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

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

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1 **Microbial changes after full-mouth tooth extraction followed by 2-stage implant**
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29 Running head: *microbiota after edentulation & implants.*
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35 extraction.
36

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Clinical relevance:

Scientific rationale: 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 full edentulism, up to 12 months 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 log₁₀ 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 (± 0.5 log₁₀). 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.

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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 one year after abutment connection, and analysed via culture, qPCR, and checkerboard technology.

Results: A reduction in 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 extend 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 detection frequencies remained very high over time.

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

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Deleted: Background: Previous studies showed that periopathogens disappear after full-mouth tooth extraction. With a more sensitive technique (qPCR) it became however obvious that most of them remain, but at very low levels. This study aimed to monitor the microbiological changes from tooth extraction, over 9 months of full edentulism, up to 12 months after reconstruction with implants.

Material and methods: Ten patients with severe periodontitis, for whom a complete edentulation was the only treatment option and with the desire of a rehabilitation via oral implants, were recruited. Six months after tooth extraction implants were inserted, and 3 to 6 months latter they were connected to abutments. Plaque samples were collected from the tongue dorsum, the saliva and the subgingival area (initially teeth, afterwards the implants), before tooth extraction, at implant insertion (6 months edentulous), at abutment connection (9-12 months of edentulous), and after 1 week, and 3 and 12 months exposure to the oral environment, respectively. The samples were analysed via: culture techniques, qPCR, and checkerboard technology.

Results: Complete edentulation resulted in a reduction in the total amount of aerobic ($\pm 0.5 \log_{10}$ in saliva, $\pm 0.4 \log_{10}$ on tongue) and anaerobic ($\leq 0.4 \log_{10}$ in saliva, $\pm 0.8 \log_{10}$ on tongue) CFU/ml in the oral cavity. The concentration of *Porphyromonas gingivalis* and *Tannerella forsythia* (qPCR and checkerboard) in the saliva, and to a lower extend on the dorsum of the tongue also reduced significantly. The detection frequencies remained however very high over time for all key-pathogens. For *Prevotella intermedia* and the changes were negligible and no changes could be detected for *Aggregatibacter actinomycetemcomitans*. The pristine subgingival niches (implants) by itself were quickly colonized by the key-periopathogens, although their final concentration remained low. The creation of these new pockets (implants) did not result in an outbreak of the pathogens in the other niches.

Conclusion: In contrast to what has been believed so far, complete edentulation did not result in full eradication of all periopathogens, but only in a significant reduction (with an exception of *Aggregatibacter actinomycetemcomitans*. The { ... [1]

INTRODUCTION

Peri-implantitis denotes an inflammatory reaction affecting the tissues surrounding osseo-integrated dental implants resulting in 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 (Bower et al. 1989, Lekholm et al. 1986, 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. 2002, 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 realise that the presence of bacteria related to periodontitis and peri-implantitis in the peri-implant sulci 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 2005).

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 (Hultin et al. 2000; Schou et al. 2008; Quirynen et al. 2007; Renvert et al. 2007); 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. 2002, Quirynen et al. 2006, De Boever & De Boever 2006), an observation that seem 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 *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. Similar observations were made after extraction of a partially erupted third molar (Rajasuo et al. 1993). These studies, so far, applied standard culture techniques. Van Assche and co-workers (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 the 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 one year after abutment connection, and all samples were analyzed by 3 different microbiological techniques (qPCR, checkerboard and culture).

MATERIAL AND METHODS.

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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 \pm 6 months later implants were inserted (4 to 7 implants in the upper or lower jaw, or both as support for a fixed full prostheses, or an overdenture). The implants (Nobel Biocare, MKIII implants) 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. Each visit, the patient was asked for eventual changes in medication and general health. Intake of antibiotics would exclude 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 with 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 with a long-cone, parallel technique (including film holders) at loading and 1 year after abutment connection. Marginal bone level was measured mesial and distal at a 7x 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 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 (4 sites pooled), always after removal of the supragingival plaque and isolation of the area. Per site, 8 paper points (Roeko[®], Roeko, Langenau, Germany)/pocket were inserted for 20 seconds and dispersed in 2ml of Reduced Transport Fluid (RTF) (Syed & Loesche 1972). Each sample was homogenized by vortexing for 30 sec and processed within 12 hours (for details see Quirynen et al. 1999).

Tongue & saliva: These niches were sampled at 5 moments: 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 un-stimulated saliva was collected from which 200 μ l was dispersed in 1800 μ l RTF.

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

Microbiological processing.

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

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. The microbial changes in the oral cavity were recorded during the entire period of treatment and up to 1 year after abutment connection. Patient

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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 keypathogens are presented separately. For the comparison between pre-extraction and later moments, a linear mixed model was fit with time as 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 was calculated to assess the influence of the number of teeth or implants on the CFU (aerobe and anaerobe) in the saliva and the tongue.

