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Microbial changes after full-mouth tooth extraction, followed by 2-stage implant placement

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***Abstract*:**

Background: Recent studies showed that qPCR could detect bacteria related to periodontitis and peri-implantitis in a low concentration after full-mouth tooth extraction. This study monitored the microbiota from tooth extraction, over 9 months of full edentulism, up to 1 year after abutment connection.

Material and methods: Ten patients with severe periodontitis were recruited. Six months after tooth extraction, implants were inserted. Three to 6 months later, they were connected to abutments. Plaque samples were collected from the tongue dorsum, saliva, and subgingival area (teeth/implants) before extraction up to 1 year after abutment connection, and analysed via culture, qPCR, and checkerboard technology.

Results: A reduction in the total amount of aerobic and anaerobic CFU/ml was observed. The concentration of *Porphyromonas gingivalis* and *Tannerella forsythia* (qPCR and checkerboard) in the saliva and, to a lower extent, on the tongue dorsum reduced. For *Prevotella intermedia*, changes were negligible and no changes could be detected for *Aggregatibacter actinomycetemcomitans*. The pristine subgingival niches were quickly colonized by key pathogens. Their final concentration remained low, while the detection frequencies remained very high over time.

Conclusion: Complete edentulation results in a significant reduction of bacteria related to periodontitis and peri-implantitis, with the exception of *A. actinomycetemcomitans*, which might indicate that key pathogens can survive without pockets.

Key words: biofilm; edentulation; implants; microbial re-colonization; subgingival colonization; tooth extraction

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Peri-implantitis is an inflammatory reaction affecting the tissues surrounding osseo-integrated dental implants, resulting in the loss of supporting bone. Healthy peri-implant sulci harbour high proportions of cocoid cells, a low ratio anaerobic–aerobic species, a low level of Gram-negative species, and low detection frequencies of bacteria related to periodontitis and peri-implantitis (Lekholm et al. 1986, Bower et al. 1989, Ong et al. 1992). Implants with signs of peri-implantitis, however, reveal a complex microbiota encompassing conventional bacteria related to periodontitis and peri-implantitis, such as *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis,* and *Tannerella forsythia* (Becker et al. 1990, van der Weijden et al. 1994, van Winkelhoff et al. 2000, Sumida et al. 2002, Heydenrijk et al. 2002, Quirynen et al. 2001, Botero et al. 2005, De Boever & De Boever 2006). Recently, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were added to the list of putative pathogens (Leonhardt et al. 1999, Renvert et al. 2007, Persson et al. 2008). One should, however, realize that the presence of bacteria related to periodontitis and peri-implantitis in the peri-implant sulci may not necessarily result in peri-implantitis or implant failure (Leonhardt et al. 1993, 2002, Papaioannou et al. 1996, Sbordone et al. 1999, De Boever & De Boever 2006).

It has been debated whether a history of periodontitis in patients receiving dental implant treatment increases the risk for peri-implantitis complications (Karoussis et al. 2007). Some studies question the relation between peri-implantitis and a history of periodontitis

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(Hultin et al. 2000, Quirynen et al. 2007, Renvert et al. 2007, Schou 2008); others show an increased incidence of peri-implantitis and implant loss in patients with a history of periodontitis compared with patients without such a history (Hardt et al. 2002, Baelum & Ellegard 2004, Karoussis et al. 2004, Roos-Jansåker et al. 2006). However, in these studies, the information on the periodontal status of the residual natural dentition in patients with a diagnosis of peri-implantitis is not always clear. A recent review based on three papers indicates that subjects with a history of periodontitis may be at a greater risk for peri-implant infections (Renvert & Persson 2009). Some studies even determined that, in partially edentulous patients, the microbiota of the oral cavity before implant placement determines the composition of the peri-implant microbiota (Heydenrijk et al. 2002, Quirynen et al. 2001, 2006, De Boever & De Boever 2006), an observation that seems to suggest a bacterial transmission from teeth to implants (Karoussis et al. 2004, Quirynen et al. 2006).

It has been suggested (Danser et al. 1994, 1997) that elimination of the subgingival environment by extraction of all teeth initiates a spontaneous disappearance of two key periodontal bacteria: *A. actinomycetemcomitans* and *P. gingivalis*. Similar observations were made after the extraction of a partially erupted third molar (Rajasuo et al. 1993). These studies, so far, have applied standard culture techniques. Van Assche et al. (2009) recently rejected the hypothesis of a spontaneous eradication of bacteria related to periodontitis and peri-implantitis after full-mouth tooth extraction, using a qPCR, a technique with, in general, a lower threshold for detection (Riggio et al. 1996, Boutaga et al. 2006). The presence of key pathogens in edentulous oral cavities has also been confirmed via the checkerboard technology (Cortelli et al. 2008, Sachdeo et al. 2008).

