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Do periodontopathogens disappear after full-mouth tooth extraction?

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Abstract

Aim: To monitor the intra-oral microbiological changes after full-mouth extraction using quantitative polymerase chain reaction (qPCR).

Material and Methods: Nine patients with severe, aggressive periodontitis, for whom a full-mouth tooth extraction was the only remaining treatment option were recruited. Before and 6 months after extraction, microbial samples were obtained (tongue, saliva and subgingival plaque) and analysed by qPCR.

Results: The elimination of subgingival niches, by extraction of all natural teeth, resulted in a 3-log reduction of *Porphyromonas gingivalis* and *Tannerella forsythia*, and more modest reductions of *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia*. However, the detection frequencies of these periodontopathogens in saliva and on the tongue remained unchanged after full-mouth tooth extraction.

Conclusion: In contrast to what has been believed so far, full-mouth tooth extraction does not result in eradication of all periodontopathogens but only in a significant reduction. The clinical consequences of this observation remain speculative.

Key words: implant; periodontitis; periodontopathogen; plaque; tooth extraction

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A plethora of studies have shown that periodontopathogens can be transmitted from teeth to implants (Quirynen et al. 2006). In partially edentulous patients, periodontal pockets serve as reservoirs for bacterial colonization of ''pristine'' implant pockets. 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).

The importance of periodontal therapy before implant placement in partially edentulous patients has been emphasized (Bragger et al. 1997). According to this concept, the colonization of the newly formed peri-implant ecological niches by periodontal pathogens could be avoided. The presence of periodontopathogens at peri-implant, as well as at periodontal sites, is associated with future attachment loss, especially in patients with an inefficient host response (Rams et al. 1991). Additionally, the microbiota of peri-implantitis lesions resembles that of chronic periodontitis lesions (Heydenrijk et al. 2002, Quirynen et al. 2002).

It has been suggested (Danser et al. 1994, 1997) that elimination of the subgingival environment by extraction of all natural 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. The aim of the current study was to verify this statement by applying for the first time the polymerase chain reaction (PCR) technology, a technique with, in general, a lower threshold for the detection of specific periopathogens (Riggio et al. 1996, Boutaga et al. 2006). If periodontopathogens remained after full-mouth tooth extraction, special care might be indicated before implant placement.

Conflicts of interest and source of funding statement

There is no conflict of interests.
These data are part of a funded study (NobelBiocare AB, Gothenburg, Sweden)

Material and Methods

Patients

Nine patients with severe, aggressive periodontitis, for whom a full-mouth tooth extraction was the only remaining treatment option (uncontrollable recur-

rent abscesses, terminal periodontal destruction, high degree of tooth mobility, cold–warm sensation, unfavourable as abutment teeth for prosthetic reconstruction, etc.), were enrolled in this study. The protocol was approved by the Ethical Committee of the Catholic University Leuven and written informed consents were obtained from all participants. Patients with a medical history of radiotherapy, chemotherapy, diabetes and/or who had taken antibiotics 3 months before the start of the study were excluded.

Plaque samples

Before and 6 months after full-mouth tooth extraction samples were obtained, approximately 5 ml of unstimulated saliva was collected. Two-hundred microlitres of the sample collected was taken and dispersed in 1800 μl of reduced transport fluid (RTF).

Next, a cotton swab was used to collect the biofilm of the tongue. The swab was wiped 10 times over the tongue starting from the dorsum. For the subgingival plaque, two pockets were selected, and sampled by eight paperpoints (Roeko®, Roeko, Langenau, Germany/pocket) (for details see Quirynen et al. 1999). All samples were dispersed in 2000 μl of RTF (Syed & Loesche 1972), homogenized by vortexing for 30 s, transferred to the microbiology laboratory and processed within 12 h.

Microbiological processing

Quantitative PCR (qPCR)

