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Microbiological shifts in intra- and extraoral habitats following mechanical periodontal therapy

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Abstract

Objectives: The aim of the present study was to analyze the intra- and extraoral colonization dynamics of periodontal pathogens following supra- and subgingival debridement.

Material and Methods: Thirty five patients with chronic periodontitis were enrolled in the study. Supra- and subgingival plaque samples, saliva, and swab samples from mucosa and extraoral sites were taken at baseline and 6 weeks, 3 months and 6 months after mechanical periodontal therapy. *Actinobacillus actinomycetemcomitans* (*Aa*), *Porphyromonas gingivalis* (*Pg*), *Eikenella corrodens* (*Ec*), *Tannerella forsythensis* (*Tf*), *Prevotella intermedia* (*Pi*), *Prevotella nigrescens* (*Pn*), and *Treponema denticola* (*Td*) were identified by PCR.

Results: Supra- and subgingival debridement decreased the number of subgingival sites infected with the analyzed pathogens only transiently, if at all. However, the detection frequencies of *Tf*, *Td*, *Ec*, *Pi*, and *Pn* in the supragingival region, of *Pg*, *Td*, and *Pn* at the oral mucosa sites (mostly the tongue), and of all pathogens except *Aa* in saliva increased over the 6-month observation period. *Td* was the only pathogen recorded in notable quantities in the extraoral habitat (external ear canal).

Conclusion: The results indicate that supra- and subgingival debridement results in a dissemination of periodontal pathogens within the oral cavity.

Key words: biofilm; dynamics; microbiology; periodontitis; prevalence; therapy

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A spectrum of mostly Gram-negative bacteria including *Actinobacillus actinomycetemcomitans* (*A. actinomycetemcomitans*), *Porphyromonas gingivalis* (*P. gingivalis*), *Eikenella corrodens* (*E. corrodens*), *Tannerella forsythensis* (*T. forsythensis*), *Prevotella intermedia* (*P. intermedia*), *Prevotella nigrescens* (*P. nigrescens*), and *Treponema denticola* (*T. denticola*) have been identified to be strongly associated with periodontal disease (Socransky & Haffajee 1992, Wolff et al. 1993, Ali et al. 1996, van Winkelhoff et al. 2002). The intraoral habitat of these pathogens is, however, not confined to the periodontal pocket but also extends to other intraoral sites, e.g. supragingival and mucosal sites like the tongue and buccal mucosa, saliva, and to oro-pharyngeal regions like the throat and tonsils (Quirynen et al. 2001). Moreover, some of these pathogens, i.e. *P. intermedia*, *P. nigrescens*, *E. corrodens*, and *P. gingivalis*, have been found in non-oral infections (Chen & Wilson 1992, Haubek et al. 1997, Matto et al. 1997, Pearce et al. 2000).

To date, there is limited information on the impact of mechanical periodontal therapy on the colonization of the above-mentioned major periodontal pathogens in the various intra- and extraoral habitats. The majority of recent studies have focused primarily on the composition of isolated intraoral niches, mainly the supra- or subgingival areas (Ximenez-Fyvie et al. 2000a, b, Socransky et al. 2002). The role of other niches in oral microbial ecology and the microbiological shifts in them, after therapy, are less clear but are of importance in identifying possible habitats that may serve as a source of subgingival reinfection following mechanical periodontal therapy.

Therefore, the present study was undertaken to analyze the intra- and extraoral prevalences of seven periodontal pathogens in periodontitis patients and to describe the colonization pattern following subgingival debridement.

Material and Methods

Study subjects

Thirty-five patients with untreated moderate to severe chronic periodontitis

were enrolled in the study. The patients were recruited from the Department of Periodontology, and signed the informed consent form approved by the Ethics Committee of the Medical Faculty, Julius Maximilian University, Würzburg.

Clinical examination and sampling schedule

Pocket probing depths (PPD) and bleeding on probing (BOP) were assessed at baseline in all patients at six sites per tooth with a periodontal probe (PCP 15, Stoma, Storz am Mark, Germany) at baseline and 6 weeks, 3 months and 6 months after completion of therapy. Supra- and subgingival debridement was performed under local anesthesia within 2 days and completeness of supra- and subgingival debridement, i.e. with all pathologically exposed subgingival root surfaces feeling hard and smooth, was determined by using a fine explorer. All patients received full-mouth supra- and subgingival debridement, and oral hygiene instruction was given after 3 and 6 months when necessary. The treatment and the clinical measurements were performed by a single trained dentist throughout the entire study. Before clinical assessment, samples for microbiological analysis were taken from all patients at the same appointment in the following order: mucosal swabs, unstimulated saliva, stimulated saliva, supragingival plaque, subgingival plaque, and samples from the extraoral sites.

