subgingival*: a pooled sample was taken from the deepest suppurating and/or bleeding periodontal pocket in each quadrant using four sterile paper points. If teeth were not present in all quadrants, the second deepest site of one of the other quadrants was sampled. This sample was only collected at T_0.
- *Prosthesis*: a sterile swab was rubbed over the internal surface of the prosthesis of both the maxilla and the mandible. This sample was collected at T_1 and T_2.

All microbiological samples were collected in separate vials containing 1.5 ml of reduced transport fluid (RTF) (Syed & Loesche 1972). In the laboratory, the samples from the buccal mucosa and the gingiva were pooled to produce an overall 'mucosa' sample. Part of the sample (0.1 ml) was used for bacterial culture. The other part was stored at -20°C and used for qPCR testing.

Outcome variables as determined by culturing were:

- Total aerobic bacterial load;

- Total anaerobic bacterial load;
- Detection frequencies and bacterial load of the periodontal pathogens *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia*, *Fusobacterium nucleatum*, *Parvimonas micra* and *Campylobacter rectus*;
- Detection frequencies and bacterial load of Gram-negative rod-shaped aerobic bacteria.

Outcome variables as determined by qPCR were:
- Detection frequencies and bacterial load of *A. actinomycetemcomitans* and *P. gingivalis*

Culturing
All microbiological samples were processed within 24 hours. Samples were vortexed for 30 s and 10-fold serial dilutions were prepared in RTF. Aliquots of 0.1 ml were plated on 5% sheep-blood agar plates supplemented with 0.05% haemin and 0.01% vitamin K1 for isolation and growth of aerobic and obligately anaerobic bacteria. MacConkey agar plates were used for selective isolation and growth of aerobic Gram-negative rod-shaped bacteria and trypticase soy serumbacitracin vancomycin plates (TSBV) (Mediaproducts BV, Groningen, the Netherlands) for selective isolation and growth of *A. actinomycetemcomitans* (Slots 1982). Blood agar plates were incubated in air for 2 days for aerobic growth and in 80% N_2, 10% H_2 and 10% CO_2 for up to 14 days for anaerobic growth. MacConkey plates were incubated in air for 2 days and TSBV plates were incubated in air with 5% CO_2 for five days (van Steenbergen et al. 1986).
Identification of the isolates was based on their colony morphology using a ring-light equipped stereomicroscope, on gram staining and on biochemical characteristics using Rapid ID 32A (bioMérieux, Marcy l'Etoile, France) (Van Winkelhoff et al. 1986; Van Winkelhoff et al. 2005). *A. actinomycetemcomitans* was identified by its typical colony morphology and catalase production upon exposure to 3% hydrogenperoxide (Slots 1982).

qPCR
Automated DNA extraction and purification of 100 µl of the microbiological samples was performed using the NucliSENS® easyMAG® (bioMérieux, Inc., Durham, NC, USA). qPCR was performed using the protocol described by Boutaga et al. (Boutaga et al. 2003, Boutaga et al. 2005). Briefly, qPCR amplification was performed in a total reaction mixture volume of 25 µl. The reaction mixture contained 12.5 µl of TaqMan® Universal PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 300 nM of pathogen-specific forward primer, 300 nM of pathogen-specific reverse primer, 100 nM of pathogen-specific probe and 5 µl of extracted sample DNA. The samples were subjected to an initial amplification cycle of 50°C for 2 min required for optimal AmpErase® UNG activity to degrade PCR products from previous PCR amplifications without degrading native nucleic acid templates and 95°C for 10 min to activate the Taq polymerase, followed by 45 cycles at 95°C for 15 s and 60°C for 1 min. Data were analyzed using 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA,

USA). To monitor extraction and amplification efficiency phocine herpes virus type 1 (PhHV-1) was added to the samples before extraction as an internal control (Niesters 2001). Positive controls of 5 µl of purified DNA isolates (1000x dilution) from *P. gingivalis* (W83) and *A. actinomycetemcomitans* (DSM 11123) and a negative control of 5 µl of sterile H_2O were added to each run.
The detection limit of the qPCR was assessed by determining the Ct-values of serial 10-fold dilutions of purified DNA isolates from *P. gingivalis* (W83) and *A. actinomycetemcomitans* (DSM 11123). Standard curves prepared with these dilutions were used to quantify the DNA from the unknown samples. The detection limit for *A. actinomycetemcomitans* was set at a Ct-value of 32.7 corresponding to 6 CFU and for *P. gingivalis* at 33.6 corresponding to 4 CFU. For comparison of qPCR results with culture results data were transformed to and expressed as CFU/ml equivalents.

Statistical methods

Sample size calculation

Sample size was randomly set at 30 patients. Patients lost during follow-up were replaced by additionally included patients, until the number of 30 patients with complete follow-up was reached. It was expected that about 50% of the patients would have a detectable level of the periodontal pathogens *A. actinomycetemcomitans* and/or *P. gingivalis* before full-mouth tooth extraction as determined by culture technique (Boutaga et al. 2003, Boutaga et al. 2005).