The aim of the current study was to follow the microbiological load within the oral cavity (saliva, tongue, subgingivally) after full-mouth edentulation and especially after the re-creation of pockets (after abutment connection). The subjects were followed up to 1 year after abutment connection, and all samples were analysed using three different microbiological techniques (qPCR, checkerboard, and culture).

Material and methods

Subject population

Ten subjects with advanced periodontitis (mean age at implant insertion: 58 years, range 47–65, 5/10 smokers, 3/10 females), for whom a full-mouth tooth extraction was the only remaining treatment option, were enrolled. They had all remaining teeth extracted, and ± 6 months later, implants were inserted (four to seven implants in the upper or the lower jaw, or both as support for fixed full prostheses or an overdenture). The implants (Nobel Biocare, MKIII implants, Göteborg, Sweden) were placed via a 2-stage protocol, and 3–6 months later, abutments were connected and a prosthetic supra-structure was prepared.

Subjects with a medical history of radiotherapy, chemotherapy, diabetes, and/or who had taken antibiotics 3 months before the start of the study were excluded. At each visit, the patient was asked for eventual changes in medication and general health. Intake of antibiotics would exclude the patient for further analysis. The protocol was approved by the Ethical Committee of the Catholic University Leuven and written informed consents were obtained from all participants.

Clinical parameters implants

At 1 year after abutment connection, pocket probing depth (PPD) was measured at six sites per implant using a periodontal probe (XP23 15, HuFriedy Chicago, IL, USA). Bleeding on probing (BOP) and plaque were scored (score 0 = absent, or 1 = present) at 6 sites per implant.

Intra-oral radiographs (Digora®, Soredex, Helsinki, Finland) were taken using a long-cone, parallel technique (including film holders) at loading and 1 year after abutment connection. Marginal bone level was measured mesially and distally at a × 7 magnification, and the threads of the implant were used for calibration. All radiographs were evaluated randomly (NVA) without patient or visit information. The shoulder of the implant served as a reference.

Samples

Subgingival samples

Just before full edentulation, and 1 week, and 3 and 12 months after abutment connection, respectively, samples were taken from the subgingival microbiota of either teeth or implants (four sites pooled), always after removal of the supragingival plaque and isolation of the area. Per site, eight paper points (Roeko®, Roeko, Langenau, Germany)/pocket were inserted for 20 s and

Table 1. Subject description

S	Smoker	Medication	Oral hygiene	nT UJ/LJ	nT PPD > 4 mm UJ/LJ	nT BL > 50% UJ/LJ	BOP %	Bone defect	nI UJ/LJ	pros UJ/LJ
1	0	1	±	1/1	1/1	1/1	83	H	4/2	R/R
2	1	–	+	4/12	2/6	2/4	50	A	0/5	D/F
3	0	–	+	0/9	0/7	0/8	85	A	4/2	R/R
4	1	–	+	8/7	8/5	5/6	67	H	4/2	R/R
5	1	–	+	0/7	0/7	0/7	62	H	0/5	D/F
6	0	–	+	5/11	5/9	3/9	58	H	0/5	D/F
7	1	–	±	0/8	0/6	0/4	75	H	0/4	D/R
8	1	–	±	14/3	12/3	13/3	88	A	4/2	R/R
9	0	2	–	9/9	9/6	9/8	82	H	4/5	R/F
10	0	–	+	9/11	7/8	7/6	26	H	4/2	R/R

S, subject; medication = 1, painkiller for slipped disc; 2, as a flow for lung embolism and cholesterol medication; oral hygiene, ± moderate, +good; T, teeth; UJ, upperjaw; LJ, lower jaw; PPD, pocket probing depth; BL, bone loss; BOP, bleeding on probing; Bone defect: H, horizontal; A, angular; I, implant; pros, prosthetic reconstruction; R, removable implant-supported denture; D, denture; F, fixed implant bridge.