Sampling procedures

At baseline the most severely affected site (MA site) per sextant was selected. Supra- and subgingival plaque samples from each of these six sites were obtained through the study with sterile curettes, and placed in 200 μl of sterile distilled water. In addition, supra- and subgingival plaque samples from six other periodontal pockets, each representing a randomly selected less affected (LA) site per sextant, were obtained with a sterile curette and pooled in 200 μl of sterile distilled water.

Samples from oral mucous membranes, i.e. dorsum of the tongue, tonsils, and left and right buccal mucosae, as well as from extraoral sites (nasal cavity, lower eyelid, and external ear canal) were collected with sterile cotton swabs and suspended individually in 1 ml sterile distilled water. The tongue was sampled by vigorous back and forth streaking with a sterile swab from the vallatae papillae to the tip of the tongue. Unstimulated whole saliva was collected from each individual by 5 min of expectorating. Stimulated saliva was achieved by paraffin chewing for 2 min. All samples were stored at -70°C until microbiological assessment.

Detection of periodontal pathogens

Total DNA was isolated with the Perfect gDNA Blood Mini Kit (Eppendorf, Hamburg, Germany) according to the manufacturer's instructions. Periodontal pathogen-specific primers amplifying a part of the *lktA* gene as described by Tonjum & Haas (1993) were used for the identification of *A. actinomycetemcomitans*. Collagenase gene *prtC* was used for the identification of *P. gingivalis*. A 548 bp fragment from the central portion of the *prtC* gene was amplified using primers coll-1 (5′-ACA ATC CAC GAC ACC ATC-3′) and coll-2 (5′-GAT TCC CTT GCC TAC ATA-3′) (Bodinka et al. 1994). *T. denticola*, *T. forsythensis*, *E. corrodens*, *P. intermedia* and *P. nigrescens* were identified with 16S rRNA-specific primers designed by Slots et al. (1995) and Ashimoto et al. (1996). The detection sensitivities of the pathogen-specific PCR reactions were 25–100 colony-forming units.

Three microliters of the isolated DNA were added to the PCR reaction samples containing 30 pmol of each specific forward and reverse primer; 200 μM of each of the 4 dNTPs, 2.5 μl of polymerase synthesis buffer (Eppendorf, Hamburg, Germany), 1.5 mM $MgCl_2$, and 2.0 U of Taq-DNA polymerase were added for a final volume of 25 μl. As positive controls, isolated DNA from *A. actinomycetemcomitans* ATCC 33384, *P. gingivalis* ATCC 53977, *E. corrodens* BCMG 00232, *T. forsythensis* ATCC 43037, *P. intermedia* ATCC 25611 and *P. nigrescens* ATCC 33563 were used. As negative control, sterile water was used. Negative and positive controls were tested with each PCR run. Each sample was amplified by 35 cycles of 30 s at 95°C, 30 s at the primer specific annealing temperature stated in the original papers (for *A. actinomycetemcomitans*: 65°C; *P. gingivalis*: 53°C; *P. intermedia*, and *P. nigrescens*: 55°C; *E. corrodens*, *T. denticola*, and *T. forsythensis*: 60°C) and 60 s at 72°C. Eighteen microliters of the PCR product were subjected to agarose gel electrophoresis on 1.8% agarose gels. The gels were stained with ethidium bromide (1 μg/ml) and assessed under UV light. Precautions as described by Kwok & Higuchi (1989) were taken to prevent contamination.