Statistical analysis

Changes in total aerobic and anaerobic bacterial load and changes in ratio of aerobic to anaerobic bacterial load across time were analyzed using repeated measures ANOVA with Bonferroni correction. Changes in mean number of *A. actinomycetemcomitans*/*P. gingivalis* positive sites per patient were analyzed with the Friedman test and changes in mean bacterial load per *A. actinomycetemcomitans*/*P. gingivalis* positive site were analyzed using the paired sample *t*-test. A significance level (α) of 0.05 was chosen. Furthermore, descriptive statistics were used. Data were analyzed using PASW® Statistics 18 (version 18.0.3, SPSS inc., Chicago, IL, USA) and Graphpad Prism 5 for Windows (version 5.04, Graphpad Software, San Diego, CA, USA).

RESULTS

The flow of participants throughout different phases of the study is depicted in Figure 1. Baseline demographic and clinical characteristics are reported in Table 1. Out of 30 patients, 13 patients were culture- and qPCR negative for both *A. actinomycetemcomitans* and *P. gingivalis* throughout the entire study.

A. actinomycetemcomitans

Culture

Four patients were culture-positive for *A. actinomycetemcomitans* at T_0 (Table 2). Full-mouth tooth extraction resulted in reduction below detection level of *A. actinomyce-*