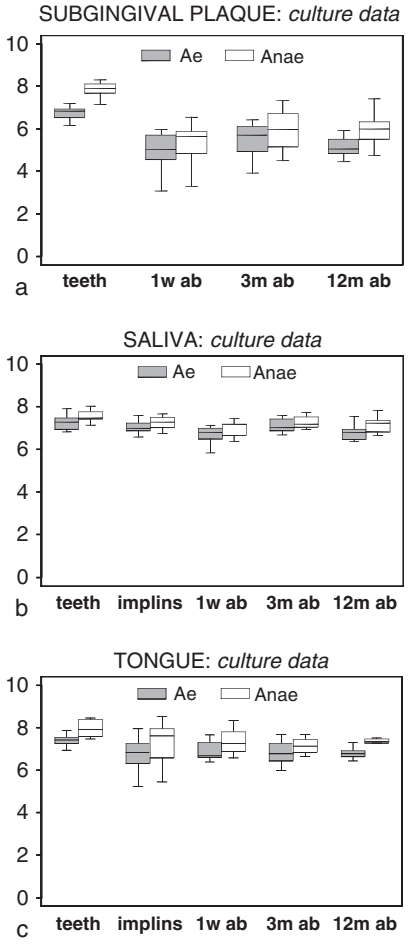

Fig. 1. Changes in the number of colony-forming units (CFU/ml) expressed in $\log_{10}$ values (aerobic and anaerobic species) over time, illustrated via Whiskers boxplots. (a) Samples from the subgingival area around teeth [before edentulation (teeth)], and around the implant abutments (abutment connection made after an edentulous interval of $\geqslant$9 months), after 1 week (1 w ab), 3 months (3 m ab), and 12 months (12 m ab) of connection with the oral environment. (b) Samples from the saliva before edentulation (teeth), at implant insertion [after 6 months of edentulism (impl ins)], and 1 week (1 w ab), 3 months (3 m ab), and 12months (12 m ab) after abutment connection (placed $\geqslant$9 months after edentulation). (c) Samples from the tongue dorsum before edentulation (teeth), at implant insertion [after 6 months of edentulism (impl ins)], and 1 week (1 w ab), 3 months (3 m ab), and 12months (12 m ab) after abutment connection (placed $\geqslant$9 months after edentulation).

dispersed in 2 ml of reduced transport fluid (RTF) (Syed & Loesche 1972). Each sample was homogenized by vortexing for 30 s and processed within 12 h (for details, see Quirynen et al. 1999).

Tongue and saliva

These niches were sampled at five time points: just before tooth extraction, at implant insertion ($\pm$ 6 months after tooth extraction) and 1 week, and 3 and 12 months after abutment connection. A cotton swab, wiped 10 times over the tongue starting from the dorsum, was used to collect the biofilm of the tongue. Approximately 5 ml of unstimulated saliva was collected, from which 200 μl was dispersed in 1800 μl RTF.

After homogenization, all samples were immediately divided into three volumes for further microbiological analysis using three different techniques.

Microbiological processing

Details on the applied culture, qPCR and checkerboard techniques have been summarized in previous papers (Quirynen et al. 1999, Van Assche et al. 2009).

All microbiological evaluations were performed blind.

Statistics

The data are presented via Whisker boxplots depicting: the lower quartile, the median, the upper quartile, and eventual outliers. The detection frequencies for specific key pathogens are presented separately. For the comparison of pre-extraction and later time points, a linear mixed model was fit with time as a fixed factor and the subject as a random factor. A normal QQ-plot was used to assess the normal distribution of the error terms and to confirm the validity of the model's assumptions. Checkerboard was analysed using the Mann–Whitney test. A statistical significant change, difference, or correlation was considered if $p<0.05$.

A Pearson correlation coefficient and its corresponding p-value were calculated to assess the influence of the number of teeth or implants on the CFU (aerobe and anaerobe) in the saliva and the tongue.

Results

Subjects

A descriptive table presents the clinical situation at the start (Table 1). No subject needed additional antibiotics for medical health. They all took antibiotics only after implant insertion. All subjects were seen 12 months after abutment connection.

Implants

No implant was lost during follow-up. The mean PPD was 2.7 mm (range: 1–4 mm), with a BOP of 15%. Plaque was recorded in 22% of the implant sites (six/implant) and pus was never diagnosed. The mean bone loss after loading was 0.6 mm (range 0–2.8 mm, SD = 0.5 mm).

Culture data (Fig. 1)

Teeth and implants

When the subgingival flora around the teeth was compared with the later implants (Fig. 1a), a significant reduction ($p<0.01$) in both the number of aerobic and of anaerobic CFU/ml could be observed ($\pm$ 1.5 $\log_{10}$ for the aerobes, $\pm$ 2 $\log_{10}$ for the anaerobes, with an increase in the proportion aerobes/anaerobes). The microflora around the implants remained relatively constant over time, with perhaps a minor increase in the total amount of anaerobes.

Saliva

The extraction of all teeth resulted in a slight reduction of aerobes ($\pm$ 0.5 $\log_{10}$) and anaerobes ($\pm$ 0.4 $\log_{10}$) in the saliva (Fig. 1b), a reduction that remained up to 1 year after abutment placement.

Tongue

On the tongue, a similar reduction of aerobes ($\pm$ 0.4 $\log_{10}$), but a more obvious reduction in anaerobes ($\pm$ 0.8 $\log_{10}$) was recorded (Fig. 1c), a reduction that again remained up to 1 year after abutment placement.