Statistics

For data analysis, the presence of a pathogen at each sampling time point in saliva was defined as positive if the pathogen was found either in stimulated or in unstimulated saliva (saliva = S). Supra- and subgingival habitats were considered to be positive for the tested pathogen, if the microorganism could be detected at least at one of the six supra- (supragingival = MSA) and subgingival MA sites (subgingival = MSB) or LA sites (supragingival = LSA, subgingival = LSB), respectively. Mucosal surfaces (mucosa = MU), e.g. tongue, tonsils, cheek, and throat, were classified as being positive, if the pathogen could be detected at least at one of the sampled mucosal surfaces. The craniofacial extraoral sites, e.g. external ear canal, upper eyelid, or nasal cavity, were defined as positive if the pathogen was detected in the assessed habitat. Differences in clinical parameters between baseline and 6-month follow-up were determined using the Wilcoxon signed ranks test. Changes in the prevalence of pathogens during the course of the study were analyzed by Cochran's Q-test. Statistical analysis was performed with SAS software (SAS Institute GmbH, Heidelberg, Germany). For data analysis an explorative approach was used; therefore, no adjustment for multiple comparisons was made. The level of significance was set at <0.05.

Results

Demographics and clinical findings

Thirty-five patients were enrolled in the study. Their demographics and periodontal status are summarized in Table 1. The percentages of sites with a PPD of 4–6 mm, of sites equal to or above 7 mm, and sites with BOP were significantly reduced 6 months after mechanical periodontal therapy.

Colonization dynamics after sub- and supragingival debridement

After therapy, the supra- and subgingival habitats of the MA sites as well as the tongue habitat showed a transient reduction of *P. gingivalis*-positive sites at 6 weeks with a rebound to baseline figures. At LA sites, however, the reduction in *P. gingivalis*-positive sites was maintained supra- and subgingivally throughout the 6-month observation period. Interestingly, the prevalence of *P. gingivalis* positive sites in saliva doubled after 6 months (28.6%) compared to baseline (14.3%). Extraorally, *P. gingivalis* could not be detected at any time point (Fig. 1).

Of all pathogens investigated, the number of *P. nigrescens* positive sites exhibited the strongest post-treatment increase at most of the analyzed habitats. The supra- and subgingival presence of *P. nigrescens* at MA sites rose significantly ($p<0.05$) from 25.7% and 11.4%, respectively, at baseline to 77.4% and 62.9%. In the supragingival region of LA sites, too, the detection frequency of *P. nigrescens* was found to exhibit a significant increase ($p<0.05$), though on a much lower level. On the mucosal surfaces, the prevalence of *P. nigrescens* increased only at the tongue, but there the increase was remarkable ($p<0.05$), rising from 2.9% at baseline to 54.3% at 6 months. *P. nigrescens* was not detected in saliva at baseline, but was found in this habitat in 31.5% of the saliva samples 6 months after therapy. The other tested sites were only slightly colonized, if at all, at baseline and underwent no relevant changes during the course of the study (Fig. 2).

Mechanical therapy resulted in an increased detection frequency of *T. forsythensis* subgingivally at the MA sites. Interestingly, this increase was preceded by a strong increase in supragingival prevalence at the LA sites. Supragingivally, at the LA sites, at mucosae and in saliva, there was a transient increase of *T. forsythensis* at 3 months, followed by a rebound to baseline levels at 6 months. *T. forsythensis* was not detected in any of the assessed extraoral habitats during the entire study (Fig. 3).

Following therapy, the subgingival *E. corrodens* colonization of the MA sites was reduced by half (22.7 at baseline to 11.4% after 6 months). At the LA sites *E. corrodens* could not be detected subgingivally at 3 months. In contrast, the supragingival prevalence of *E. corrodens* was drastically increased at MA sites from 31.4% at baseline to 71.4% at 6 months ($p<0.05$). At the other analyzed sites, only minor changes were registered in *E. corrodens* prevalence. No colonization with *E. corrodens* was detected at the investigated extraoral sites (Fig. 4).

The colonization dynamics of *T. denticola* showed no major changes in the subgingival habitat of the MA and LA sites. However, in contrast to the

Table 1. Demographics and periodontal status of the study subjects

	BL	6 months
subjects (*n*)	35	
age (years) ± SD	54.0 ± 12.9	
females	14	
smokers	4	
mean no. of teeth/subject ± SD	22.4 ± 5.9	22.2 ± 6.0
mean % of sites with PPD 4–6 mm ± SD	23.1 ± 12.7	10.8 ± 9.2*
mean % of sites with PPD ⩾7 mm ± SD	3.1 ± 4.5	1.9 ± 2.9*
mean % of sites with BOP ± SD	30.8 ± 16.4	6.9 ± 6.8*
mean PPD of MA sites ± SD	5.1 ± 2.64	4.32 ± 2.52
mean % of MA sites with BOP ± SD	63.7 ± 34.5	19.8 ± 16.3

SD, standard deviation; BL, baseline; PPD, pocket probing depths; BOP, bleeding on probing; MA site, most severely affected site.