Figure 1. Flow-diagram

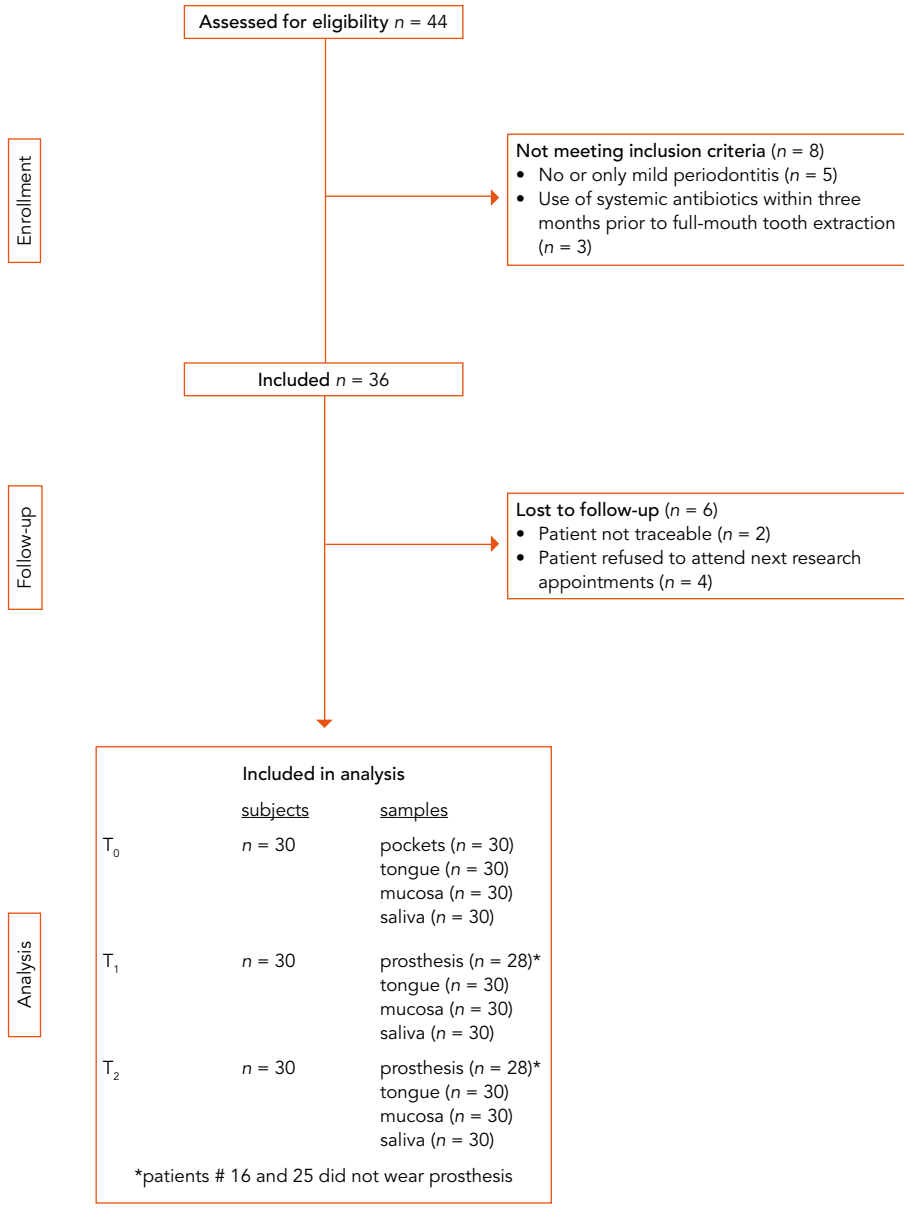


Table 1. Baseline demographic and clinical characteristics of included subjects

Characteristics	
Number of patients	30
Age (years; mean (SD))	52.6 (12.1)
Gender; M (male), F (female)	M 19, F 11
Smoking; *n* subjects (%)	
never or former smoker	10 (33.3%)
current smoker	20 (66.7%)
Use of daily medication; *n* subjects (%)	
no	11 (36.7%)
yes	19 (63.3%)
Total number of teeth before full-mouth tooth extraction (range)	12 (4-28)
Mean probing pocket depth of all teeth (SD)	3.69 (0.95)
Mean probing pocket depth of sampled sites (SD)	5.97 (1.36)
Use of systemic antibiotics during study; *n* subjects (%)	
between T_0 and T_1 (patient #21)	1 (3.3%)
between T_1 and T_2 (patient #6)	1 (3.3%)

Table 2. Log-transformed bacterial load (CFU/ml) of *A. actinomycetemcomitans* per patient. Culture and qPCR data of samples obtained before and after full-mouth tooth extraction.

A. actinomycetemcomitans - Culture												
	T_0				T_1				T_2			
Patient number	SG	T	M	S	P	T	M	S	P	T	M	S
7	3.91											
11	1.00			1.85								
17	2.38	2.51	1.30	1.78								
23	4.81	4.26	3.34	4.15	2.85	2.30	1.90	2.85				
mean	3.02	3.38		2.59	2.85	2.30	1.90	2.85				
SD	1.68	1.24		1.35								
A. actinomycetemcomitans – qPCR												
	T_0				T_1				T_2			
Patient number	SG	T	M	S	P	T	M	S	P	T	M	S
7	3.99	3.27		4.14								
11	4.33	4.55		5.37								
17				3.15								
23	4.46	6.04		6.05	4.46	4.53		4.72	3.49	3.77		3.85
mean	4.26	4.62		4.68	4.46	4.53		4.72	3.49	3.77		3.85
SD	0.24	1.38		1.29								

T_0 = prior to full-mouth tooth extraction; T_1 = four weeks after full-mouth tooth extraction; T_2 = three months after full-mouth tooth extraction; SG = subgingival sample; T = tongue sample; M = mucosal sample; S = saliva sample; P = prosthesis sample; SD = standard deviation; CFU = colony forming unit.

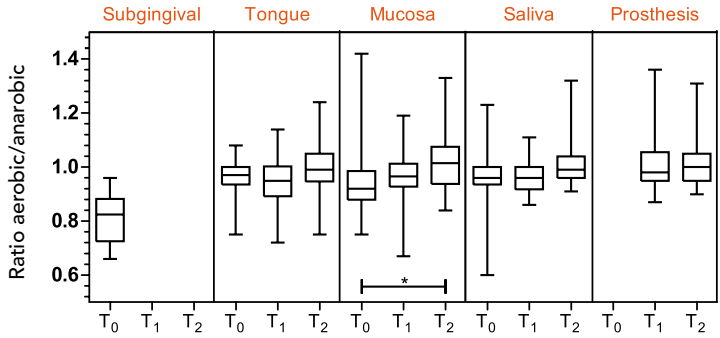
Table 3. Log-transformed bacterial load (CFU/ml) of *P. gingivalis* per patient. Culture and qPCR data of samples obtained before and after full-mouth tooth extraction.

P. gingivalis - Culture												
	T_0				T_1				T_2			
Patient number	SG	T	M	S	P	T	M	S	P	T	M	S
1	7.62											
9	5.64											
10	5.60											
13	7.64											
14		5.30										
18	5.78											
19	7.90	7.60	5.48									
20	6.04											
21	5.30											
24	7.70			6.78							4.00	
25	8.21	8.20										
27	5.85											
mean	6.66	7.04	5.48	6.78							4.00	
SD	1.13	1.53										

P. gingivalis - qPCR												
	T_0				T_1				T_2			
Patient number	SG	T	M	S	P	T	M	S	P	T	M	S
1	7.92	6.08	4.85	7.33	4.23	5.25	3.83	6.00	3.91	5.18		6.66
9	7.02	5.41	3.87	6.31		3.88				4.84	3.43	
10	6.53	5.35	5.94	7.44	3.75	4.22	4.75	5.03				
13	8.10	5.36	4.60	5.81		4.56		5.44	3.69	4.14		3.36
16	7.24	7.05	6.74	7.65								
17	4.21	5.80	3.80	5.07								
18	5.73	5.93	6.22	5.03								
19	7.36	6.96	4.08	7.56	4.79	4.04	5.04	3.80	6.52	4.96	3.80	7.80
20	7.89	6.92	5.78	7.59		3.43		3.39		3.04		3.86
21	7.32	6.74	6.13	5.99								
24	7.17	6.72	6.04	7.15	3.75	3.24		3.88		4.59		4.92
25	7.59	6.80	5.94	6.98								
27	7.73	6.44	5.55	7.60	3.88	5.27	4.00	6.63		4.12	3.55	5.50
mean	7.06	6.27	5.35	6.73	4.08	4.24	4.40	4.88	4.71	4.41	3.59	5.35
SD	1.06	0.65	0.99	0.97	0.44	0.76	0.58	1.23	1.57	0.72	0.19	1.68

