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Analysis of supra- and subgingival long-term biofilm formation on orthodontic bands

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SUMMARY Insertion of fixed orthodontic appliances induces increased biofilm formation caused by a higher number of plaque-retentive sites. The purpose of the study was to perform a quantitative analysis of supra- and subgingival long-term biofilm formation on orthodontic bands.

Ten patients (five females and five males, aged 18.3 ± 5.4 years) who had received therapy with fixed orthodontic appliances for 24 ± 9 months were enrolled in the study. Biofilm formation on 28 orthodontic bands was analyzed quantitatively with the Rutherford backscattering detection method, a scanning electron microscopy technique. The biofilm formation for the supra- and subgingival surfaces was calculated from the grey values. Statistical analysis was performed with a mixed model with the patient as the random factor. A *P*-value <0.05 was considered significant.

A biofilm was found on 16.1 ± 9.2 per cent of supragingival surfaces and on 3.6 ± 4.4 per cent of subgingival surfaces. Differences in biofilm formation in supra- and subgingival surfaces were statistically significant ($P < 0.05$) and formed a distinct demarcation line. Despite the presence of supragingival biofilm, no mature subgingival biofilm was found on the tested orthodontic bands.

Introduction

The insertion of fixed orthodontic appliances induces an increase in plaque formation associated with a qualitative bacterial shift from aerobic to anaerobic microflora that endangers the integrity of hard and soft tissues. In clinical studies, an increasing incidence of incipient carious lesions and generalized gingival inflammation have been found in patients undergoing fixed orthodontic appliance therapy (Boyd *et al.*, 1989; Øgaard, 1989; Atack *et al.*, 1996; Naranjo *et al.*, 2006). The ecological changes in oral microbiota affect the composition, metabolic activity and pathogenicity of the biofilm (Diamanti-Kipioti *et al.*, 1987; Paolantonio *et al.*, 1997, 1999; Chang *et al.*, 1999; Hägg *et al.*, 2004) and can be explained by a higher number of plaque-retentive sites and impaired mechanical plaque removal (Boyd, 1983). Furthermore, it has been observed that the material and surface properties of the bracket can influence bacterial attachment, plaque retaining capacity and microbial diversity (Eliades *et al.*, 1995; Anhoury *et al.*, 2002).

After exposure of all hard and soft tissues to the oral cavity, an acquired pellicle is formed from adsorbed salivary biopolymers (Hannig, 1997, 1999). This initial coating forms the interface between the surface and the colonizing microorganisms (Busscher and van der Mei, 1997; Bos *et al.*, 1999). Initial bacterial colonization is a pre-condition for the development of a mature biofilm, a factor associated with the progression of tooth decay and periodontitis (Liljemark and Bloomquist, 1996).

Clinical studies have shown that the gingiva surrounding natural teeth has the potential to prevent subgingival biofilm formation and to respond to early biofilm accumulation (Berglundh *et al.*, 1992). Protective mechanisms include structural components, such as a well-keratinized oral epithelium (Berglundh *et al.*, 1991). In a recent study, it was found that subgingival biofilm during initial bacterial colonization was absent around implant abutments, despite increased supragingival biofilm formation (Heuer *et al.*, 2007).

Orthodontic bands are also seated in supra- and subgingival areas. This seating can compromise the health of the surrounding periodontal tissues and can be associated with the occurrence of periodontopathogenic bacteria (Diamanti-Kipioti *et al.*, 1987; Huser *et al.*, 1990). Consequently, gingival inflammation can be induced that causes gingival enlargement, which is largely reversible (Alexander, 1991; Kouraki *et al.*, 2005). This gingival enlargement increases the amount of the subgingival surface.

Little is known about the adherent subgingival environment around orthodontic bands and the potential of mucosal components to prevent long-term subgingival biofilm formation on these surfaces. Therefore, the purpose of the present research was to perform a quantitative analysis of biofilm formation on supra- and subgingival surfaces of orthodontic bands, employing a scanning electron microscopy (SEM) technique.

Subjects and methods

The present study was approved by the Ethics Committee of Hannover Medical School (no. 4347). The examination was performed with the understanding and written consent of each subject.

Ten patients (five females and five males, aged between 14 and 32 years, mean 18.3 ± 5.4 years) who had received therapy with fixed orthodontic appliances for 24 ± 9 months were included in the study. The exclusion criteria were systemic diseases, missing teeth in the permanent dentition, antibiotic therapy 6 weeks before removal of the appliance and a history of periodontal disease, excluding gingivitis and carious lesions.

