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Reduction of biofilm on orthodontic brackets with the use of a polytetrafluoroethylene coating

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SUMMARY Treatment with fixed orthodontic appliances can cause enamel demineralization by increased biofilm adhesion. The purpose of the present study was to investigate whether a polytetrafluoroethylene (PTFE) coating reduces biofilm formation on orthodontic brackets.

One PTFE-coated bracket and one uncoated stainless steel bracket were bonded symmetrically on the first or second (four maxillary and nine mandibular) primary molars in 13 adolescent patients (five females and eight males, aged 11.2±2.8 years; four dropouts) for 8 weeks. Quantitative biofilm formation on brackets was analysed with the Rutherford backscattering detection (RBSD) method, a scanning electron microscopy technique. A total of five RBSD micrographs were obtained per bracket with views from the buccal, mesial, distal, cervical, and occlusal aspects. A two-sided paired *t*-test was used to compare data. A *P*-value less than 0.05 was considered significant.

Total biofilm formation was 4.0±3.6 per cent of the surface on the PTFE-coated brackets and 22.2±5.4 per cent on uncoated brackets. Differences between the two groups were statistically significant ($P<0.05$). Pairwise comparison of biofilm formation with respect to location (buccal, mesial, distal, cervical, and occlusal) revealed a significantly lower biofilm accumulation on PTFE-coated brackets on all surfaces.

The results indicate that PTFE coating of brackets reduces biofilm adhesion to a minimum and might have the potential to reduce iatrogenic side effects, e.g. decalcification during orthodontic treatment with fixed appliances.

Introduction

Treatment with fixed orthodontic appliances allows three-dimensional correction of malocclusions. Compared to treatment with removable appliances, fixed orthodontic therapy affords non-compliance treatment and superior efficacy in terms of treatment time and treatment results (Tang and Wei, 1990).

However, the detrimental effects of fixed therapy can be observed in the short- and long-term. Bracket insertion induces ecological changes of the oral microbiota by affecting its amount, composition, metabolic activity, and pathogenicity, which clinically results in a higher incidence of periodontal disease and incipient carious lesions (Atack *et al.*, 1996; Naranjo *et al.*, 2006; Ahn *et al.*, 2007; van Gastel *et al.*, 2008). In contrast to periodontal and microbial side-effects of fixed orthodontic treatment, which are considered to be largely reversible, signs of previous decalcification resulting in persisting white spot lesions can be observed even long-term after completion of treatment (Øgaard, 1989; Sallum *et al.*, 2004).

These side-effects of fixed orthodontic therapy can be explained by the higher number of plaque-retentive sites and impaired mechanical plaque removal after bracket insertion (Boyd, 1983). In clinical studies, an influence of the bracket material used was also shown to affect intraoral biofilm formation, as well as surface properties of the brackets used (Fournier *et al.*, 1998; Anhoury *et al.*, 2002).

Current strategies to avoid the clinical side-effects of fixed orthodontic treatment are professional tooth cleaning, the local application of fluorides, and the use of antimicrobial mouth rinses (Alves *et al.*, 2008; Shafi, 2008; Tufekci *et al.*, 2008). Furthermore, sealants are used to reduce enamel decalcification (Buren *et al.*, 2008). All these strategies are focused on reducing or removing oral biofilm and are aimed at increasing the resistance of hard tissues against bacterial metabolic waste products, i.e. bacterial acids.

Despite increased preventive efforts, fixed orthodontic treatment still entails the risk of enamel demineralization (Attin *et al.*, 2005; Lovrov *et al.*, 2007). Furthermore, none of the preventive strategies have the potential to inhibit bacterial adhesion on bracket surfaces *ab initio*. Consequently, a preferable method to inhibit the detrimental effects of fixed orthodontic therapy is the development and clinical implementation of bracket surfaces with anti-adhesive characteristics.

Therefore, the objective of the present study was to compare biofilm formation on uncoated brackets with those coated with polytetrafluoroethylene (PTFE).

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 participant and his/her parents.