T_0 = prior to full-mouth tooth extraction; T_1 = four weeks after full-mouth tooth extraction; T_2 = three months after full-mouth tooth extraction; SG = subgingival sample; T = tongue sample; M = mucosal sample; S = saliva sample; P = prosthesis sample; SD = standard deviation; CFU = colony forming unit.

Figure 2. Ratio of aerobic to anaerobic bacterial load before and after full-mouth tooth extraction.



T_0 = prior to full-mouth tooth extraction; T_1 = four weeks after full-mouth tooth extraction; T_2 = three months after full-mouth tooth extraction; * = statistically significant difference (repeated measures ANOVA with Bonferroni correction, $p < 0.05$).

temcomitans in three patients, whereas one patient remained culture-positive at T_1 (#23). At T_2, *A. actinomycetemcomitans* could no longer be detected by culture from any of the sample sites in any patient.

qPCR

Using qPCR, *A. actinomycetemcomitans* was detected in the same four patients at T_0 as identified by culture. Extraction of all teeth resulted in reduction below detection level of *A. actinomycetemcomitans* in three out of four patients. One patient (#23) remained positive for *A. actinomycetemcomitans* throughout the study.

P. gingivalis

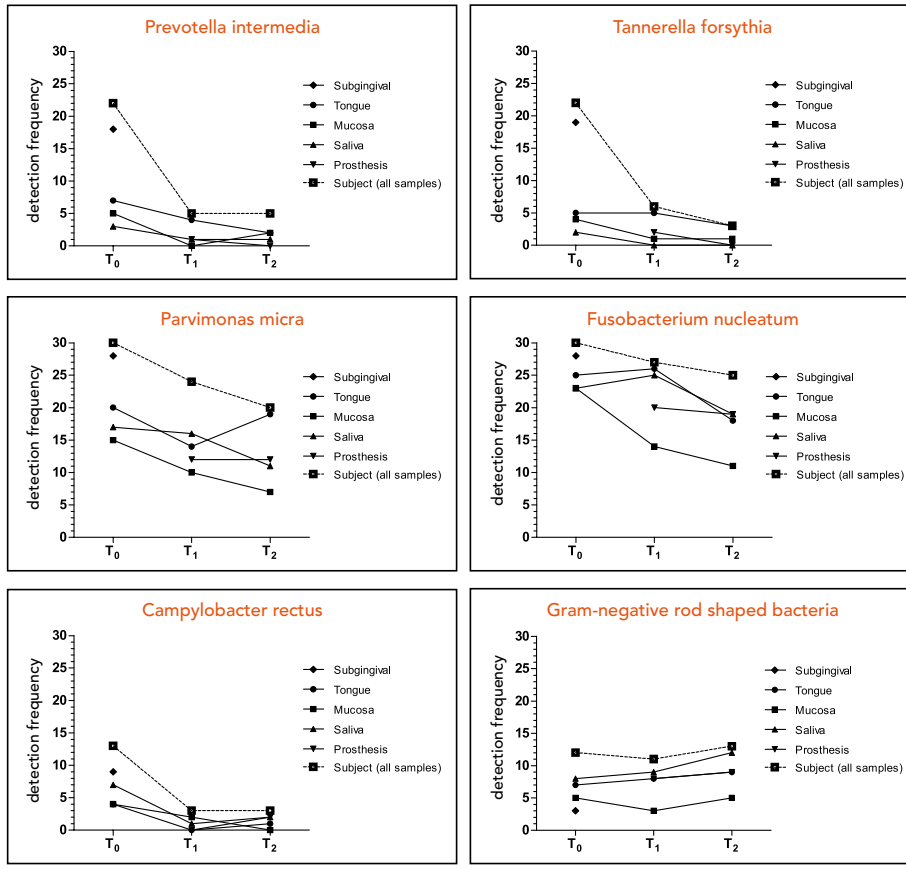
Culture

Twelve patients were culture-positive for *P. gingivalis* at T_0 (Table 3). In all of these patients, full-mouth tooth extraction resulted in eradication or reduction below detection level of *P. gingivalis*. However, one patient (#24) who was culture-negative at T_1 turned out to be culture positive again at T_2.

qPCR

At T_0 two patients (#16 and #17) were qPCR positive, but culture negative for *P. gingivalis* and one patient (#14) was culture positive, but qPCR negative. The number of patients positive for *P. gingivalis* as determined by qPCR decreased from 13 at T_0 to 8 at $T_1$and 7 at T_2. In the patients remaining *P. gingivalis* -positive after full-mouth tooth extraction, a significant reduction was seen in the number of positive sites per patient (4 ± 0 at T_0; 3 ± 1.2 at T_1 and 2 ± 1.2 at T_2, $p = 0.014$) and in the bacterial load per positive site (10log 6.45 ± 1.15 at T_0; 10log 4.42 ± 0.86 at T_1; 10log 4.63 ± 1.27 at T_2, $p < 0.001$).