Correlation between number of teeth or implant *versus* microbiota in the saliva and tongue (Table 2)

There was no correlation between the number of teeth and the aerobe and anaerobe species in the saliva before tooth extraction. The same result was found for the tongue. When the correlation was assessed between the number of implants and the aerobe and anaerobe species in the saliva and the tongue (3 and 12 months), only a significant negative correlation ($r = -0.87$, $p = 0.001$)

Table 2. Correlation between number (teeth or implant) and aerobe or anaerobe species (CFU), before extraction and at 12 months

S	nT	Sal Aer pre	Sal Anaer pre	Ton Aer pre	Ton Anaer pre	nI	Sal Aer 12m	Sal Anaer 12m	Ton Aer 12m	Ton Anaer 12m
1	2	6.9	7.5	7.5	8.5	6	6.7	7.5	7.4	7.5
2	16	7.9	8.3	7.4	7.8	5	7.6	7.9	7.3	7.5
3	9	6.9	7.4	7.9	8.4	6	6.7	6.8	6.8	7.3
4	15	7.2	7.5	7.4	7.6	6	5.4	5.9	5.1	5.5
5	7	7.4	7.8	7.5	8.4	5	6.9	7.3	6.8	7.5
6	16	6.8	7.1	7.4	8.1	5	6.5	6.9	6.6	7.4
7	8	7.5	7.7	7.3	7.5	4	7.0	7.3	6.4	6.7
8	17	7.6	8.0	7.7	8.4	6	6.4	6.7	6.7	7.4
9	18	7.4	7.5	7.2	7.6	9	6.9	7.2	6.9	7.5
10	20	7.0	7.4	6.9	7.5	6	7.3	7.4	6.8	7.3
	r	0.2	0.1	−0.4	−0.5	*r*	−0.1	−0.2	−0.1	−0.1
	P	0.56	0.82	0.24	0.11	*P*	0.77	0.72	0.84	0.75

S, subject; T, teeth; CFU, colony-forming units (log10); Sal, saliva; Ton, tongue; Aer, aerobe; Anaer, anaerobe; pre, pre extraction; *r*, correlation.

was found for the aerobes in the tongue at 3 months.

qPCR (Fig. 2, Table 3)

Teeth and implants

Before tooth extraction, the deep periodontal pockets were heavily colonized with key pathogens. All pockets were positive for *P. gingivalis*, *T. forsythia*, and *Prevotella intermedia*, and 7 of 10 for *A. actinomycetemcomitans*, with high total numbers. For the pristine pockets around the implants, nearly the same detection frequencies were observed, already after 1 week, and up to 1 year (Table 3). In general, however, the total amount of these pathogens in the peri-implant pockets (Fig. 2a) was significantly ($p<0.01$) lower ($\pm 4\log_{10}$ *for P. gingivalis*, $\pm 2\log_{10}$ for *P. intermedia*, $\pm 4\log_{10}$ for *T. forsythia*, and this reduction remained consistent over time), except for *A. actinomycetemcomitans*, which did not show a significant change.

Saliva

The detection frequencies of key pathogens at this niche did not change significantly (Table 3) either after tooth extraction or after implant insertion. For some species (except for *A. actinomycetemcomitans*), however, a clear reduction in the total amount (Fig. 2b) could be recorded ($\pm 3\log_{10}$ for *P. gingivalis* and *T. forsythia,* and $\pm 1.5\log_{10}$ for *P. intermedia*). These reductions remained over time.

Tongue

The detection frequencies of key pathogens at these niches did not change significantly (Table 3) either after tooth extraction or after implant insertion. For some species (not *A. actinomycetemcomitans or P. intermedia*), however, a clear reduction in the total amount (Fig. 2c) could be recorded ($\pm 2\log_{10}$ for *P. gingivalis* and $\pm 1.5\log_{10}$ for *T. forsythia*). The later reductions remained constant over time.

Checkerboard: (Fig. 3, Table 4)

Teeth and implants

The checkerboard data confirm the observations above. Compared with the teeth with severe periodontitis, the implants showed a clear overall reduction of the bacteria, and especially those of the red and orange complex. For some species, this reduction was statistically significant (Fig. 3a). The changes in the composition of the subgingival plaque around the implants during the 1-year follow-up are negligible.

Saliva and tongue

The changes over time within the saliva and on the tongue are small, both in amount as well as in detection frequencies. For some species of the orange and red complex, small reductions could be observed, especially in the saliva. A few statistically significant reductions were only observed after extraction (Fig. 3b).