Samples for qPCR were immediately frozen at -80°C. Four hundred microlitres of each sample was centrifuged. A remaining volume of 50 μl with the pellet was dispersed in 200 μl instagene. DNA was extracted with InstaGene matrix (Bio-Rad Life Science Research, Hercules, CA, USA) according to the instructions of the manufacturer. Five microlitres of the purified DNA was used for the quantification of *Tannerella forsythia* (Shelburne et al. 2000), *P. gingivalis* (Boutaga et al. 2003), *A. actinomycetemcomitans* and *Prevotella intermedia* by qPCR as described previously (Boutaga et al. 2005). As a standard for the qPCR, a fragment of the 16S rRNA gene of *T. forsythia* ATCC 43037, *P. gingivalis* ATCC 33277, *A. actinomycetemcomitans* ATCC 43718 and *P. intermedia* ATCC 25611 was amplified with primers flanking the annealing site of the qPCR primers. This fragment was ligated into the pGEM-T easy vector system (Promega, Madison, WI, USA) and used to transform *Escherichia coli* DH5α. Plasmids were isolated from the clones with the High Pure Plasmid Isolation Kit (Roche Diagnostics GmbH, Mannheim, Germany). The concentration of the plasmid was determined using a GeneQuant RNA/DNA calculator (Amersham Pharmacia Biotech, Roosendaal, the Netherland) at a wavelength of 260 nm. A 10-fold dilution series of the plasmid was used in each qPCR run to construct the standard curve. Primers, probes and qPCR mastermix were synthesized by Eurogentec (Seraing, Belgium). qPCR was performed on the ABI 7700 Sequence Detection System platform (Applied Biosystems, Foster City, CA, USA). Data were collected during each annealing phase. In each run, no template controls were included. Results were expressed as log 10 genome equivalents (gEq)/ml or as number of bacterial genome/ml.

All microbiological evaluations were performed blind.

Statistics

Only descriptive statistics are used in this paper.

All data are expressed in log 10. The mean and the standard deviation were calculated using the log 10 data.

Results

Before tooth extraction, the periodontal pockets were heavily colonized with periodontopathogens (Fig. 1). All patients were found to harbour *P. gingivalis* and *T. forsythia* subgingivally, and eight patients harboured *P. intermedia* and/or *A. actinomycetemcomitans*. Pre-extraction, alomg with the saliva and the tongue showed large amounts of these species when qPCR was used. In comparison with the subgingival samples, similar detection frequencies were observed in these niches, but the levels of these periodontopathogens were slightly lower, especially for *P. gingivalis* and *A. actinomycetemcomitans*.

After full-mouth tooth extraction (Figs 1 and 2), most patients remained positive for the four key periodontopathogens (before and after extraction, respectively: 9/9 for *P. gingivalis*, 8/7 for *P. intermedia*, 9/9 for *A. actinomycetemcomitans* and 9/9 *for T. forsythia*), but the number of species was clearly lower, especially for *P. gingivalis* (3 log) and *T. forsythia* (3 log). This was the case for samples from the saliva as well as for samples taken from the tongue.

Discussion

Because of the similarity between the microbiota causing periodontitis and microbiota found in in peri-implantitis, periodontopathogens are considered to cause peri-implant infections (Mombelli et al. 1987, Mombelli & Lang 1992). Therefore, a reduction or elimination of periodontopathogens before implant installation (or abutment connection) is thought to be helpful in preventing peri-implantitis.

Based on microbial culturing techniques, several studies showed that an elimination of subgingival niches by extraction of all teeth resulted in the disappearance of the two key periodontopathogens *A. actinomycetemcomitans* and *P. gingivalis* (Mombelli et al. 1988, Danser et al. 1994, 1997). Similar observations were made after extraction of a partially erupted third molar (Rajasuo et al. 1993). Therefore, many clinicians decided to extract all (mostly hopeless) teeth before implant insertion, in order to reduce the chance for peri-implantitis.

In contrast to previous studies, the current study, using qPCR techniques, revealed that periodontopathogens do not disappear from the oral cavity after full-mouth extraction. They remain present within the saliva and on the tongue dorsum albeit in lower concentrations. The reductions were modest for *P. intermedia* and *A. actinomycetemcomitans* and more pronounced for *P. gingivalis* and *T. forsythia*. These data are in line with Devides & Franco (2006), who analysed the microbiota in completely edentulous patients, before and after implant insertion using PCR. Of the 15 completely edentulous patients included in their study, seven patients were positive for *P. intermedia*, two for *A. actinomycetemcomitans* and none for *P. gingivalis*. The lower detection frequencies in this study, compared with our study, can be explained by the longer period of edentulism.

