Periodontitis is associated with a loss of colonization by *Streptococcus sanguinis*

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The aim of this study was to estimate differences in the prevalence of oral streptococcal species in the subgingival biofilm of patients with aggressive periodontitis and of healthy controls. Thirty-three patients with clinical and radiological proof of aggressive periodontitis and 20 healthy subjects were enrolled in this study. Clinical indices were recorded in a six-point measurement per tooth. Samples of the subgingival biofilm were taken with paper points from four teeth of each individual. Alpha- and non-haemolytic, small and catalase-negative colonies were biochemically identified using a rapid ID 32 STREP system and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. A total of 118 strains of oral streptococci (11 species) were identified and *Streptococcus sanguinis* was found significantly more often in healthy subjects (\(P<0.001\)). Conversely, the absence of *S. sanguinis* was associated with high values of clinical indices (\(P=0.001–0.002\)). Aggressive periodontitis seems to be associated with a loss of colonization of *S. sanguinis*. Whether or not *S. sanguinis* offers protection against aggressive periodontitis needs to be determined. Otherwise, there were no significant differences in the distribution of oral streptococcal species in patients and healthy subjects.

**INTRODUCTION**

Healthy gingivae are associated with a simple supragingival biofilm composition: a few (1–20) layers of oral streptococci, Gram-positive rods and very few Gram-negative cocci. These bacteria are early colonizers that are able to survive in an aerated environment. In contrast, clinical gingivitis is associated with the development of a more organized dental plaque of 100–300 layers, with anaerobic Gram-negative rods and filaments being predominant. The species involved in biofilm formation may vary depending on local environmental characteristics, but the colonization pattern is always the same (Marsh & Bradshaw, 1999; Marsh, 2004).

Bacterial communities from dental biofilms tend to be grouped in clusters (complexes) according to nutritional and atmospheric requirements. The initiation and progression of periodontitis is thought to be caused by several species belonging to ‘red’ and ‘orange’ complexes (*Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*, and *Prevotella intermedia* and *Fusobacterium nucleatum*, respectively) (Socransky et al., 1998). However, according to the ‘ecological plaque hypothesis’ (Marsh, 1991), the lack of so-called ‘protective bacteria’ is also thought to play an important role. These are microbial species that can occupy a niche that could shelter pathogenic organisms or that can inhibit some pathogens through metabolic antagonism or by directly inactivating them (Quirynen et al., 2001). Some of the members of the ‘yellow’ complex of oral streptococci are candidates for this position. Plaque samples from healthy gingival sulci normally contain a large number of oral streptococci (Theilade et al., 1966). Socransky et al. (1998) previously showed that yellow complexes, as a total, were associated with shallow pockets (probing depth of <3 mm). Thus colonization of certain oral streptococci might be one factor offering protection against periodontitis.

However, few data are available about the distribution of oral streptococci in subgingival biofilms of patients with aggressive periodontitis. Previous studies have focused on

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certain species of oral streptococci (Streptococcus sanguinis, Streptococcus mutans, Streptococcus intermedius and Streptococcus oralis) (Van der Reijden et al., 2001; Dowsett et al., 2002) or described the composition of subgingival biofilm in oral streptococci in healthy subjects (Frandsen et al., 1991), in diabetic patients with periodontitis (Hintao et al., 2007) or in patients with periodontitis.

The aim of this study was to estimate differences in the prevalence of oral streptococcal species in the subgingival biofilm of patients with aggressive periodontitis and of healthy controls.

**METHODS**

**Patients and healthy control subjects.** Thirty-three patients with aggressive periodontitis and 20 healthy subjects were enrolled in this prospective study. Diagnosis of aggressive periodontitis was made on clinical and radiographic findings, which showed rapid attachment loss and bone destruction (Wiebe & Putnins, 2000). The patients included had at least 14 natural teeth, and at least four pockets (one in each quadrant) with a probing depth (PD) and clinical attachment level (CAL) of $\geq 4$ mm. Healthy controls had a PD of $\leq 3$ mm and no attachment loss, and were matched with the patients for age, sex and smoking status. Patients were excluded from the study if they were pregnant, had other infectious diseases, had a history of previous periodontal treatment or if they had had antimicrobial therapy 6 months prior to the study. Demographic parameters and the history of smoking were obtained using a questionnaire. The Ethical Committee of the Faculty of Medicine, University of Leipzig, Germany, approved the protocol, including clinical measurements and the sampling procedure. All subjects were informed of the nature, potential risks and benefits of participation in the study, and signed informed consent prior to entry into the study.

**Clinical measurements.** Measurements of approximate plaque index (API; $+/−$), oral hygiene index (OHI; 0–3), gingival index (Lobene; 0–4), bleeding on probing (BOP; $+/−$), PD and CAL were recorded in a six-point measurement per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) for all teeth. For quadrant) of each individual. The teeth were chosen based on clinical and bacteriological data. To determine differences in the streptococci identified by PCR and sequence analysis of the 16S rRNA gene showed that all of the spectra belonging to the same species. Similarity analysis between peak lists was carried out using a support vector machine algorithm. Support vector machines are a class of statistical learning algorithm whose theoretical basis was first presented by Vapnik (1995).