Using nQuery Advisor 5.0 (Statistical Solutions, Saugus, Massachusetts, USA), power and sample sizes were calculated. Power calculation revealed that a sample size of four would have a power of 80 per cent to detect a difference in means of 18.2 [e.g. a first condition mean (μ_1) of 22.2 and a second condition mean (μ_2) of 4.0], assuming that the standard deviation of the differences was 7.8. In the case of the bracket debonding, the subject was considered as a dropout.

A total of 13 consecutive adolescent patients (five females and eight males, aged between 8 and 16 years, mean 11.2±2.8 years) who received orthodontic treatment were included in the present study. Criteria for inclusion were need for orthodontic treatment in the late mixed dentition with symmetric primary teeth in at least one jaw. Criteria for exclusion were systemic diseases, pharmacological, or antibiotic therapy 6 weeks before the start of and/or during the study, a history of periodontal disease, excluding gingivitis, the presence of carious lesions, and congenitally missing permanent teeth. The patients were instructed not to seek professional tooth cleaning during the study and not to use antibacterial mouth rinses.

Of a total of 26 sterile brackets (Victory Series; 3M Unitek, Monrovia, California, USA), 13 were coated with food-safe PTFE after sandblasting with 54 μm Al_2O_3 (Adelhelm Kunststoffbeschichtungen GmbH, Eningen, Germany). To avoid bracket debonding, PTFE was removed from the mesh by sandblasting with 110 μm Al_2O_3. During this procedure, a dental silicone embedding material (Silagum; DMG Chemisch Pharmazeutische Fabrik GmbH, Hamburg, Germany) was used to protect the PTFE-coated facial surfaces. For all 26 brackets, a 0.016×0.022 inch stainless steel segment of archwire (OrthoForm; 3M Unitek) was fixed with of two elastomeric ligatures (Alastik; 3M Unitek).

In all patients, one PTFE-coated bracket and one uncoated bracket were temporarily and symmetrically bonded (Transbond; 3M Unitek) to the first or second primary molars (four maxillary and nine mandibular) for a period of 8 weeks (Figure 1a and b). Selection of the right and left quadrants was randomized using a random list. Before bracket bonding, the teeth were sandblasted for 3–4 seconds with 50 μm Al_2O_3 (Microetcher; Danville Engineering, San Ramon, California, USA) and etched with orthophosphoric acid for 30 seconds (Conditioner 36; DeTrey, Konstanz, Germany).

After 8 weeks, the brackets were debonded with orthodontic pliers and the archwire, including the elastomeric ligatures, was removed. The brackets were then gently rinsed with sterile water and air-dried.

Analysis of quantitative biofilm formation was performed with the Rutherford backscattering detection (RBSD) method, a scanning electron microscopy (SEM) technique (Leo 1455 VP; Carl Zeiss SMT AG, Oberkochen, Germany). For each bracket, a total of five RBSD photomicrographs were obtained with identical views from the mesial, distal, occlusal, cervical, and buccal aspects. After obtaining the RBSD micrographs, positive findings of biofilm coverage were validated using SEM at high magnification.

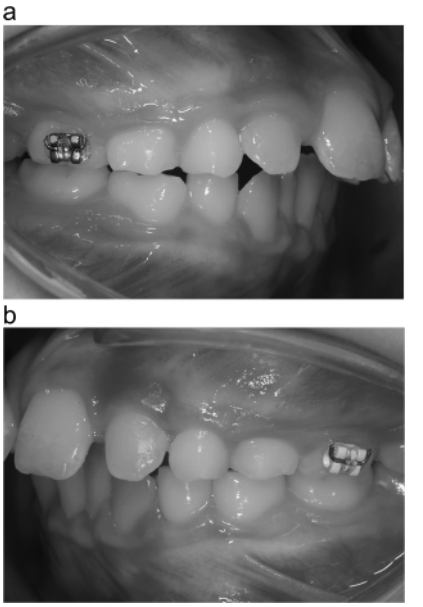

Figure 1 Intraoral view of the uncoated (a) and polytetrafluoroethylene-coated (b) premolar bracket bonded to primary second molars, including a segmented 0.016×0.022 inch stainless steel archwire and two elastic modules per bracket.