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RESULTS

Subjects:

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

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Implants:

No implant was lost during follow up. Mean PPD was 2.7 mm (range: 1 – 4 mm), with a BOP of 15%. Plaque was recorded in 22% of the implant sites (6/implant) and pus was never diagnosed. Mean bone loss after loading was 0.6 mm (range 0 – 2.8 mm, SD= 0.5 mm).

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Culture data (Figure 1):

Teeth & implants: When the subgingival flora around the teeth was compared to the later implants (Figure 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 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 (Figure 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 (Figure 1c), a reduction that again remained up to 1 year after abutment placement.

Correlation between number of teeth or implant versus microbiota in 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 ($r=-0.87$, $p=0.001$) was found for the aerobes in the tongue at three months.

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qPCR (Figure 2, Table 3):

Teeth & implants: Before tooth extraction, the deep periodontal pockets were heavily colonized with key-pathogens. All pockets were positive for *P. gingivalis*, *T. forsythia*, and *P. intermedia*, and 7/10 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 (Figure 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* that did not show a significant change.

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Saliva: The detection frequencies of key-pathogens at this niche did not change significantly (Table 3), neither after tooth extraction, nor after implant insertion. For some species (except for *A. actinomycetemcomitans*), however, a clear reduction in total amount (Figure 2b) could be recorded (± 3

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1 log₁₀ for *P. gingivalis* and *T. forsythia*, and for ± 1.5 log₁₀ *P. intermedia*). These reductions remained over
2 time.

3
4 **Tongue:** The detection frequencies of key-pathogens at these niche did not change significantly (Table 3),
5 neither after tooth extraction, nor after implant insertion. For some species (not *A. actinomycetemcomitans*
6 or *P. intermedia*), however, a clear reduction in total amount (Figure 2c) could be recorded (± 2 log₁₀ for *P.*
7 *gingivalis* and ± 1.5 log₁₀ for *T. forsythia*). The later reductions remained constant over time.

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9 **Checkerboard: (Figure 3, Table 4):**

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

15
16 **Saliva & tongue:** The changes over time within the saliva and on the tongue are small, both in amount as
17 well as in detection frequencies. For some species of the orange and red complex, small reductions could
18 be observed, especially in the saliva. A few statistical significant reductions were only observed after
19 extraction (Figure 3b).

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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 were provided (Danser et al. 1995, 1997, Könönen 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 et al. 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 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. $\geq 10^6$) was used. While culture techniques may be considered the gold standard by some, many reports suggest that the newer microbiological techniques may improve the predictability and accuracy of 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, Fürst et al. 2007, Salvi et al. 2008) and is now also confirmed for full edentulous patients. The initial colonization of peri-implant pockets in full edentulous, without teeth as bacterial reservoir, has previously not been examined extensively. Nakou and co-workers (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 and co-workers (1997) examined dental implants in 20 edentulous patients and with a history of periodontitis. They were not able to identify neither *A. actinomycetemcomitans* nor *P. gingivalis* after one 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 co-workers (2006) took samples in 15 full edentulous patients, before and 4 and 6 months after implant insertion, and analyzed 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 put another light on oral microbiology. Questions arise like: “Is it possible to eradicate 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?”. Since 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 depend on the size of the pocket.

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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 out flow of 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.

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The question arise 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 can cope with low numbers of bacteria, but it is currently not defined whether the number of bacterial define their virulence, or whether it is the (in)ability of the host to defend. Indeed, Haffajee and co-workers (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 split the data into subgroups with different initial bacterial loads, or subgroups with different host susceptibilities.

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It is obvious that the initially pristine peri-implant pockets might slowly become colonized by bacteria related to periodontitis, in a way 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 long presence of the implants in the oral cavity (>5 years in his study). When the subgingival samples are considered (teeth vs implants) again only negligible changes in detection frequencies were seen, but clear changes in 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.

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The fact that the *A. actinomycetemcomitans* concentration did not change in the subgingival plaque after edentulation could be seen as a surprise. 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 than 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 to the levels in the inflamed periodontal pockets around the extracted teeth.