Discussion

Some investigators have stated that *A. actinomycetemcomitans* and *P. gingivalis* disappear from the oral cavity after edentulation, and do not reappear even when hard surfaces such as dentures are provided (Danser et al. 1995, 1997, Kononen et al. 2007). Our high detection frequency for bacteria related to periodontitis and peri-implantitis in ''fully edentulous'' patients might therefore be surprising; however, several papers already reported similar observations using the checkerboard technology (Quirynen et al. 2005, Sachdeo et al. 2008) or qPCR (Devides & Franco 2006, Cortelli et al. 2008, Van Assche et al. 2009, Fernandes et al. 2010). The differences between more recent observations and those of studies using microbial culturing (Mombelli et al. 1988, Danser et al. 1994, 1997) can be explained by differences in the detection limits for the different technologies. Several papers have compared the detection sensitivity and specificity of checkerboard DNA–DNA hybridization with culture techniques and PCR (Papapanou et al. 1997, Sunde et al. 2000, Siqueira et al. 2001, 2002, Watson et al. 2004). In general, these studies reported a clearly higher detection frequency for *P. gingivalis* and *A. actinomycetemcomitans* when using the two molecular tests. When quantitative PCR techniques were compared with conventional culture techniques, a higher detection sensitivity and specificity was obtained (Riggio et al. 1996, Boutaga et al. 2003). Leonhardt et al. (2003) compared culture techniques with checkerboard DNA–DNA hybridization for samples from Brånemark implants. They reported clearly higher detection frequencies for the latter, even when a high cut-off point (e.g. $\geqslant 10^6$) was used. While culture techniques may be considered the gold standard by some,

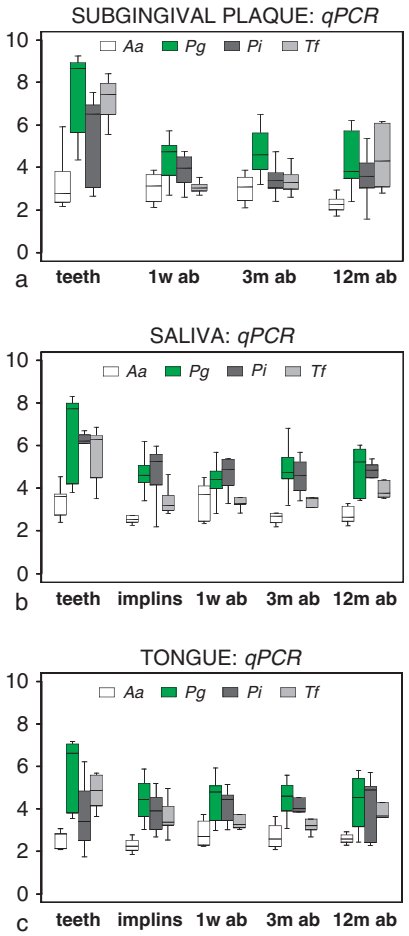

Fig. 2. Changes in qPCR values (expressed in $\log_{10}$ values) over time, for four key pathogens (*Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, and *Tannerella forsythia*), illustrated via Whiskers boxplots. (a) Samples from the subgingival area around teeth [before edentulation (teeth)], and around the implant abutments (abutment connection made after an edentulous interval of $\geqslant$9 months), after 1 week (1 w ab), 3 months (3 m ab), and 12 months (12 m ab) of connection with the oral environment. (b) Samples from the saliva before edentulation (teeth), at implant insertion [after 6 months of edentulism (impl ins)], and 1 week (1 w ab), 3 months (3 m ab), and 12months (12 m ab) after abutment connection (placed $\geqslant$9 months after edentulation). (c) Samples from the tongue dorsum before edentulation (teeth), at implant insertion [after 6 months of edentulism (impl ins)], and 1 week (1 w ab), 3 months (3 m ab), and 12months (12 m ab) after abutment connection (placed $\geqslant$9 months after edentulation).

Table 3. Detection frequency with qPCR technology for key periodontopathogens: *A. actinomycetemcomitans* (*A.a.*), *P. gingivalis* (*P.g.*), *P. intermedia* (*P.i.*), *and T. forsythia* (*T.f.*), in samples from the subgingival area (teeth or implants, Sub), the saliva (Sal), and the tongue (Ton) at different time intervals

	Teeth	Impl ins	1 w ab	3 m ab	12 m ab
A.a.					
Sub	7	–	8	6	5
Sal	6	7	6	4	5
Ton	6	4	5	4	4
P.g.					
Sub	10	–	10	10	10
Sal	10	10	10	8	7
Ton	9	10	10	10	7
P.i.					
Sub	10	–	10	8	7
Sal	7	7	7	4	5
Ton	8	9	8	5	6
T.f.					
Sub	10	–	7	6	5
Sal	10	8	6	3	6
Ton	8	6	7	6	3

Number of positive sites/10 sites.
–, no samples taken because no teeth/implants present at this appointment.

many reports suggest that the newer microbiological techniques may improve the predictability and accuracy of a microbiological test in relation to disease. Additionally, PCR will detect not only viable but also moribund and dead cells (Sanz et al. 2004).