Six weeks before removal of the orthodontic bands (Ormco, Amersfoort, The Netherlands), parameters of oral hygiene were obtained. First, the approximal plaque index (API) was determined by probing of interdental spaces on the buccal sites of the upper left and lower right quadrants and the oral sites of the upper right and lower left quadrants (Lange, 1975). Removal of plaque after probing was recorded as a positive finding. The API was calculated by dividing the number of positive findings by the number of examined sites, resulting in a percentage index. Furthermore, the sulcus bleeding index (SBI) was determined by gently guiding a periodontal probe through the sulcus of the same examination sites as in the API (Muehlemann and Son, 1971). The SBI was calculated by dividing the number of bleeding points by the number of probing sites, again resulting in a percentage index.

After removal of the first molar orthodontic bands using band-removing pliers, these were cleaned by rinsing with a sterile water solution and dried by air. Iatrogenic biofilm dislodgement during the removal process was avoided by placing the pliers only on supragingival attachments. The quantitative analysis of biofilm formation was based on 28 orthodontic bands (six patients with two bands and four patients with four bands), employing the Rutherford backscattering detection (RBSD) method, a SEM procedure (Leo 1455 VP, Leo Electron Microscopy Ltd, Cambridge, UK). The photomicrographs were obtained after rotating each sample approximately 45 degrees, generating a total of eight photographs per sample.

The RBSD photomicrographs allowed the detection of biofilm-coated surfaces. Biofilm coverage was validated by using SEM with high magnification and energy dispersive X-ray spectroscopy that allowed elemental characterization of the biofilm as an organic material. On the RBSD micrographs, plaque-covered surfaces appeared dark and non-covered surfaces bright (Figure 1). Plaque- and non-plaque-covered surfaces were differentiated on the basis of the grey values after conversion to a binary display (Figure 2). The extent of coverage with biofilm was calculated using surface analysis software (Image J 10.2 for Apple, National Institutes of Health, Bethesda, Maryland, USA). The demarcation line between supra- and subgingival regions was reproduced on casts manufactured 6 weeks prior to debonding. On these casts, the distances from the gingival margin to the occlusal border of the orthodontic bands were determined continuously by multiple measurements. These distances were later transferred to the RBSD photomicrographs to reproduce the localization of supra- and subgingival surfaces.

Power and sample sizes were calculated using nQuery Advisor 5.0 (Statistical Solutions, Saugus, Massachusetts, USA). Power calculation revealed that a sample size of 10 would have an 80 per cent power to detect a difference in means of 15 per cent, assuming that the standard deviation of the differences was 15 per cent. Documentation and evaluation of the data were performed with the Statistical Package for Social Sciences Version 14.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Comparison of supra- and subgingival biofilm coverage was undertaken with the mixed model, with the patient as the random factor. All tests were performed two tailed with a significance level of $P < 0.05$.

Results

Six weeks before removal of the orthodontic bands, the API was positive for 53.3 ± 22.8 per cent of the examined sites and SBI for 33.0 ± 11.1 per cent.

Biofilm was detected on all orthodontic bands after removal from the oral cavity. A total surface of 2805 mm^2 was analyzed. Eighty per cent of the analyzed surfaces were located supragingivally and 20.0 per cent subgingivally. Figure 3 summarizes the results of the quantitative analysis of biofilm formation with respect to location. Biofilm was found on 16.1 ± 9.2 per cent of supragingival surfaces and was mostly located at the gingival margin and around the attachments (Figure 4). Biofilm formation was observed on only 3.6 ± 4.4 per cent of subgingival surfaces. The difference

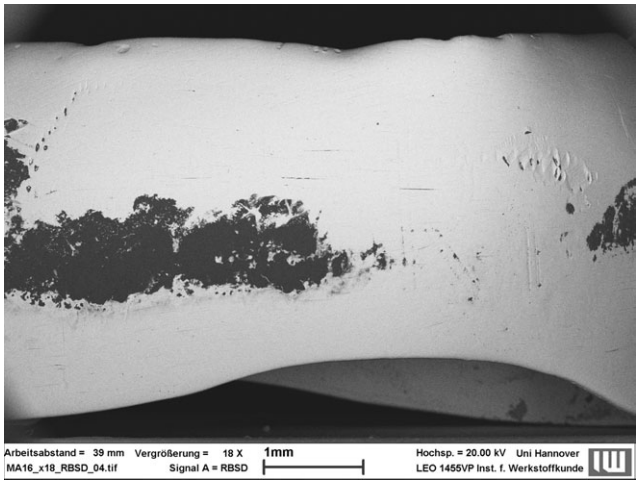

Figure 1 Rutherford backscattering detection micrograph of an orthodontic band.