Data analysis. A univariate description was used to analyse all clinical and bacteriological data. To determine differences in the prevalence of oral streptococcal species in the subgingival biofilm of patients with aggressive periodontitis and of healthy controls, $\chi^2$ analysis and a Mann–Whitney U-test were used. To account for multiplicity, the alpha level was lowered from 0.05 to 0.003 using Bonferroni adjustment.

**RESULTS AND DISCUSSION**

The clinical data of the aggressive periodontitis patients and periodontally healthy subjects are summarized in Table 1. The two groups of patients had comparable mean ages, the range being 20–69 years of age. Thirty-seven (69.8%) were women and 20 (37.7%) were smokers. The mean PD and attachment loss of sampled sites were higher than the mean for all teeth in all patients with aggressive periodontitis.
From both groups, a total of 134 isolates were tested using the rapid ID 32 STREP system (bioMérieux). Strains that were not identified as belonging to the genus Streptococcus were not studied further, and the remaining 118 streptococcal isolates (73 isolates from patients, 45 isolates from control subjects) were subjected to MALDI-TOF-MS analysis. The distribution of species of oral streptococci was shown in Table 2. Among the 11 streptococcal species isolated, S. oralis (38 isolates), S. sanguinis (33 isolates) and S. mitis (20 isolates) were the most prevalent. S. sanguinis was found in 15 patients and 18 healthy subjects. The $\chi^2$ test showed that the difference in prevalence of streptococcal strains between periodontitis patients and healthy controls was significant only for S. sanguinis, which was found significantly more often in healthy subjects ($P=0.001$). Significant differences between males and females and between smokers and non-smokers with respect to prevalence of oral streptococci were not found.

The relationship with age and periodontal indices of streptococcal strains was analysed using a Mann–Whitney test. Table 3 presents the $P$ values for the significant association between the absence of S. sanguinis and high values of API, gingival index, BOP, CAL and PD. This study analysed the prevalence of oral streptococci in patients suffering from aggressive periodontitis and in healthy control individuals. Special emphasis was laid on the correct species identification of the streptococci. As it is known that conventional biochemical tests may fail to discern certain species of viridans streptococci, the recently established MALDI-TOF-MS methodology (Smole et al., 2002; Rupf et al., 2005; Friedrichs et al., 2007) was used and adjusted for this study.

The results indicated that, among the 11 species cultured from patients and healthy individuals, only one species – S. sanguinis – showed significant differences in colonization rates for the two groups, and was associated with health rather than disease.

It has been demonstrated previously that S. sanguinis is a beneficial bacterium in the prevention of dental caries. The antagonism with S. mutans has been known for many years. Early colonization with S. sanguinis is significantly correlated with a delay in colonization by S. mutans. After S. mutans colonization, the levels of S. sanguinis decrease (Caufield et al., 2000; Kreth et al., 2005).

Several other studies have focused on the in vitro relationship between S. sanguinis and some of the periodontal pathogens. Hillman et al. (1985) showed that, in samples from oral sites where S. sanguinis was detected, Tannerella forsythia was present in 1 % of the cases, and in samples where S. sanguinis was not detected, Tannerella forsythia was present in 10 % of the oral sites. They also showed that one-third of S. sanguinis strains tested were able to inhibit Prevotella intermedia B56. In vitro growth of Aggregatibacter actinomycetemcomitans is inhibited by the

### Table 1. Mean clinical parameters of the subjects enrolled in this study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Periodontitis patients (mean ± sd, n=33)</th>
<th>Healthy subjects (mean ± sd, n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>39.39 ± 10.47</td>
<td>37.65 ± 10.88</td>
</tr>
<tr>
<td>API (%)</td>
<td>41.35 ± 30.58</td>
<td>15.77 ± 12.56</td>
</tr>
<tr>
<td>OHI (0–3)</td>
<td>0.41 ± 0.39</td>
<td>0.06 ± 0.07</td>
</tr>
<tr>
<td>Lobene (0–4)</td>
<td>0.82 ± 0.62</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>BOP (%)</td>
<td>44.20 ± 24.96</td>
<td>3.09 ± 3.32</td>
</tr>
<tr>
<td>CAL (total)*</td>
<td>3.73 ± 0.85</td>
<td></td>
</tr>
<tr>
<td>CAL (sites)†</td>
<td>4.79 ± 1.28</td>
<td></td>
</tr>
<tr>
<td>PD (total)*</td>
<td>3.50 ± 0.73</td>
<td>1.69 ± 0.28</td>
</tr>
<tr>
<td>PD (sites)†</td>
<td>4.36 ± 1.10</td>
<td>1.39 ± 0.24</td>
</tr>
</tbody>
</table>

*Mean of all teeth.
†Mean of the four sampling sites.

### Table 2. Distribution of oral streptococcal species in patients and healthy subjects

$n$, Number of patients/healthy subjects colonized with oral streptococci.