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1 A second remarkable observation was that the concentration of *A. actinomycetemcomitans* on the
 2 tongue and in the saliva did not change after edentulation, whereas the levels of *P. gingivalis* and *T.*
 3 *forsythia* dropped dramatically. A similar lack of decrease was observed for *P. intermedia* levels on the
 4 tongue. Similarly, the salivary levels of *P. intermedia* decreased to a much lesser extent than the levels of
 5 *P. gingivalis* and *T. forsythia* after edentulation. These opposite effects of edentulation on *A.*
 6 *actinomycetemcomitans* and *P. intermedia* versus the effects on *P. gingivalis* and *T. forsythia* can be
 7 explained by the tissue tropism of these different species. Already in 1999, Socransky and coworkers
 8 reported that the most dominantly colonized niche in the oral cavity for *P. gingivalis* is the subgingival
 9 pocket whereas for *A. actinomycetemcomitans*, the soft tissues are more dominantly colonized. Similar
 10 observations can be derived from Mager and coworkers (2003) who showed, next to confirming the
 11 aforementioned tissue tropisms for *A. actinomycetemcomitans* and *P. gingivalis*, that the subgingival
 12 plaque is the preferred niche for *T. forsythia* and the soft tissues for *P. intermedia*. Based upon these
 13 proportional observations, both studies seem to indicate that *P. gingivalis* and *T. forsythia* are more
 14 dentotropic bacteria whereas *A. actinomycetemcomitans* and *P. intermedia* are more epitheliotropic. With
 15 this in mind it then becomes obvious that the effect of removing teeth will have a more pronounced effect
 16 on salivary and tongue levels of the dentotropic species, than on the salivary and tongue levels of
 17 epitheliotropic species. The latter was confirmed by this study and gives at the moment a reasonable
 18 explanation for the observations. Due to differences in sampling technology (paper point versus cotton
 19 swab versus pure saliva), a direct comparison between different niches could not be made. We therefore
 20 advise to concentrate on changes within the same niche, or to concentrate on relative
 21 changes/proportional changes over the niches. **In conclusion.** The data of the present investigation
 22 suggests that periodontal pathogens may persist for a long period of time in the oral cavity of edentulous
 23 subjects with a history of periodontitis, even in the absence of other hard subgingival surfaces in the
 24 mouth.

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Legends:**Figure 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 (prior to edentulation (teeth)), and around the implant abutments (abutment connection made after an edentulous interval of ≥ 9 months), after 1 week (1w ab), 3 months (3m ab), and 12 months (12m ab) of connection with the oral environment.

b: Samples from the saliva prior to edentulation (teeth), at implant insertion (after 6 months of edentulism (impl ins)), and 1 week (1w ab), 3 months (3m ab), and 12months (12m ab) after abutment connection (placed ≥ 9 months after edentulation).

c: Samples from the tongue dorsum prior to edentulation (teeth), at implant insertion (after 6 months of edentulism (impl ins)), and 1 week (1w ab), 3 months (3m ab), and 12months (12m ab) after abutment connection (placed ≥ 9 months after edentulation).

Figure 2:

Changes in qPCR values (expressed in \log_{10} values) over time, for 4 key-pathogens (*A.*

actinomycetemscomitans, *P. gingivalis*, *P. intermedia* and *T. forsythia*), illustrated via Whiskers boxplots.

a: Samples from the subgingival area around teeth (prior to edentulation (teeth)), and around the implant abutments (abutment connection made after an edentulous interval of ≥ 9 months), after 1 week (1w ab), 3 months (3m ab), and 12 months (12m ab) of connection with the oral environment.

b: Samples from the saliva prior to edentulation (teeth), at implant insertion (after 6 months of edentulism (impl ins)), and 1 week (1w ab), 3 months (3m ab), and 12months (12m ab) after abutment connection (placed ≥ 9 months after edentulation).

c: Samples from the tongue dorsum prior to edentulation (teeth), at implant insertion (after 6 months of edentulism (impl ins)), and 1 week (1w ab), 3 months (3m ab), and 12months (12m ab) after abutment connection (placed ≥ 9 months after edentulation).

Figure 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 (prior to edentulation (teeth)), and around the implant abutments (abutment connection made after an edentulous interval of ≥ 9 months), after 1 week (1w ab), 3 months (3m ab), and 12 months (12m ab) of connection with the oral environment

Asterisks in pink means statistical difference for "teeth moment" vs 1 wk

Asterisks in green means statistical difference for "teeth moment" vs 12m ab

b: Samples from the saliva and the tongue prior to edentulation (teeth), and 1 week (1w ab), and 12 months (12m ab) after abutment connection (placed ≥ 9 months after edentulation).

Asterisks in pink indicates a statistical difference for "teeth-moment" vs 1 wk in saliva

Square in pink indicates a statistical difference for "teeth-moment" vs 1 wk at the tongue

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Nele Van Assche

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Background: Previous studies showed that periopathogens disappear after full-mouth tooth extraction. With a more sensitive technique (qPCR) it became however obvious that most of them remain, but at very low levels. This study aimed to monitor the microbiological changes from tooth extraction, over 9 months of full edentulism, up to 12 months after reconstruction with implants.