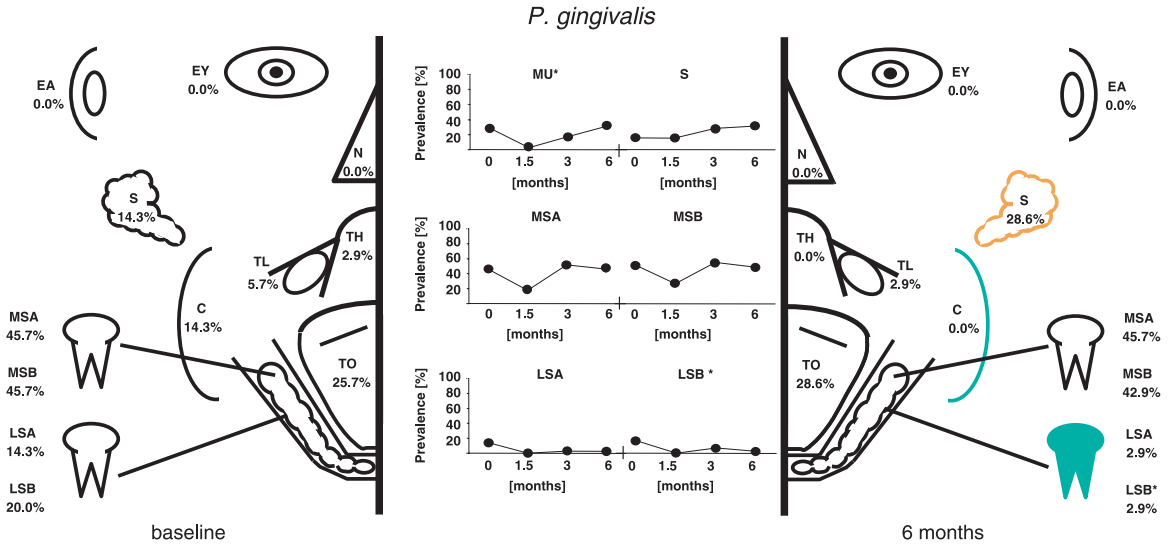

Fig. 1. Microbiological dynamics in the different habitats 1.5 months, 3 months, and 6 months after therapy for *P. gingivalis*. EA, external ear canal; Ey, lower eyelid; C, cheek; LSA, supragingival region of less affected sites, LSB, subgingival region of less affected sites; MSA, supragingival region of most severely affected sites, MSB, subgingival region of most severely affected sites, MU, all mucosal surfaces; N, nasal cavity, TH, throat, TL, tonsils, TO, tongue, S, saliva. *Significant ($p<0.05$) changes in detection frequency throughout the study period. Green, any decrease in prevalence, black, increase ⩽10% from baseline (BL), orange, increase ⩾20% from baseline (BL), red, increase ⩾30% from BL.