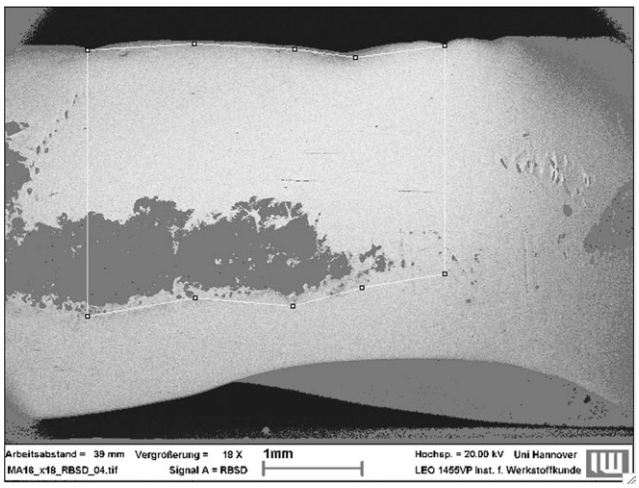

Figure 2 Conversion of grey values to a binary display and marked supragingival areas.

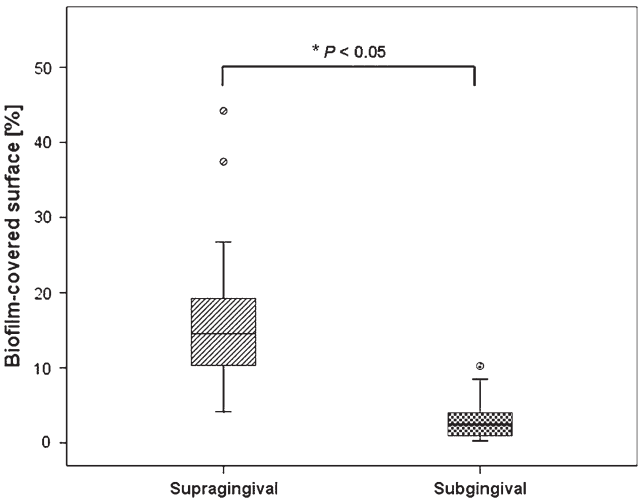

Figure 3 Percentage frequency of adherent biofilm on supra- and subgingival surfaces.

in biofilm formation was statistically significant ($P < 0.05$) and resulted in a demarcation line between supra- and subgingival surfaces. This demarcation line was formed by the presence of supragingival biofilm and biofilm-free subgingival surfaces and was colinear with the reproduced gingival margin.

On indented surfaces (indentation for the identification of orthodontic bands, scratched surfaces), more adherent biofilm was found than on adjacent surfaces. This phenomenon was observed on all analyzed bands in the supragingival areas. On some subgingival areas, there was ingrowth of bacteria via the observed indented surfaces (Figure 4).

Discussion

Seating cemented orthodontic bands compromises oral health by increasing plaque formation on banded teeth (Boyd and Baumrind, 1992). Therefore, in the present study quantitative analysis of biofilm formation on orthodontic bands was performed using the RBSD technique and surface analysis software. Identification of biofilm-covered surfaces on orthodontic bands was possible with these methods. In the RBSD technique, primary electrons leave the sample as a consequence of an elastic scattering process depending on atomic weight (Rutherford backscattering). In these element-contrast photomicrographs, surfaces covered with elements of low atomic weight (such as oral biofilms) give darker images than surfaces with a higher atomic weight (such as stainless steel) and software-assisted analysis of biofilm coverage can be performed on the basis of the resulting differences in grey values.

The representation of curved surfaces in two-dimensional photographs results is distortion. In the present study, measurement error during surface analysis was reduced by taking eight photomicrographs of each orthodontic band.

To perform an analysis of biofilm formation with respect to localization, it is necessary to differentiate supra- from subgingival areas without destroying the biofilm on these surfaces. Therefore, 6 weeks prior to debonding, casts were manufactured and clinical parameters were assessed. To avoid the removal or diversion of plaque, no probing was performed when obtaining the bands.

Analysis of clinical parameters showed a high percentage of plaque accumulation and bleeding of the sulcus, indicating that 6 weeks prior to removal of the orthodontic bands a plaque-associated gingivitis was present. These findings can be explained by the impairment of plaque removal during orthodontic treatment (Atack *et al.*, 1996).