Material and methods: Ten patients with severe periodontitis, for whom a complete edentulation was the only treatment option and with the desire of a rehabilitation via oral implants, were recruited. Six months after tooth extraction implants were inserted, and 3 to 6 months later they were connected to abutments. Plaque samples were collected from the tongue dorsum, the saliva and the subgingival area (initially teeth, afterwards the implants), before tooth extraction, at implant insertion (6 months edentulous), at abutment connection (9-12 months of edentulous), and after 1 week, and 3 and 12 months exposure to the oral environment, respectively. The samples were analysed via: culture techniques, qPCR, and checkerboard technology.

Results: Complete edentulation resulted in a reduction in the total amount of aerobic ($\pm 0.5 \log_{10}$ in saliva, $\pm 0.4 \log_{10}$ on tongue) and anaerobic ($\leq 0.4 \log_{10}$ in saliva, $\pm 0.8 \log_{10}$ on tongue) CFU/ml in the oral cavity. The concentration of *Porphyromonas gingivalis* and *Tannerella forsythia* (qPCR and checkerboard) in the saliva, and to a lower extent on the dorsum of the tongue also reduced significantly. The detection frequencies remained however very high over time for all key-pathogens. For *Prevotella intermedia* and the changes were negligible and no changes could be detected for *Aggregatibacter actinomycetemcomitans*. The pristine subgingival niches (implants) by itself were quickly colonized by the key-periopathogens, although their final concentration remained low. The creation of these new pockets (implants) did not result in an outbreak of the pathogens in the other niches.

Conclusion: In contrast to what has been believed so far, complete edentulation did not result in full eradication of all periopathogens, but only in a significant reduction (with an exception of *Aggregatibacter actinomycetemcomitans*). The pristine subgingival niches along the implants were colonized quickly. These observations might indicate that the periodontal pocket is an important source for the periopathogens on the tongue and in the saliva, but also that these species can survive in the oral cavity without pockets.

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Nele Van Assche

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Culture. The samples were cultured under aerobic (3 days) and anaerobic conditions on selective and non-selective agar plates in order to quantify the colony forming units (CFU/ml) under aerobic and anaerobic conditions. Details concerning the protocol have been summarized previously (Quirynen et al. 1999).

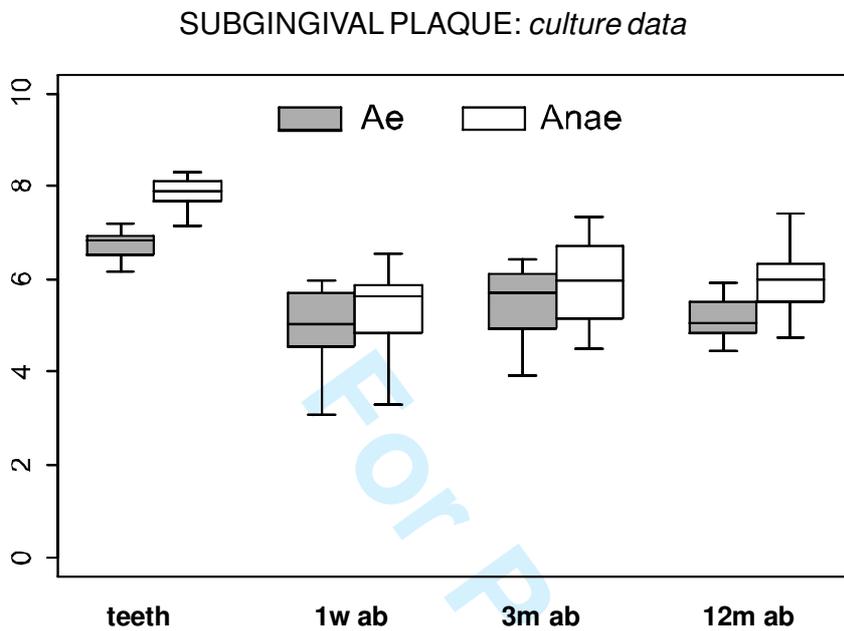
qPCR. Samples for qPCR were frozen at -80°C until the DNA was extracted with InstaGene matrix (Bio-Rad Life Science Research, Hercules, CA, USA) according to the instructions of the manufacturer. $5\mu\text{l}$ of the purified DNA was used for the quantification of *Tannerella forsythia* (*T.f.*) (Shelburne et al. 2000), *P. gingivalis* (*P.g.*) (Boutaga et al. 2003), *A. actinomycetemcomitans* (*A.a.*) and *P. intermedia* (*P.i.*) (Boutaga et al. 2005) by qPCR as described. As a standard for the