Figure 3. Detection frequencies of *P. intermedia*, *T. forsythia*, *P. micra*, *F. nucleatum*, *C. rectus* and Gram-negative rod shaped bacteria before and after full-mouth tooth extraction in tongue, mucosa and saliva samples and number of culture positive subjects for the selected bacteria.



Subject (all samples) = patients culture positive on at least one of the sampled sites; T_0 = prior to full-mouth tooth extraction; T_1 = four weeks after full-mouth tooth extraction; T_2 = three months after full-mouth tooth extraction.

Other culture data

No differences were observed in total aerobic and anaerobic bacterial load of the tongue, mucosa and saliva samples between different time points. Only the mean total anaerobic bacterial load of the mucosa samples showed a significant reduction between T_0 and T_2 ($p = 0.027$). The corresponding ratio of aerobic to anaerobic bacterial load was significantly higher at T_2 compared to T_0 ($p = 0.004$, Figure 2). The detection frequencies (on subject level) of the putative periodontal pathogens *P. intermedia*, *T. forsythia*, *P. micra*, *F. nucleatum* and *C. rectus* were lower after full-mouth tooth extraction than before (Figure 3). The detection frequencies of Gram-negative rod-shaped bacteria remained constant over time.

DISCUSSION

The effect of full mouth extraction on the composition of the oral microflora was investigated using both aerobic and anaerobic culture techniques and qPCR. Using culture technique, full-mouth tooth extraction resulted in reduction below detection level of *A. actinomycetemcomitans* and *P. gingivalis* in almost all patients (15 out of 16) previously culture positive for one/both of these pathogens. Using qPCR technique, 8 out of 16 patients became negative for these species after full-mouth tooth extraction. Moreover, patients remaining qPCR positive for *A. actinomycetemcomitans* or *P. gingivalis* showed a reduction in bacterial load and in number of positive sites.

The culture results of this study are in line with a previous culture study evaluating the effect of full-mouth tooth extraction (Danser et al. 1994) and with culture studies evaluating the oral microflora in fully edentulous patients (Könönen et al. 1991, Danser et al. 1997, Danser et al. 1995). Danser et al. (1994) could no longer detect *A. actinomycetemcomitans* and *P. gingivalis* in the oral cavity of patients previously positive for these pathogens up to 3 months after extraction. In line with this, Könönen et al. (1991) failed to detect *A. actinomycetemcomitans* and *P. gingivalis* on any site in the oral cavity of 51 fully edentulous subjects. Similarly, Danser et al. (1995) could not detect *A. actinomycetemcomitans*, and *P. gingivalis* only rarely on oral mucous membranes in 26 fully edentulous subjects with a past history of periodontitis.

However, researchers using molecular DNA techniques to detect target species have disputed conclusions based on culture techniques (Sachdeo et al. 2008, Cortelli et al. 2008, Fernandes et al. 2010, Van Assche et al. 2009, Quirynen & Van Assche 2011). With checkerboard technique, Sachdeo et al. (2008) found *A. actinomycetemcomitans* and *P. gingivalis* in significant numbers in saliva, and on soft tissue and denture samples in subjects who were edentulous for at least one year. With PCR-technique, Cortelli et al. (2008) and Fernandes et al. (2010) also detected *A. actinomycetemcomitans* and *P. gingivalis* in the edentulous oral cavity, but only in a limited number of patients. The results of these studies are compatible with the qPCR results of our study, showing that *A. actinomycetemcomitans* and *P. gingivalis* may be present in the oral cavity of fully edentulous subjects, but only in a relatively small proportion of subjects. Contrastingly, Van Assche et al. (2009) and Quirynen & Van Assche (2011) found nearly all study subjects positive for *A. actinomycetemcomitans* and *P. gingivalis* and did not find an effect of full-mouth tooth extraction on the detection frequency of these pathogens. Only a reduction in load of these bacteria was noted.

From the literature it is known that not all patients with periodontitis harbor *A. actinomycetemcomitans* or *P. gingivalis*. Prevalence of *A. actinomycetemcomitans* in periodontitis patients varies from 20 to 30% using culture technique and is about 30% using PCR (Ashimoto et al. 1996, Van Winkelhoff et al. 2002, Boutaga et al. 2006). Prevalence for *P. gingivalis* varies from 18 to 60% using culture technique and from 46 to 79% using PCR (Ashimoto et al. 1996, Griffen et al. 1998, Van Winkelhoff et al. 2002, Boutaga et al. 2006). It seems that the 'before-extraction' prevalence figures found in the present study correspond well to the prevalence figures that are generally found in the literature.