On 16.1 ± 9.2 per cent of supragingival surfaces, a biofilm was found, mostly located at the gingival margin and around the attachments. This amount of biofilm was comparable with supragingival biofilm formation on other intraoral alloplastic materials. In a recent study, similar supragingival biofilm formation was found on intraoral titanium surfaces after 14 days of exposure to the oral cavity (Heuer *et al.*, 2007). The amount of supragingival biofilm was similar in both studies, indicating that the effect of time on biofilm formation on alloplastic intraoral surfaces appears, after an initial period, to be negligible. The results of the present investigation showed a high standard deviation, corresponding to interindividual differences in the amount of biofilm-covered surfaces. This can be explained by covariables, such as nutrition, tongue activity, and oral hygiene.

In the present study, supragingival biofilm was mostly located at the gingival margin, around orthodontic attachments and on indented surfaces. These findings can be explained by reduced shear forces in these regions, where bacteria are protected from mechanical and hydrodynamic effects such as tongue movement and saliva flow (Quirynen and Bollen, 1995; Hannig, 1999).

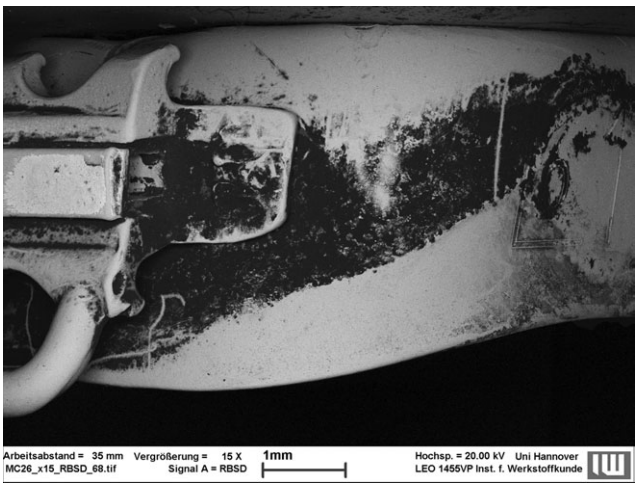

Figure 4 Demarcation line formed by adherent biofilm in supragingival areas and the absence of biofilm in subgingival areas with ingrowth in subgingival located indentations. Scratches are the result of band removal.

Only 3.6 ± 4.4 per cent of subgingival surfaces were covered by an adherent biofilm. A line separating supra- and subgingival areas was apparent from the presence of supragingival surfaces covered with biofilm and subgingival surfaces, which were almost free of biofilm. These results were unexpected, as unfavourable clinical parameters, occurrence of periodontal pathogens, and retention of bacterial plaque on supragingival surfaces during therapy with fixed orthodontic appliances is well described in the literature (Petti *et al.*, 1997; Ong and Wang, 2002; Hägg *et al.*, 2004). Even in the present investigation, high plaque and bleeding indices indicated the presence of a biofilm-induced gingivitis. As almost no mature biofilm was observed on the subgingival surfaces of the orthodontic bands, the impaired clinical parameters might have been caused by the presence, composition, and metabolic activity of supragingival biofilm. Subgingival colonization with microorganisms should have been possible, as there was long-standing biofilm and bacterial proliferation in supragingival areas.

On titanium surfaces, adhesion of fibroblasts can provide a tight peri-implant barrier, by forming circular collagen fibres, adherent hemidesmosoma, actin filaments, and microvilli (Gould *et al.*, 1984). *In vitro* studies have found similar non-cytotoxic effects of titanium and stainless steel on periodontal ligament and gingival fibroblasts (Mockers *et al.*, 2002; Eliades *et al.*, 2004). However, a structural barrier cannot be expected around orthodontic bands, as there is no direct contact between fibroblasts and stainless steel surface when seating orthodontic bands. Consequently, effects such as an immunological response to microbiota could play a key role in the inhibition of subgingival biofilm formation.

In vivo studies have shown the presence and activation of T- and B-lymphocytes in subjects with periodontal disease (Yamazaki *et al.*, 1993). The crevicular bacteriostatic effects might be due to the release of immunomodulators which specifically enhance neutrophil priming and emigration, phagocytosis, and bacterial death (Niederman *et al.*, 2002).

Conclusions

On all bands, a demarcation line was found, corresponding to a supragingival biofilm and the absence of a mature subgingival biofilm. These findings indicate that factors such as crevicular fluid and immunological response seem to inhibit bacterial adhesion on subgingival surfaces.

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