An important finding of the current investigations was that soon after implant insertion, the ''pristine'' peri-implant niches in these ''full edentulous'' subjects became colonized by bacteria related to periodontitis and peri-implantitis. This early colonization by pathogens has been reported for ''partially'' edentulous patients, where teeth were considered as the source for transmission (van Winkelhoff et al. 2000, Sumida et al. 2002, Quirynen et al. 2005, Quirynen et al. 2006, De Boever & De Boever 2006, Furst et al. 2007, Salvi et al. 2008) and is now also confirmed for fully edentulous patients. The initial colonization of peri-implant pockets in full edentulous, without teeth as a bacterial reservoir, has previously not been examined extensively. Nakou et al. (1987) examined the subgingival flora along implants, 10 weeks after insertion, via dark-field microscopy and anaerobic culturing, and identified several potential periodontal pathogens, but no black-pigmented Bacteroides species. Danser et al. (1997) examined dental implants in 20 edentulous patients and with a history of periodontitis. They were not able to identify either *A. actinomycetemcomitans* or *P. gingivalis* after 1 year. A recent single case report, however, mentions the presence of the entire gamma of bacteria related to periodontitis in an edentulous subject soon after implant placement (Emrani et al. 2009). Devides and Franco (2006) took samples in 15 full edentulous patients, before and 4 and 6 months after implant insertion, and analysed them via PCR. Before implant placement, *A. actinomycetemcomitans* could be detected in 2/15 and *P. gingivalis* in 0/15 patients, but soon after implant placement, these proportions increased to 11/15 and 8/15, respectively.

These observations provide another perspective regarding oral microbiology. Questions arise like: ''Is it possible to eradicated bacteria related to periodontitis from the oral cavity?'' or ''What is important, the presence of bacteria related to periodontitis or their concentration, of course related to the efficiency of the host immune response?''. As the amount of bacteria is partially dependent on the size of the pocket (probing depth), one could even argue that the number of pathogens simply depends on the size of the pocket.

This study shows only minor changes in the detection frequency of several key pathogens in the saliva and on the tongue, before and after edentulation, and after abutment connection. The absolute amount, however, reduced significantly, especially in the saliva, slightly less on the tongue, and not for *A. actinomycetemcomitans*. The latter indicates that bacteria related to periodontitis can survive in the oral cavity without the presence of subgingival niches, but also that periodontal pockets are an important source for those species (and even for the commensal flora) on the tongue and in the saliva. Whether there is a direct link (ejections of bacteria from the pocket) or an indirect link with the outflow of a nutritional source via the pocket is not clear. On the other hand, the oral cavity is not an isolated area but part of both the respiratory as well as the gastro-intestinal tract. Many of the species found in the oral cavity are not unique for this area. Therefore, other niches can be considered as possible sources in future research.

The question arises as to whether these residual low concentrations of bacteria related to periodontitis after full-mouth extraction constitute a potential risk for the long-term survival of the implants. It seems that the human host