<table>
<thead>
<tr>
<th>Oral streptococci</th>
<th>Colonized periodontitis patients</th>
<th>Colonized healthy subjects</th>
<th>$P$ value ($\chi^2$ test)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$n$</td>
<td>%</td>
<td>$n$</td>
</tr>
<tr>
<td>S. mitis</td>
<td>12</td>
<td>36.36</td>
<td>8</td>
</tr>
<tr>
<td>S. sanguinis</td>
<td>15</td>
<td>45.45</td>
<td>18</td>
</tr>
<tr>
<td>S. oralis</td>
<td>24</td>
<td>72.72</td>
<td>14</td>
</tr>
<tr>
<td>S. gordonii</td>
<td>8</td>
<td>24.24</td>
<td>0</td>
</tr>
<tr>
<td>S. cristatus</td>
<td>1</td>
<td>3.03</td>
<td>1</td>
</tr>
<tr>
<td>S. sinensis</td>
<td>1</td>
<td>3.03</td>
<td>0</td>
</tr>
<tr>
<td>S. anginosus</td>
<td>3</td>
<td>9.09</td>
<td>1</td>
</tr>
<tr>
<td>S. salivarius</td>
<td>1</td>
<td>3.03</td>
<td>0</td>
</tr>
<tr>
<td>S. constellatus</td>
<td>7</td>
<td>21.21</td>
<td>1</td>
</tr>
<tr>
<td>S. parasanguinis</td>
<td>1</td>
<td>3.03</td>
<td>1</td>
</tr>
<tr>
<td>S. mutans</td>
<td>0</td>
<td>0.00</td>
<td>1</td>
</tr>
</tbody>
</table>
hydrogen peroxide produced by *S. sanguinis* (Hillman et al., 1985; Hillman & Socransky, 1982). However, *A. actinomycetemcomitans* may produce a bacteriocin that can kill *S. sanguinis* (Hammond et al., 1984), so there is an inverse relationship between these bacteria. In a complex ecosystem such as dental biofilm, these relationships may contribute to the transition from health to disease. Also, Teughels et al. (2007) showed in an *in vitro* study that *S. sanguinis* (as well as *S. mitis* and *Streptococcus salivarius*) has protective properties that interfere with *A. actinomyctetemcomitans* colonization of epithelial cells. All of these data suggest that *S. sanguinis* may serve as a ‘protective’ bacterium as defined by Quirynen et al. (2001). The effect of *S. sanguinis* on the presence of *Porphyromonas gingivalis* is still under debate. Whilst Hillman et al. (1985) showed that *S. sanguinis* had a minimal effect on the presence of *Porphyromonas gingivalis*, another study from China showed that pre-colonization and superinfection with *S. sanguinis* reduced the level of *Porphyromonas gingivalis* in experimental rats (Zhang et al., 2000).

In clinical studies, Haffajee et al. (1998) showed that *S. sanguinis* and *S. oralis* did not differ among the subject group of periodontitis patients, healthy controls and elders with a well-maintained periodontitis, and Dowsett et al. (2002) reported that *S. sanguinis* was more prevalent in shallow sites but was not the species associated with health. These discrepancies could be due to different subject inclusion and exclusion criteria and work protocols. These latter two clinical studies based their identification on a chequerboard DNA–DNA hybridization assay, whilst previously mentioned *in vitro* studies used cultivation. Our study combined cultivation with MALDI-TOF-MS.

A recent study (Maestre et al., 2007) showed that all of the bacteria from subgingival plaques (including anaerobic bacteria), *S. oralis* and *S. mitis* were the most prevalent species, with a frequency of detection of approximately 70%, whilst *S. sanguinis* was isolated in only 23% of cases. The three species were the only oral streptococci identified in this study, and 33/125 strains could not be identified to the species level. This is in keeping with the majority of earlier studies which focused on two or three species of oral streptococci only. The reason could be that only conventional biochemical methods were used for identification. With our study, we were able to employ MALDI-TOF-MS methodology, which allowed us to identify a larger number of different species. In our study, *S. oralis* was also the most prevalent oral streptococcus in both groups of subjects (present in 70% of cases), but *S. sanguinis* was the second most common isolate (present in 90% of healthy subjects and 45% of periodontitis patients). *S. mitis* was present in both groups in 36–40% of cases. Interestingly, all eight strains of *Streptococcus gordoni* were isolated from periodontitis patients, but the difference between the two groups was not significant (*P* >0.003). Lamont et al. (2002) and Daep et al. (2006) demonstrated that *S. gordoni* plays a role in colonization with *Porphyromonas gingivalis*, whilst Haffajee et al. (2006) found this species to be more prevalent in healthy subgingival sites. Further studies are needed to clarify the possible relationship between *S. gordoni* and periodontal status.

In conclusion, aggressive periodontitis seems to be associated with a loss of colonization with *S. sanguinis*. Whether or not *S. sanguinis* offers protection against aggressive periodontitis needs to be determined. Otherwise, there were no significant differences in the distribution of oral streptococcal species between patients and healthy subjects.

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**REFERENCES**