The differences between our observations and those of studies using microbial culturing (Mombelli et al. 1988, Danser et al. 1994, 1997) can be explai-

PCR																					
		pre extraction											6 months post extraction									
	sample	Geq		patient number									Geq		patient number							
		mean	*SD*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*mean*	*SD*	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*
P.g.	***Saliva***	8.31	2.03	8.69	9.15	9.89	10.17	5.28	9.44	5.82	10.37	6.00	5.48	2.21	7.31	5.59	0.00	6.49	4.80	5.43	7.13	6.04	**6.55**
	Tongue	7.49	1.73	7.69	6.73	9.10	9.34	5.32	8.84	5.54	9.27	5.54	5.04	1.97	5.75	0.00	5.92	5.61	5.11	4.82	5.32	6.53	**6.28**
	Subgingival	9.17	2.17	11.10	8.71	10.88	10.77	7.38	10.57	5.71	11.03	6.41											
P.i.	***Saliva***	4.98	4.10	0.00	0.00	9.39	3.71	6.60	9.31	0.00	8.42	7.39	3.91	3.37	0.00	0.00	7.84	0.86	**7.48**	7.99	**5.10**	1.76	4.11
	Tongue	3.24	3.32	0.00	0.00	8.94	2.41	4.49	6.87	0.00	5.32	1.15	3.83	2.84	**3.97**	0.00	7.32	0.36	**8.20**	5.78	**3.04**	3.31	**2.51**
	Subgingival	6.36	3.05	0.00	4.29	9.59	5.54	6.65	9.48	5.35	9.04	7.27											
A.a.	***Saliva***	4.38	2.01	3.68	0.00	4.12	4.45	7.15	6.19	3.85	4.52	5.49	3.75	1.24	**3.88**	**3.44**	3.23	1.69	5.33	4.56	**4.54**	**4.91**	2.18
	Tongue	3.36	1.62	3.28	3.49	0.00	3.67	5.87	4.43	3.06	4.05	4.86	2.86	1.39	2.99	2.73	**3.25**	0.00	3.44	3.80	**3.69**	**4.54**	1.29
	Subgingival	4.15	2.29	0.00	4.14	3.02	3.94	8.62	4.44	3.34	4.06	5.74											
T.f.	***Saliva***	7.79	1.70	7.43	8.88	8.51	9.37	4.41	9.15	5.91	7.31	9.12	4.43	1.30	4.76	4.02	4.17	5.68	2.09	6.09	4.23	3.13	5.72
	Tongue	6.50	1.90	6.56	6.31	7.66	8.45	2.09	8.01	5.58	6.31	7.50	3.27	1.97	4.39	0.00	4.89	3.16	**4.75**	5.06	0.00	3.21	3.98
	Subgingival	7.98	3.12	10.18	9.64	10.56	10.28	8.45	1.04	6.90	5.36	9.40											

0 - <1 log10
>1 - <2 log10
>2 - <3 log10
>3 - <4 log10
≥4 log10
bold = increase

Fig. 1. Polymerase chain reaction (PCR) data for samples before and after tooth extraction. The colours in the right column show the change between the pre- and post-extraction values. *A.a*, *Aggregatibacter actinomycetemcomitans*; *P.g.*, *Porphyromonas gingivalis*; *P.i.*, *Prevotella intermedia*; *T.f.*, *Tannerella forsythia*; gEq, genome equivalents; SD, standard deviation.

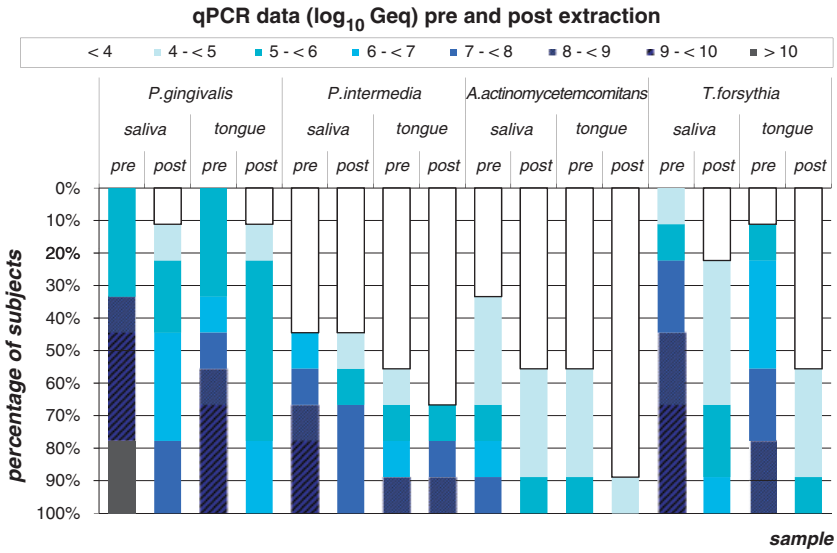

Fig. 2. Percentage of subjects positive for *Porphyromonas gingivalis*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans* and *Tannerella forsythia* pre- and post-extraction in saliva and tongue samples evaluated using quantitative polymerase chain reaction (qPCR).