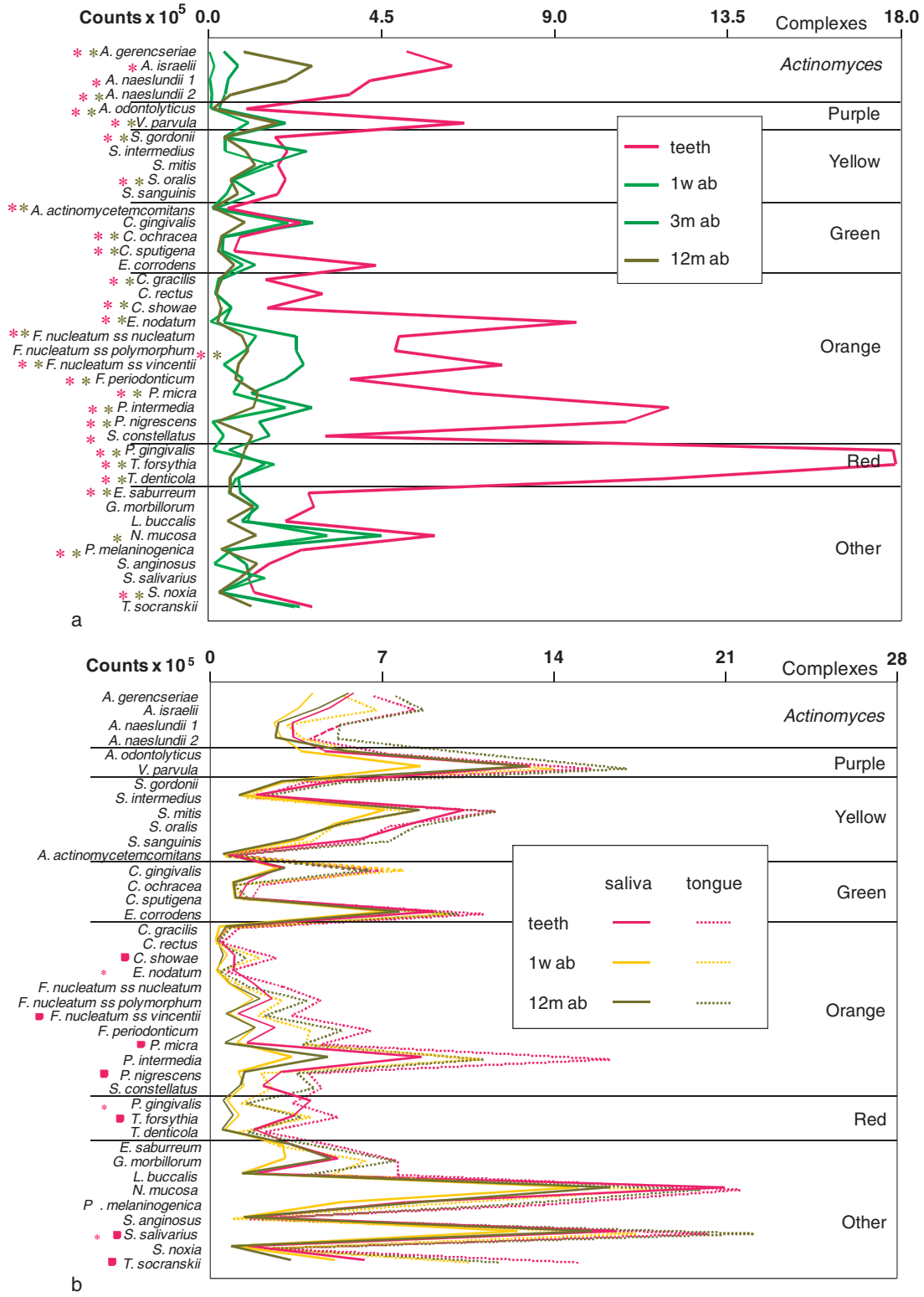

Fig. 3. Changes in DNA counts (expressed in $\log_{10}$ scores) for the different microbial complexes (Socransky et al. 1998) over time, using the checkerboard technology. (a) Samples from the subgingival area around teeth [before edentulation (teeth)], and around the implant abutments (abutment connection made after an edentulous interval of $\geqslant$9 months), after 1 week (1 w ab), 3 months (3 m ab), and 12 months (12 m ab) of connection with the oral environment Asterisks in pink means statistical difference for ''teeth moment'' *versus* 1 week Asterisks in green means statistical difference for ''teeth moment'' *versus* 12 m ab (b) Samples from the saliva and the tongue prior to edentulation (teeth), and 1 week (1 w ab), and 12 months (12 m ab) after abutment connection (placed $\geqslant$9 months after edentulation). Asterisks in pink indicates a statistical difference for ''teeth-moment'' *versus* 1 week in saliva Square in pink indicates a statistical difference for ''teeth-moment'' *versus* 1 week at the tongue.

Table 4. Detection frequency with checkerboard technology for key periodontopathogens: *A. actinomycetemcomitans* (*A.a.*), *P. gingivalis* (*P.g.*), *P. intermedia* (*P.i.*), *and T. forsythia* (*T.f.*) in samples from the subgingival area (teeth or implants, Sub), the saliva (Sal), and the tongue (Ton) at different time intervals

	Teeth	Impl ins	1 w ab	3 m ab	12 m ab
A.a.					
Sub	10	No	9	8	9
Sal	10	10	10	10	10
Ton	10	8	9	10	10
P.g.					
Sub	10	No	6	8	7
Sal	10	9	7	10	9
Ton	10	8	8	10	10
P.i.					
Sub	10	No	10	10	10
Sal	10	10	10	9	10
Ton	10	10	10	10	10
T.f.					
Sub	10	No	10	10	10
Sal	10	10	9	10	10
Ton	10	10	9	10	10

Number of positive sites/10 sites.
No samples taken because no teeth/implants present at this appointment.

can cope with low numbers of bacteria, but it is currently not defined whether the numbers of bacteria define their virulence or whether it is the (in)ability of the host to defend. Indeed, Haffajee et al. (1998) showed high detection frequencies but low detection numbers for most bacteria related to periodontitis in patients with a healthy periodontium. Similar observations have been made for completely edentulous patients who had been rehabilitated for 10 years with an overdenture on two implants (Quirynen et al. 2005). Even though these implants were clinically healthy, high detection frequencies for most bacteria related to periodontitis and peri-implantitis were observed. The small number of subjects and the short follow-up period are limitations of this study. Therefore, it was not possible to divide the data into subgroups with different initial bacterial loads or subgroups with different host susceptibilities. It is obvious that the initially pristine peri-implant pockets might slowly become colonized by bacteria related to periodontitis, in a manner similar to natural teeth; otherwise, peri-implantitis would be unlikely to occur in edentulous patients. Several authors indeed identified several periodontitis related bacteria (including *P. gingivalis* and *A. actinomycetemcomitans*) in the subgingival plaque of implants in fully edentulous patients, even with culture techniques, particularly around implants with symptoms of peri-implantitis (Rosenberg et al. 1991, Mombelli & Lang 1992, Leonhardt et al. 1999). Leonhardt et al. (1999) explained the occurrence of these species partially by the prolonged presence of the implants in the oral cavity (>5 years in his study). When the subgingival samples are considered (teeth *versus* implants) again only negligible changes in the detection frequencies were seen, but clear changes in the total amounts were present. It is not clear whether this simply reflects the difference in probing depth or the fact that more time is needed before these pathogens obtain higher amounts.