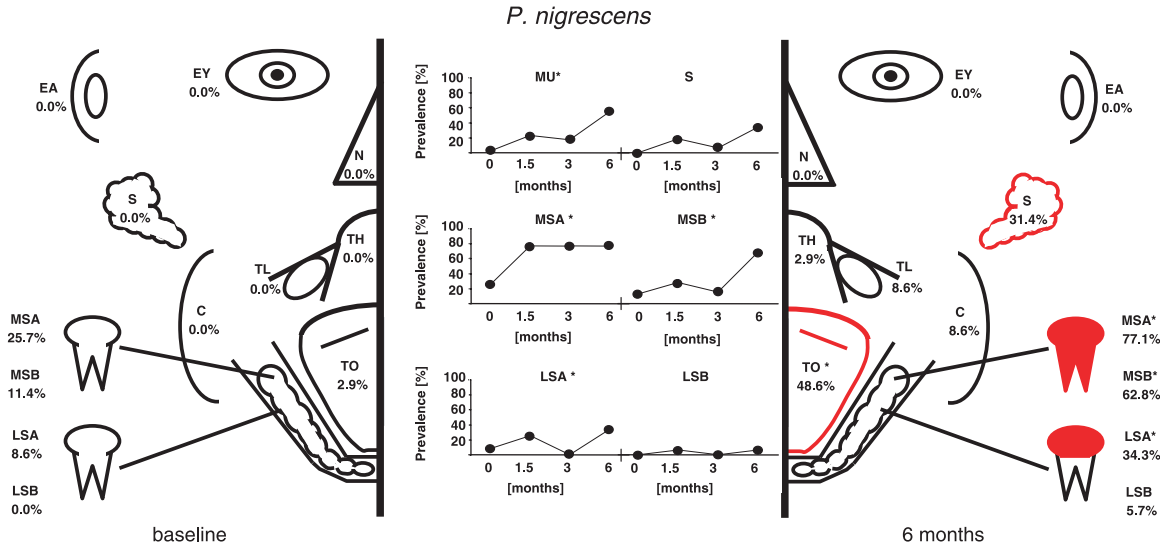

Fig. 2. Microbiological dynamics in the different habitats 1.5 months, 3 months, and 6 months after therapy for *P. nigrescens*. Legend as in Fig. 1.

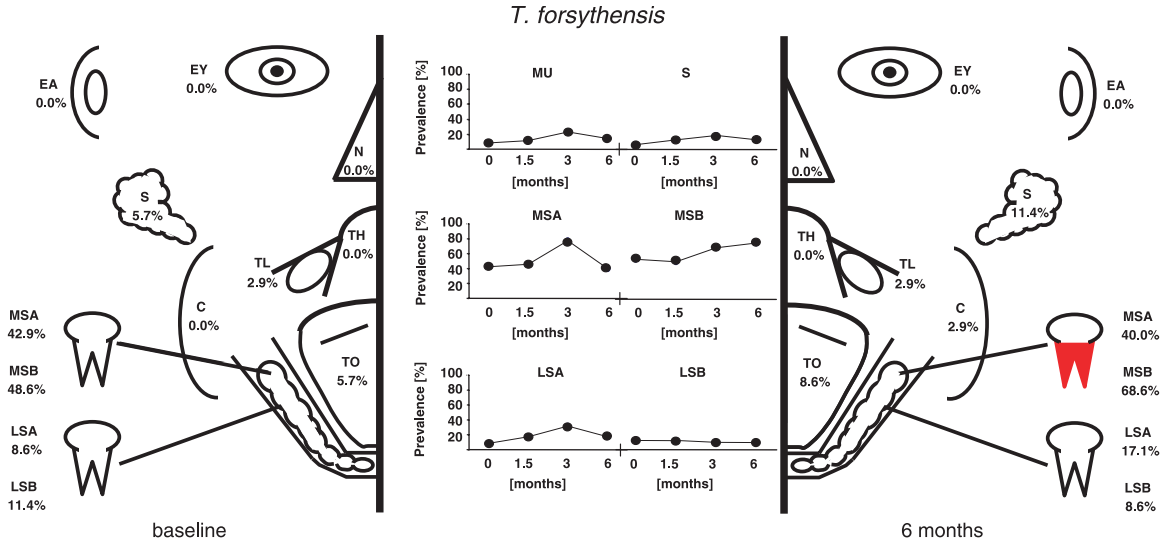

Fig. 3. Microbiological dynamics in the different habitats 1.5 months, 3 months, and 6 months after therapy for *T. forsythensis*. Legend as in Fig. 1.

MA sites, there was an increased detection rate at the supragingival LA sites following treatment ($p<0.05$). Moreover, the prevalence of *T. denticola* in saliva underwent a continuous increase throughout the study period from 17.1% to 85.7% ($p<0.05$). On the mucosal surfaces of the cheeks, tongue, and tonsils, the prevalences of *T. denticola* were found to be increased following therapy. *T. denticola* was the only periopathogen to be detected in the external ear canal. All other extraoral sites with the exeption of the lower eyelid (2.9% 6 months after therapy) were tested negative throughout the observation period (Fig. 5).