In the present study, no large differences were observed between culture and qPCR in

the ability to detect *A. actinomycetemcomitans* and *P. gingivalis* at baseline on a subject level. However, at site level and at T_1 and T_2 large differences existed, with qPCR showing higher detection frequencies. These differences might be explained by the higher sensitivity of qPCR and thus its ability to detect small numbers of pathogens which are under the detection limit by culture technique (Boutaga et al. 2006, Zambon & Haraszthy 1995). Another explanation may be the presence of dead bacterial cells or free bacterial DNA, which can be detected by PCR but not by culture techniques. Also, cross reaction of the primers and probe with related species can not be ruled, although this has been thoroughly tested (Boutaga et al. 2003, Boutaga et al. 2005).
A. actinomycetemcomitans, P. gingivalis and other periodontal species were recovered from all examined intra-oral sites, but most frequently from the subgingival area. After tooth extraction, these species were most frequently found on the tongue and in saliva, and less frequently on mucosa and the prosthesis. Apparently, the rough surface of the tongue and the continually forming tongue coating provide a more protective habitat for these species than the relatively smooth surfaces of the buccal and alveolar mucosa. In addition to intra-oral reservoirs, one could also think of other potential sources that prevent 'elimination' of periodontal pathogens from the oral cavity after edentulation, such as other parts of the respiratory and gastro-intestinal tract, *e.g.* tonsils (Danser et al. 1995) or tonsilloliths (Stoodley et al. 2009), or exogenous sources (Petit et al. 1993, Von Troil-Lindén et al. 1995).
All patients included in the present study had been scheduled for full-mouth tooth extraction by their dentist and suffered from moderate or severe periodontitis. In the majority of the patients periodontitis was the main reason for full-mouth tooth extraction. Generally however, periodontal destruction is seldom the sole reason for full-mouth tooth extraction, especially not in older patients. Indeed in some patients of the present study other dental conditions, such as caries, restorative issues or endodontic infections, significantly contributed to the final decision of full-mouth tooth extraction. This might have influenced the microbiological results. Furthermore, some of the included patients had received some form of periodontal treatment in the past, whereas others had not. Also, oral hygiene levels and use of antiseptics varied among the patients. These factors, amongst others, could have additionally influenced the microbiological results.
The persistence of (small numbers of) *A. actinomycetemcomitans*, *P. gingivalis* and other putative periodontal pathogens in the edentulous oral cavity raises the question whether this constitutes a risk factor for peri-implant disease if dental implants are being installed. Implant placement in the edentulous oral cavity establishes a submucosal subgingival-like environment. This might increase detection frequencies of periodontal pathogens in the oral cavity (Devides & Franco 2006). However, presence of periodontal pathogens in fully edentulous implant patients may not reach pre-tooth-extraction levels (Quirynen & Van Assche 2011) and levels seem to remain lower compared to partially edentulous implant patients (De Waal et al. 2014). These generally lower levels of periodontal pathogens might explain why implants in fully edentulous subjects, despite significantly higher plaque levels, clinically seem to perform at least as good as implants in partially edentulous subjects (De Waal et al. 2013).
Unfortunately, no prospective long term studies are available evaluating the specific

role of bacterial species as potential risk factors for the onset and progression of peri-implant disease. Generally, peri-implant disease is associated with a microflora similar to the subgingival flora of chronic periodontitis, *i.e.* dominated by Gram-negative bacteria, but it is unclear whether the associated microflora is the cause or the result of the disease process (Mombelli & Décaillet 2011). However, as high detection frequencies of periodontal pathogens can be observed at implants without peri-implant pathology (De Waal et al. 2014), it seems evident that implant prognosis is not necessarily jeopardized by the mere presence of putative periodontal pathogens. The ability of the host's immune system to cope with the bacterial challenge seems to be of critical importance. One should keep in mind however that the current knowledge of peri-implant infections and the assumed microbiological associations are based on studies with implants in function for only 10 to maximum 20 years. Compared to teeth, which are generally present in the mouth many more years before periodontitis becomes evident, this is a relatively short period of time.

Conclusion

This study shows that significant changes occur in the oral microflora as a result of full-mouth tooth extraction. *A. actinomycetemcomitans*, *P. gingivalis* and other putative periodontal pathogens can be reduced to levels below detection threshold. However, in a considerable proportion of patients, these species may persist in the edentulous oral cavity up to three months after full-mouth tooth extraction. Factors that enable these bacterial species to persist in the edentulous oral cavity are currently unknown. Whether persisting periodontal pathogens comprise a risk factor for peri-implant infection when dental implants are being placed, remains to be established. Long-term prospective studies comparing implant treatment in patients with different initial microbial profiles are needed to elucidate this matter.