The fact that the *A. actinomycetemcomitans* concentration did not change in the subgingival plaque after edentulation is surprising. However, from the subgingival microbiological profile of the extracted teeth, it is quite obvious that all of these subjects, due to the high levels, suffered from an advanced *P. gingivalis–P. intermedia–T. forsythia*-associated periodontitis. One could hypothesize that the levels of *A. actinomycetemcomitans* that were detected in the pockets of the extracted teeth merely represent a commensal colonization of the species. Under this hypothesis, it is then understandable that once a new and healthy subgingival niche is created by connecting the abutment, *P. gingivalis, P. intermedia*, and *T. forsythia* would decrease while the *A. actinomycetemcomitans* levels were not changed when compared with the levels in the inflamed periodontal pockets around the extracted teeth.

A second remarkable observation was that the concentration of *A. actinomycetemcomitans* on the tongue and in the saliva did not change after edentulation, whereas the levels of *P. gingivalis* and *T. forsythia* declined dramatically. A similar lack of decrease was observed for *P. intermedia* levels on the tongue. Similarly, the salivary levels of *P. intermedia* decreased to a much lesser extent than the levels of *P. gingivalis* and *T. forsythia* after edentulation. These opposite effects of edentulation on *A. actinomycetemcomitans* and *P. intermedia versus* the effects on *P. gingivalis* and *T. forsythia* can be explained by the tissue tropism of these different species. Already in 1999, Socransky and co-workers reported that the most dominantly colonized niche in the oral cavity for *P. gingivalis* is the subgingival pocket, whereas for *A. actinomycetemcomitans*, the soft tissues are more dominantly colonized. Similar observations can be derived from Mager et al. (2003), who showed, next to confirming the aforementioned tissue tropisms for *A. actinomycetemcomitans* and *P. gingivalis*, that the subgingival plaque is the preferred niche for *T. forsythia* and the soft tissues for *P. intermedia*. Based on these proportional observations, both studies seem to indicate that *P. gingivalis* and *T. forsythia* are more dentotropic bacteria, whereas *A. actinomycetemcomitans* and *P. intermedia* are more epitheliotropic. With this in mind, it then becomes obvious that the effect of removing teeth will have a more pronounced effect on salivary and tongue levels of the dentotropic species than on the salivary and tongue levels of epitheliotropic species. The latter was confirmed by this study and provides at present a reasonable explanation for the observations. Because of differences in sampling technology (paper point *versus* cotton swab *versus* pure saliva), a direct comparison between different niches could not be made. We therefore recommend focusing on changes within the same niche or on relative changes/proportional changes over the niches.

Conclusion

The data of the present investigation suggest that periodontal pathogens may persist for a long period of time in the oral cavity of edentulous subjects with a history of periodontitis, even in the absence of other hard subgingival surfaces in the mouth.

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Clinical Relevance

Scientific rationale for the study: Previous studies suggested, via microbial culture techniques, that bacteria related to periodontitis and peri-implantitis disappear after full-mouth tooth extraction. One can question whether this change represents an actual disappearance or a reduction below the detection limit of culture techniques. Therefore, this study aimed to document the entire scala of microbiological changes from before tooth extraction, over 9 months of full edentulism, up to 12 months of implant loading via culture techniques, and via more sensitive techniques such as qPCR and checkerboard technology.

Principal findings: Complete edentulation resulted in significant reductions (2–4 $\log_{10}$ values) in the concentration of bacteria related to periodontitis and peri-implantitis in the saliva as well as on the tongue, even though the overall reductions of aerobic and anaerobic bacteria (culture technique) were small ($\pm$ 0.5 $\log_{10}$). The subgingival niches are thus a major reservoir for the bacteria related to periodontitis and peri-implantitis on the tongue and in the saliva, even though these pathogens can survive on the latter without subgingival niches. The pristine subgingival niches (implants) were, even in this group of ''full edentulous'' subjects, quickly colonized by bacteria related to periodontitis and peri-implantitis, but at low concentrations.

Practical implication: Bacteria related to periodontitis and peri-implantitis remain in the oral cavity after full-mouth tooth extraction, and they do not necessarily increase again after implant insertion.