The subgingival prevalence of *A. actinomycetemcomitans* at the MA sites remained largely unchanged throughout the study period. At the LA sites, however, the prevalence of *A. actinomycetemcomitans* was reduced supra- and subgingivally following therapy. The detection frequency on the mucosal surfaces showed only minor changes throughout the observation period. With the exeption of the lower eyelid (2.9% at baseline) the extraoral sites were not colonized by this pathogen (Fig. 6).

The prevalence of *P. intermedia* showed only minor changes throughout the study period. Only in the supra- and subgingival region of the MA sites was an increase in detection frequencies recorded (Fig. 7). The assessed extraoral sites showed no colonization with this pathogen.

Discussion

The results of this study show that the prevalences of the analyzed periodontal pathogens differ between the various habitats. They further indicate that supra- and subgingival debridement results in an increased colonization of intraoral habitats that were not preferential niches before therapy. This may indicate the existence of a direct and

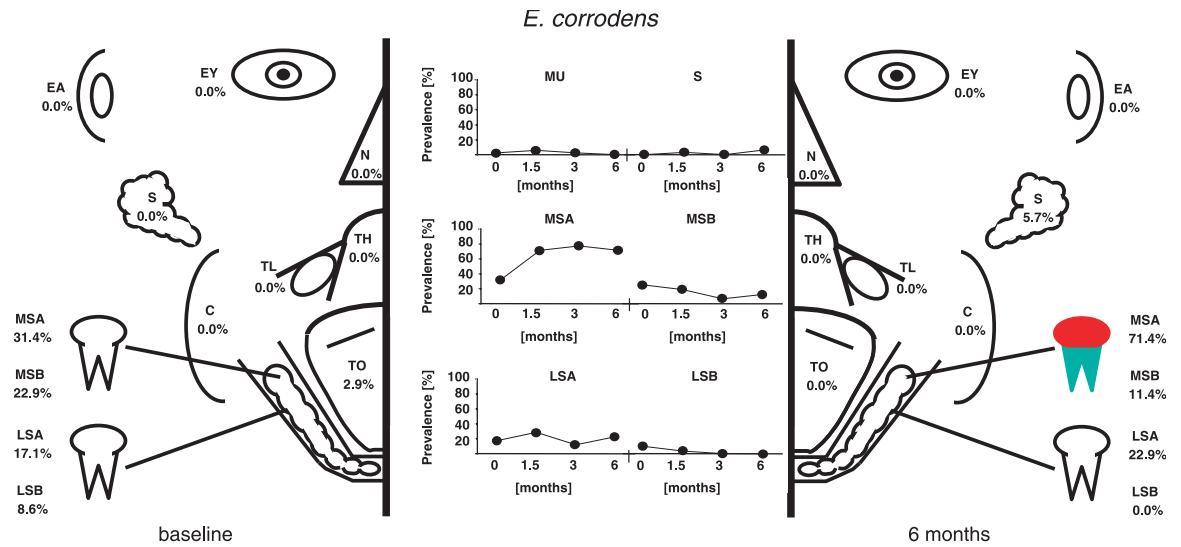

Fig. 4. Microbiological dynamics in the different habitats 1.5 months, 3 months, and 6 months after therapy for *E. corrodens*. Legend as in Fig. 1.