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4 qPCR, a fragment of the 16S rRNA gene of *T. forsythia* ATCC 43037, *P. gingivalis* ATCC 33277,
5 *A. actinomycetemcomitans* ATCC 43718 and *P. intermedia* ATCC 25611 was amplified with
6 primers flanking the annealing site of the qPCR primers. This fragment was ligated into the
7 pGEM-T easy vector system (Promega, Madison, WI, USA) and used to transform *E. coli* DH5 α .
8 Plasmids were isolated from the clones with the High Pure Plasmid Isolation Kit (Roche
9 Diagnostics GmbH, Mannheim, Germany). The concentration of the plasmid was determined
10 with the GeneQuant RNA/DNA calculator (Amersham Pharmacia Biotech) at a wavelength of 260
11 nm. A 10-fold dilution series of the plasmid was used in each qPCR run to construct the standard
12 curve. Primers, probes and qPCR mastermix were synthesized by Eurogentec (Seraing,
13 Belgium). qPCR was performed on the ABI 7700 Sequence Detection System platform (Applied
14 Biosystems, Foster City, CA, USA). Data was collected during each annealing phase. In each
15 run no template controls were included. Results were expressed in log₁₀ Genome Equivalents
16 (Geq)/ ml or number of bacterial genome/ml.
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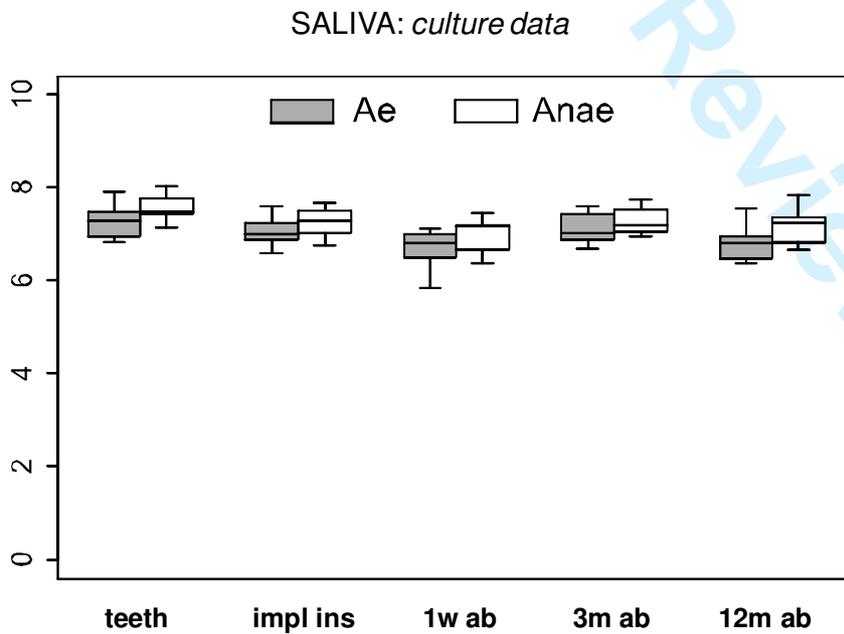
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21 **Checkerboard:** Briefly, the samples were lysed and the DNA was placed in lanes on a nylon
22 membrane using a Minislot device (Immunelect, Cambridge, MA). After fixation of the DNA to the
23 membrane, the membrane was placed in a Miniblotter 45 (Immunelect) with the lanes of DNA at
24 90° to the lanes of the device. Digoxigenin-labelled whole genomic DNA probes to 38 bacterial
25 species were hybridized in individual lanes of the Miniblotter. After hybridization, the DNA probes
26 presented the target DNA using chemifluorescence detection. A computer-linked instrument read
27 the intensity of the fluorescent signals resulting from the probe-target hybridization. Two lanes in
28 each run contained standards at concentrations of 10⁵ and 10⁶ cells of each species. The
29 sensitivity of the assay was adjusted to permit the detection of 10⁴ cells of a given species by
30 adjusting the concentration of each DNA probe. Signals were converted to absolute counts by
31 comparison with the standards on the same membrane. Failure to detect a signal was recorded
32 as zero. All samples were analyzed on the same checker board, in order to optimize their
33 reading.
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Figure 1:

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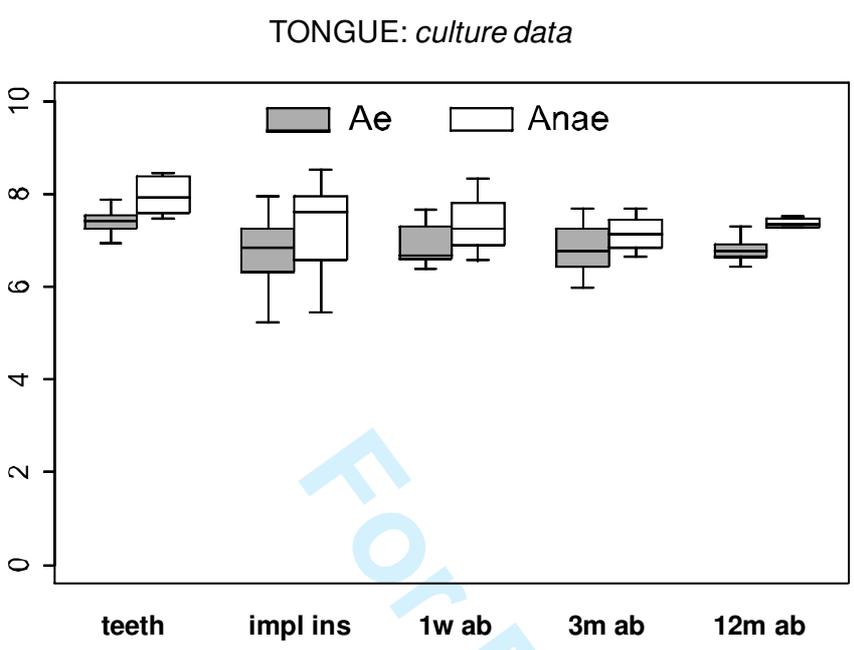
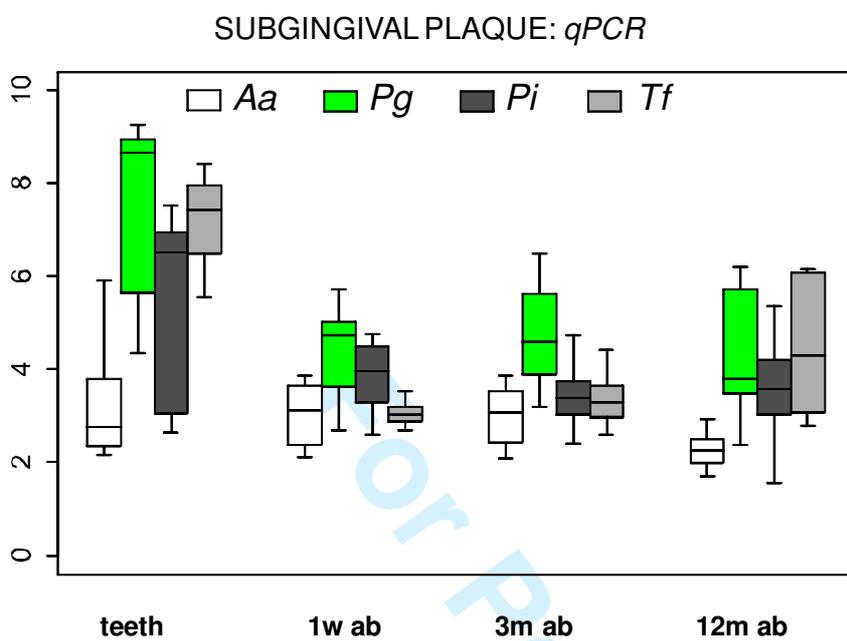
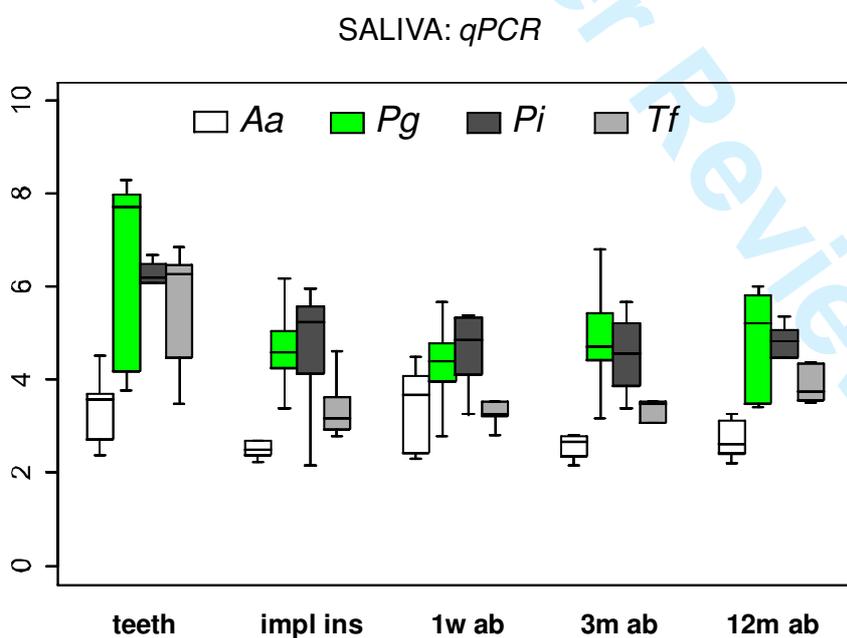