Conflict of interest and source of funding statement

Co-authors E.G. Winkel and A.J. van Winkelhoff declare stock ownership of LabOral International and LabOral Diagnostics, service laboratories for microbial diagnostics in dentistry. The other authors declare no potential conflict of interest. The study was supported by a grand of Dentaid SL (Cerdanyola, Spain).

References

Ashimoto A., Chen C., Bakker I., Slots J. (1996) Polymerase chain reaction detection of 8 putative periodontal pathogens in subgingival plaque of gingivitis and advanced periodontitis. *Oral Microbiology and Immunology* 11, 266-273.

Boutaga K., Van Winkelhoff A.J., Vandenbroucke-Grauls C.M. & Savelkoul P.H. (2006) The additional value of real-time PCR in the quantitative detection of periodontal pathogens. *Journal of Clinical Periodontology* 33, 427-433.

Boutaga K., Van Winkelhoff A.J., Vandenbroucke-Grauls C.M. & Savelkoul P.H. (2005) Periodontal pathogens: a quantitative comparison of anaerobic culture and real-time PCR. *FEMS Immunology and Medical Microbiology* 45, 191-199.

Boutaga K., Van Winkelhoff A.J., Vandenbroucke-Grauls C.M. & Savelkoul P.H. (2003) Comparison of real-time PCR and culture for detection of Porphyromonas gingivalis in subgingival plaque samples. *Journal of Clinical Microbiology* 41, 4950-4954.

Cortelli J.R., Aquino D.R., Cortelli S.C., Nobre Franco G.C., Fernandes C.B., Roman-Torres C.V. & Costa F.O. (2008) Detection of periodontal pathogens in oral mucous membranes of edentulous individuals. *Journal of Periodontology* 79, 1962-1965.

Danser M.M., Van Winkelhoff A.J. & Van der Velden U. (1997) Periodontal bacteria colonizing oral mucous membranes in edentulous patients wearing dental implants. *Journal of Periodontology* 68, 209-216.

Danser M.M., Van Winkelhoff A.J., De Graaff J. & Van der Velden U. (1995) Putative periodontal pathogens colonizing oral mucous membranes in denture-wearing subjects with a past history of periodontitis. *Journal of Clinical Periodontology* 22, 854-859.

Danser M.M., Van Winkelhoff A.J., De Graaff J., Loos B.G. & Van der Velden U. (1994) Short-term effect of full-mouth extraction on periodontal pathogens colonizing the oral mucous membranes. *Journal of Clinical Periodontology* 21, 484-489.

De Waal Y.C., Winkel E.G., Meijer H.J., Raghoebar G.M. & Van Winkelhoff A.J. (2014) Differences in peri-implant microflora between fully and partially edentulous patients: a systematic review. *Journal of Periodontology* 85, 68-82.

De Waal Y.C., Van Winkelhoff A.J., Meijer H.J., Raghoebar G.M. & Winkel E.G. (2013) Differences in peri-implant conditions between fully and partially edentulous subjects: a systematic review. *Journal of Clinical Periodontology* 40, 266-286.

Devides S.L. & Franco A.T. (2006) Evaluation of peri-implant microbiota using the polymerase chain reaction in completely edentulous patients before and after placement of implant-supported prostheses submitted to immediate load. *International Journal of Oral and Maxillofacial Implants* 21, 262-269.

Fernandes C.B., Aquino D.R., Franco G.C., Cortelli S.C., Costa F.O. & Cortelli J.R. (2010) Do elderly edentulous patients with a history of periodontitis harbor periodontal pathogens? *Clinical Oral Implants Research* 21, 618-623.

Griffen A.L., Becker M.R., Lyons S.R., Moeschberger M.L., Leys E.J. (1998) Prevalence of *Porphyromonas gingivalis* and periodontal health status. *Journal of Clinical Microbiology* 36, 3239-3242.

Jenkinson H.F. & Lamont R.J. (2006) Oral microbial ecology. In *Oral Microbiology and Immunology*, eds. Lamont, R.J., Burne, R.A., Lantz, M.S. & LeBlanc, D.J. , pp. 90-91. ASM press.

Kamma J.J., Diamanti-Kipioti A., Nakou M. & Mitsis F.J. (2000a) Profile of subgingival microbiota in children with mixed dentition. *Oral Microbiology and Immunology* 15, 103-111.

Kamma J.J., Diamanti-Kipioti A., Nakou M. & Mitsis F.J. (2000b) Profile of subgingival microbiota in children with primary dentition. *Journal of Periodontal Research* 35, 33-41.

Könönen E., Asikainen S., Saarela M., Karjalainen J. & Jousimies-Somer H. (1994) The oral gram-negative anaerobic microflora in young children: longitudinal changes from edentulous to dentate mouth. *Oral Microbiology and Immunology* 9, 136-141.