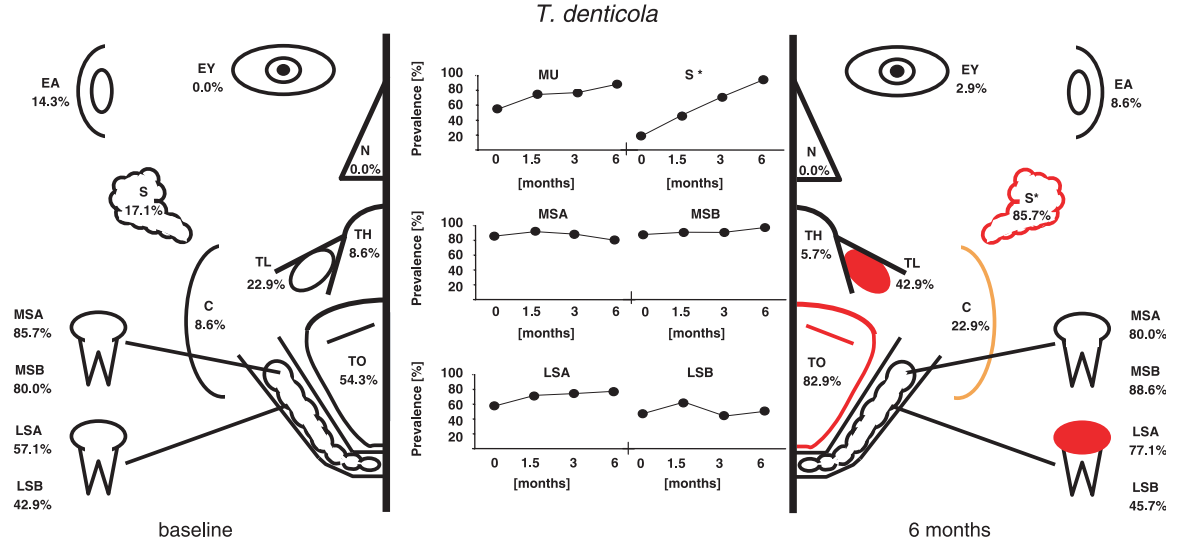

Fig. 5. Microbiological dynamics in the different habitats 1.5 months, 3 months, and 6 months after therapy for *T. denticola*. Legend as in Fig. 1.

dynamic relationship between environment and bacteria that can be influenced by supra- and subgingival debridement. In order to explain the maintenance of microbial communities with a distinctive composition in the newly evolved habitats, it has to be assumed that each of these habitats differs in terms of key ecological factors that enable certain populations to dominate at one region while being suppressed at others. Such factors include expression of receptors for attachment (Amano et al. 1999) and essential nutrients and cofactors for growth, as well as an appropriate pH or redox potential produced by the ecosystem itself or by other bacteria in the biofilm (Marsh & Bradshaw 1997, Bowden & Li 1997).

It is interesting to note that, following therapy, the detection frequencies of all pathogens except *A. actinomycetemcomitans* increased in saliva, suggesting that this habitat may be of particular interest in the process of bacterial transition between different intraoral habitats. The fact that supra- and subgingival debridement results in intraoral spreading of bacteria from a sessile phase, e.g. supra- and subgingival plaque, to a planktonic phase, e.g. in saliva might suggest opportunities for selection. For instance, some bacterial species may be prevented from establishing themselves in biofilm by mechanisms such as clearance by aggregation, mucosal shedding or killing by antibacterial proteins (Douglas 1994, Slomiany et al. 1996, Rudney 2000). In contrast, salivary proteins on oral surfaces may result in a positive selection by providing ligands for adherence. Moreover, bacteria that catabolize salivary components may likewise have a selective advantage during biofilm formation. The increased prevalence in saliva may further indicate the developing of mucosal biofilms from which the planktonic bacteria were detached by shearing forces.