Figure 2:

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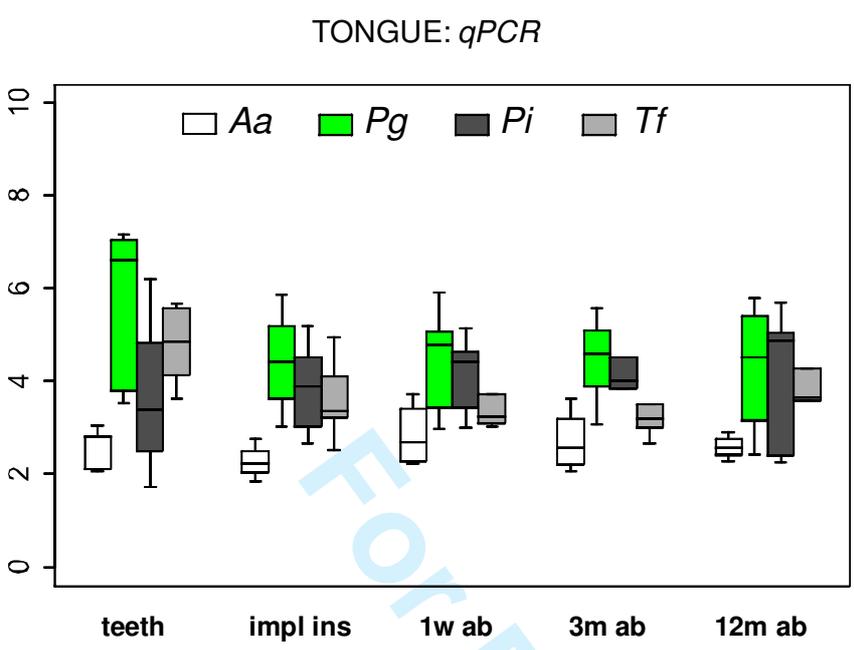
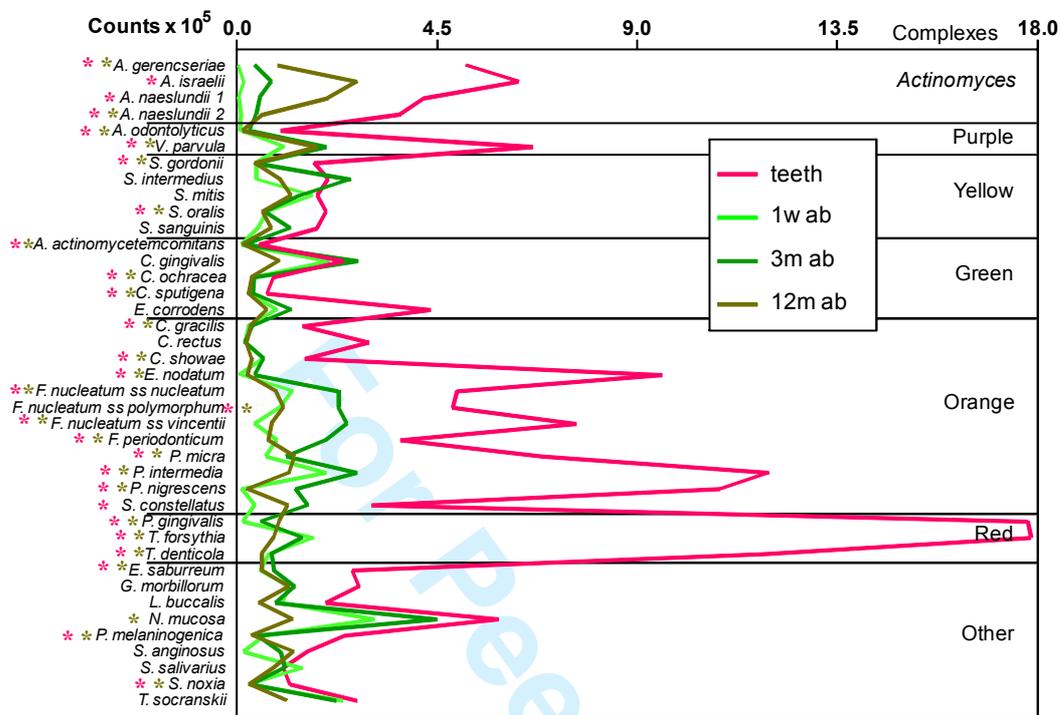


Figure 3:

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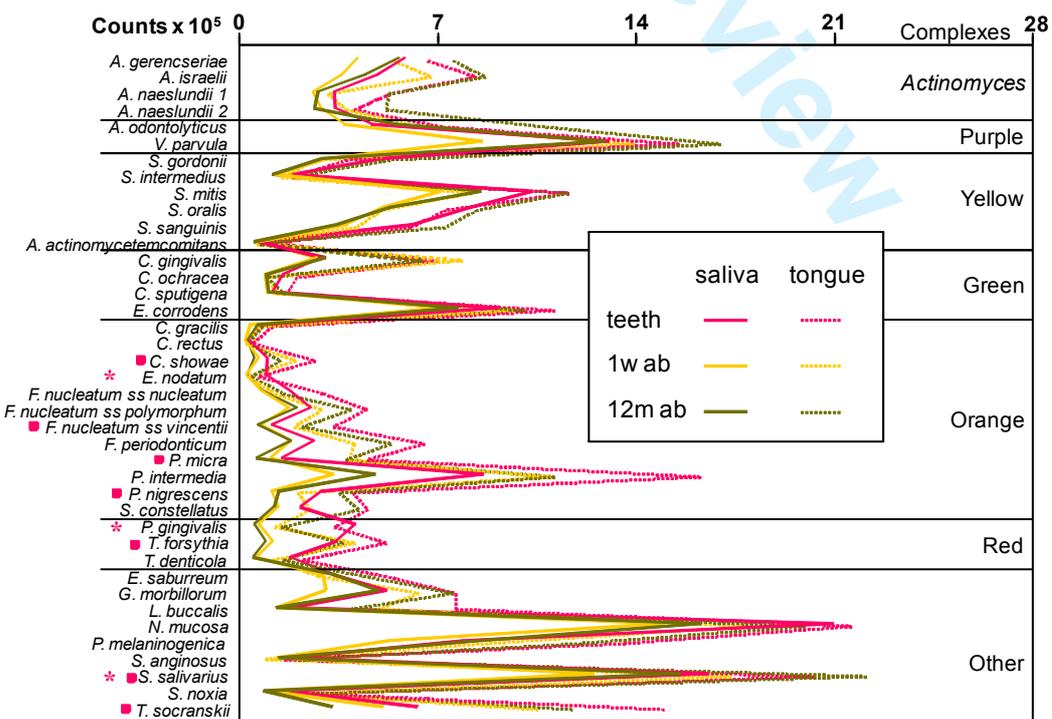


Table 1: Subject description:

S	smoker	medication	oral hygiene	nT UJ & LJ	nT PPD>4mm UJ & LJ	nT BL>50% UJ&LJ	BOP %	bone defect	nl 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

Legend: S= subject, medication= 1:painkiller for slipped disc, 2: asaflow 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

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

S	nT	CFU Sal Aer pre	CFU Sal Anaer pre	CFU Ton Aer pre	CFU Ton Anaer	nl	CFU Sal Aer 12m	CFU Sal Anaer 12m	CFU Ton Aer 12m	CFU Ton Anaer
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

Legend: S= subject, T= teeth, CFU= colony forming units (log10), Sal= saliva, Ton= tongue, Aer= aerobe, Anaer: anaerobe, pre= pre extraction, r= correlation

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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.

Number of positive sites/ 10 sites.

		teeth	impl ins	1w ab	3m ab	12m ab
A.a.	<i>SUB</i>	7	-	8	6	5
	<i>SAL</i>	6	7	6	4	5
	<i>TON</i>	6	4	5	4	4
P.g.	<i>SUB</i>	10	-	10	10	10
	<i>SAL</i>	10	10	10	8	7
	<i>TON</i>	9	10	10	10	7
P.i.	<i>SUB</i>	10	-	10	8	7
	<i>SAL</i>	7	7	7	4	5
	<i>TON</i>	8	9	8	5	6
T.f.	<i>SUB</i>	10	-	7	6	5
	<i>SAL</i>	10	8	6	3	6
	<i>TON</i>	8	6	7	6	3

-: no samples taken because no teeth/implants present at this appointment

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.

Number of positive sites/ 10 sites.

		teeth	impl ins	1w ab	3m ab	12m ab
<i>A.a.</i>	<i>SUB</i>	10	no	9	8	9
	<i>SAL</i>	10	10	10	10	10
	<i>TON</i>	10	8	9	10	10
<i>P.g.</i>	<i>SUB</i>	10	no	6	8	7
	<i>SAL</i>	10	9	7	10	9
	<i>TON</i>	10	8	8	10	10
<i>P.i.</i>	<i>SUB</i>	10	no	10	10	10
	<i>SAL</i>	10	10	10	9	10
	<i>TON</i>	10	10	10	10	10
<i>T.f.</i>	<i>SUB</i>	10	no	10	10	10
	<i>SAL</i>	10	10	9	10	10
	<i>TON</i>	10	10	9	10	10

-: no samples taken because no teeth/implants present at this appointment