Könönen E., Asikainen S., Alaluusua S., Könönen M., Summanen P., Kanervo A. & Jousimies-Somer H. (1991) Are certain oral pathogens part of normal oral flora in denture-wearing edentulous subjects? *Oral Microbiology and Immunology* 6, 119-122.

Kumar P.S. (2013) Sex and the subgingival microbiome: do female sex steroids affect periodontal bacteria? *Periodontology 2000* 61, 103-124.

Leonhardt Å., Renvert S. & Dahlén G. (1999) Microbial findings at failing implants. *Clinical Oral Implants Research* 10, 339-345.

Lindhe J., Meyle J. & Group D of European Workshop on Periodontology. (2008) Peri-implant diseases: Consensus Report of the Sixth European Workshop

on Periodontology. *Journal of Clinical Periodontology* 35, 282-285.

Mombelli A. & Décaillet F. (2011) The characteristics of biofilms in peri-implant disease. *Journal of Clinical Periodontology* 38 Suppl 11, 203-213.

Mombelli A., van Oosten M.A., Schurch E.,Jr & Land N.P. (1987) The microbiota associated with successful or failing osseointegrated titanium implants. *Oral Microbiology and Immunology* 2, 145-151.

Niesters H.G. (2001) Quantitation of viral load using real-time amplification techniques. *Methods* 25, 419-429.

Page R.C. & Eke P.I. (2007) Case definitions for use in population-based surveillance of periodontitis. *Journal of Periodontology* 78, 1387-1399.

Petit M.D., Van Steenbergen T.J., De Graaff J. & Van der Velden U. (1993) Transmission of *Actinobacillus actinomycetemcomitans* in families of adult periodontitis patients. *Journal of Periodontal Research* 28, 335-345.

Quirynen M. & Van Assche N. (2011) Microbial changes after full-mouth tooth extraction, followed by 2-stage implant placement. *Journal of Clinical Periodontology* 38, 581-589.

Sachdeo A., Haffajee A.D. & Socransky S.S. (2008) Biofilms in the edentulous oral cavity. *Journal of Prosthodontics* 17, 348-356.

Shibli J.A., Melo L., Ferrari D.S., Figueiredo L.C., Faveri M. & Feres M. (2008) Composition of supra- and subgingival biofilm of subjects with healthy and diseased implants. *Clinical Oral Implants Research* 19, 975-982.

Slots J. (1982) Selective medium for isolation of *Actinobacillus actinomycetemcomitans*. *Journal of Clinical Microbiology* 15, 606-609.

Socransky S.S. & Haffajee A.D. (2005) Periodontal microbial ecology. *Periodontology 2000* 38, 135-187.

Stoodley P., Debeer D., Longwell M., Nistico L., Hall-Stoodley L., Wenig B. & Krespi Y.P. (2009) Tonsillolith: not just a stone but a living biofilm. *Otolaryngology - Head and Neck surgery* 141, 316-321.

Syed S.A. & Loesche W.J. (1972) Survival of human dental plaque flora in various transport media. *Applied Microbiology* 24, 638-644.

Tsuruda K., Miyake Y., Suginaka H., Okamoto H. & Iwamoto Y. (1995) Microbiological features of gingivitis in pubertal children. *Journal of Clinical Periodontology* 22, 316-320.

Van Assche N., Van Essche M., Pauwels M., Teughels W. & Quirynen M. (2009) Do periodontopathogens disappear after full-mouth tooth extraction? *Journal of Clinical Periodontology* 36, 1043-1047.

Van Steenbergen T.J., Van Winkelhoff A.J., Van der Mispel L., Van der Velden U., Abbas F. & De Graaff J. (1986) Comparison of two selective media for *Actinobacillus actinomycetemcomitans*. *Journal of Clinical Microbiology* 24, 636-638

Von Troil-Lindén B., Torkko H., Alaluusua S., Wolf J., Jousimies-Somer H. & Asikainen S. (1995) Periodontal findings in spouses. A clinical, radiographic and microbiological study. *Journal of Clinical Periodontology* 22, 93-99.

Van Winkelhoff A.J., Van Steenbergen T.J., Kippuw N., De Graaff J. (1986) Enzymatic characterization of oral and non-oral black-pigmented Bacteroides species. *Antonie Van Leeuwenhoek* 52,163-171.

Van Winkelhoff A.J., Loos B.G., Van der Reijden W.A., Van der Velden U. (2002) *Porphyromonas gingivalis*, *Bacteroides forsythus* and other putative periodontal pathogens in subjects with and without periodontal destruction. *Journal of Clinical Periodontology* 29, 1023-1028.

Van Winkelhoff A.J., Boutaga K. (2005) Transmission of periodontal bacteria and models of infection. *Journal of Clinical Periodontology* 32 Suppl 6, 16-27.

Zambon J.J. (1996) Periodontal diseases: microbial factors. *Annals of Periodontology* 1, 879-925.

Zambon J.J. & Haraszthy V.I. (1995) The laboratory diagnosis of periodontal infections. *Periodontology 2000* 7, 69-82.