ned by several factors. The discrepancy between PCR-based and culture-based studies could be explained by the lower detection limit of PCR. For PCR, the detection limit is typically 25–100 cells, whereas, for culturing 10^3–10^4 bacteria are needed before detection. The sensitivity of bacterial culturing is rather low, especially for non-selective media, and therefore, low numbers of a specific pathogen in a subgingival sample will remain undetected. Another potential source of error in the culturing procedure for anaerobic bacteria resides in the processing of samples, which is often carried out under aerobic conditions (Riggio et al. 1996). Additionally, PCR will detect not only viable but also moribund and dead cells (Sanz et al. 2004). All these factors explain why in our study qPCR detected 100 × more *P. gingivalis* and *P. intermedia* species than microbial culturing, even though the same samples had been used. The same observation of a lower prevalence by culture technique was found when analysing *Enterococcus faecalis* in endodontic lesions (Sedgley et al. 2006) and oral rinse samples (Sedgley et al. 2005). *E. faecalis* was detected in 10.2% *versus* 79.5% of endodontic samples and 7% *versus* 17% in oral rinse samples for culture *versus* qPCR techniques, respectively.

A study by Sakamoto & coworkers (2001) examined the concentrations of four periodontopathogens in saliva and subgingival plaque of five patients; three patients suffered from rapidly progressive periodontitis, and two patients from adult periodontitis. The concentrations of periodontopathogens in the saliva were lower compared with our results (e.g. for *P. gingivalis* 2.0×10^4–3.0×10^7 cells/ml *versus* 1.9×10^5–2.3×10^{10} gEq). The reason for the higher counts might be that in the present study a plasmid standard was used to quantify the number of bacteria in a sample, and not a bacterial culture-based standard. Therefore, the data were expressed as gEq (number of bacterial genomes detected) and not as colony forming unit (number of bacteria

that will form a colony on an agar plate). This can additionally explain the relative higher quantitative numbers detected in this study, when compared with the relative numbers in studies that use culture-based quantification systems.

The question whether these residual low concentrations of periodontopathogens after full-mouth extraction are of clinical importance remains unanswered. It seems that the human host can cope with low numbers of periodontopathogens. Indeed, Haffajee et al. (1998) showed high detection frequencies but low detection numbers for most periodontopathogens in patients with a healthy periodontium. Similar observations have been made for completely edentulous patients who had been rehabilitated with an overdenture on two implants (Quirynen et al. 2005). Even though these implants were clinically healthy, high detection frequencies for most periodontopathogens were observed.

Cortelli & coworkers (2008) detected periodontopathogens in edentulous patients (edentulous for >12 months, <36 months) using a checkerboard technique. Only prevalence data (e.g. *P. gingivalis* was recovered from tongue and cheek in 3.2%) were published. Because of the absence of quantitative information, it was not possible to compare with our results. Another study (Sachdeo et al. 2008), also using the checkerboard, looking for 41 different species in edentulous (at least 1 year) patients, detected *A. actinomycetemcomitans* and *P. gingivalis* in the saliva around 10^5 counts.

Whether these low numbers of remaining periodontopathogens after full-mouth tooth extraction will increase again when new suitable environmental conditions are re-created (such as pockets after implant insertion) is still a matter of debate (Devides & Franco 2006). It remains questionable whether low concentrations of periodontopathogens can jeopardize the future of oral implants. It is likely that the outcome, periodontitis/peri-implantitis, is because of not only the presence and/or levels of pathogens but also the ability of the immune system to cope with these species. The latter has of course to be confirmed by long-term clinical trials.

The data of the present investigation could re-open the debate concerning the use of antibiotics before implant placement. The success of implant therapy and the low incidence of peri-implantitis, however, does not seem to justify a general use of antibiotics before implant insertion. Additionally, it has not yet been proven that with antibiotics one can really eradicate the pathogens below PCR detection levels.

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Clinical Relevance

Clinical relevance: Previous study showed that periodontopathogens disappear after tooth extraction. This conclusion was based on culture technique. For the first time, a more precise technique (PCR) was used to verify whether periodontopathogens could be detected, 6 months after tooth extraction.

Principle findings: Six months after full-mouth tooth extraction, periodontopathogens could be detected by PCR in a low concentration.

Practical implication: If periodontopathogens remained after full-mouth tooth extraction, special care might be indicated before implant placement.