Since no data were available on the craniofacial–extraoral prevalences of

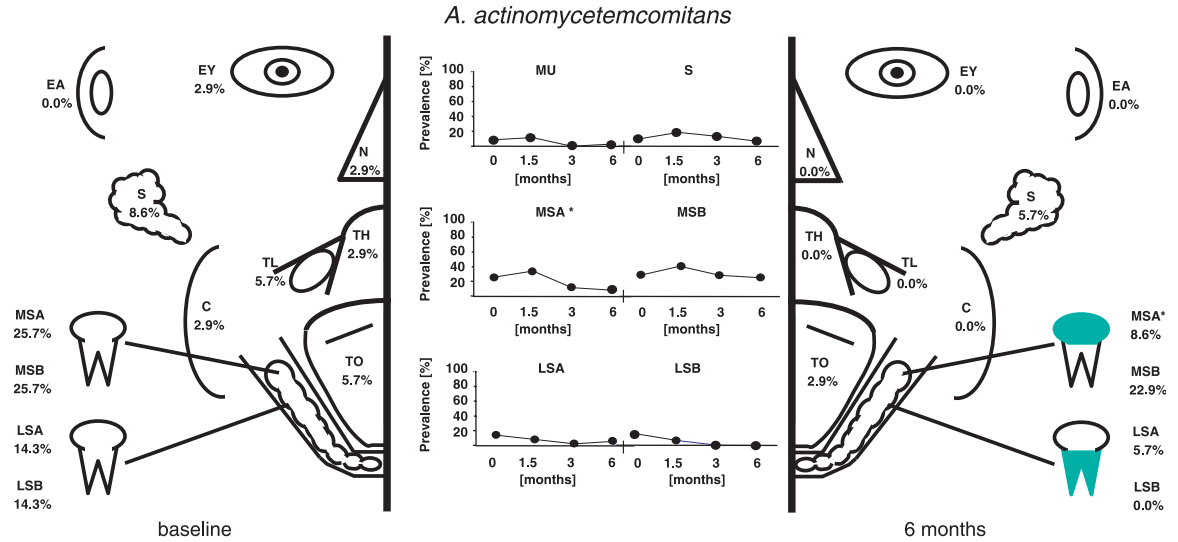

Fig. 6. Microbiological dynamics in the different habitats 1.5 months, 3 months, and 6 months after therapy for *A. actinomycetemcomitans*. Legend as in Fig. 1.

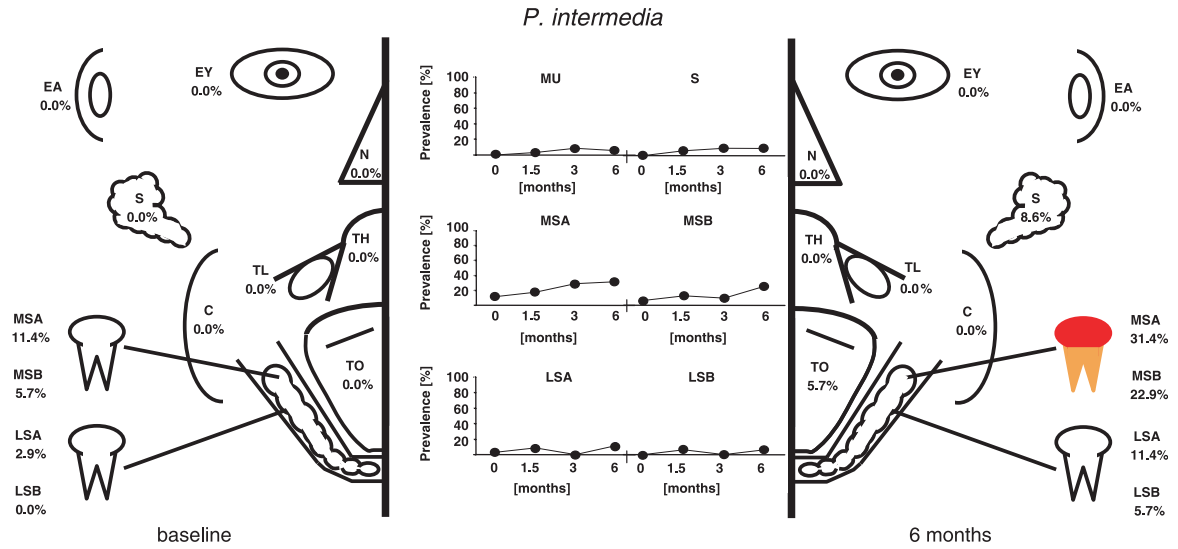

Fig. 7. Microbiological dynamics in the different habitats 1.5 months, 3 months, and 6 months after therapy for *P. intermedia*. Legend as in Fig. 1.

the analyzed pathogens, the nose, eye, and ear were selected as locations because of their potential role as a source of intraoral reinfection. These sites are anatomically adjacent to the oral cavity and the bacteria may be easily transferred from intraoral sites to these cranio-extraoral sites and vice versa. Although the analyzed periodontal pathogens are known to favor an anaerobic environment, the colonization of aerobic sites like those mentioned above could not be ruled out a priori, especially since anaerobic organisms can cope with the toxic effects of oxygen by interacting with oxygen-consuming species that can reduce the environmental levels of oxygen sufficiently to enable them to detoxify the residual low levels with a range of protective enzyme systems (Marquis 1995). However, with the exception of the colonization of the external ear canal with *T. denticola*, no notable prevalences of the other investigated pathogens were observed, indicating that their favored habitat is the oral cavity and that the studied craniofacial-extraoral sites are not an important source for recolonization.

The results of this study indicate that supra- and subgingival debridement results in a microbiological shift from subgingival regions to regions formerly not constituting preferential habitats, and thus may be of great importance in re-establishing and maintaining oral biofilms following therapy.

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