The extent of biofilm-covered surfaces on the RBSD photomicrographs was calculated using surface analysis software (Image J 10.2 for Apple; National Institutes of Health, Bethesda, Maryland, USA). On these photomicrographs, biofilm coverage appears as dark areas and uncoated surfaces as bright areas as a result of different atomic weights (Figure 2a and b). Firstly, the regions of interest on each photomicrograph were marked. The threshold value for ideal representation of biofilm-covered surface in the region of interest was then determined. Depending on these different grey values, biofilm coverage was calculated for each photomicrograph after conversion to a binary display (Figure 3a–d; Chin *et al.*, 2006).

Documentation and evaluation of the data were performed with the Statistical Package for Social Sciences Version 16.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Reproducibility of surface analysis was assessed by repeated measurements of 10 randomly selected photomicrographs applying the method of Bland and Altman (1986). Firstly, the means and standard deviations of absolute and relative biofilm coverage were calculated for each view of a bracket. Furthermore, total biofilm formation was calculated for both surface modifications. Data were compared using a two-sided paired *t*-test. For multiple tests, Bonferroni corrected levels of *P*-values were calculated. All tests were performed at a significance level of $\alpha = 0.05$.

Results

A total of four dropouts were recorded during the study, due to bracket loss. Therefore, analysis of quantitative biofilm

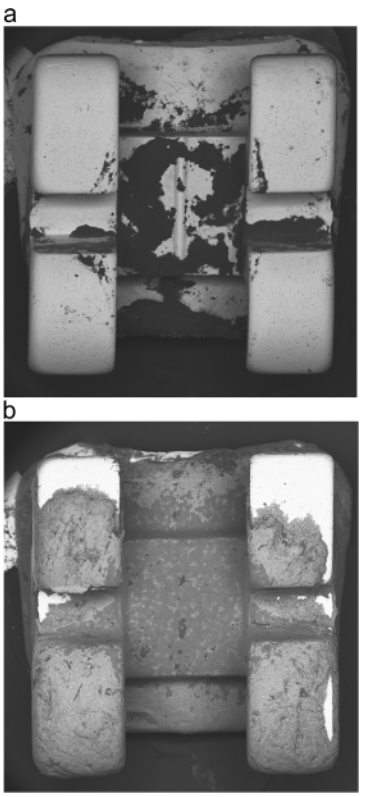

Figure 2 Rutherford backscattering detection method photomicrographs of an uncoated (a) and a polytetrafluoroethylene-coated bracket (b) as viewed from the buccal aspect. Both brackets were obtained from the same patient. Dark areas indicate adherent biofilm. The bright surfaces in Figure 2b represent abraded surfaces due to high shear forces.

formation was based on a total of 90 photomicrographs. A total area of 21.7 ± 0.7 mm^2 per bracket was investigated. The results of total biofilm analysis on brackets are shown in Figure 4. On PTFE-coated brackets, total biofilm formation was 4.0 ± 3.6 per cent (0.9 ± 0.8 mm^2) of the bracket surface and on uncoated brackets 22.2 ± 5.4 per cent (4.8 ± 1.2 mm^2). The results of pairwise comparison with respect to location are shown in Table 1. For all locations, significantly less biofilm formation was found on PTFE-coated surfaces compared with stainless steel surfaces. After Bonferroni correction, the difference in biofilm formation was not significant for the cervical surfaces.

For PTFE-coated brackets, the highest values of biofilm formation were found on the mesial surfaces (6.8 ± 4.4 per cent) and the lowest on the occlusal surfaces (1.2 ± 1.2 per cent). On uncoated brackets, the greatest biofilm accumulation was present on the distal surfaces (29.7 ± 17.0 per cent), whereas the lowest values were found on the occlusal surfaces (16.0 ± 7.2 per cent).

A total of 13.9 ± 5.2 per cent (3.0 ± 1.2 mm^2) of PTFE-coated brackets were abraded. Abrasion was found in regions with high shear forces (occlusal bracket wings).

As regards the reproducibility of surface analysis, the empirical standard deviation for biofilm coverage was 0.5 ± 1.2 per cent, indicating good reproducibility.

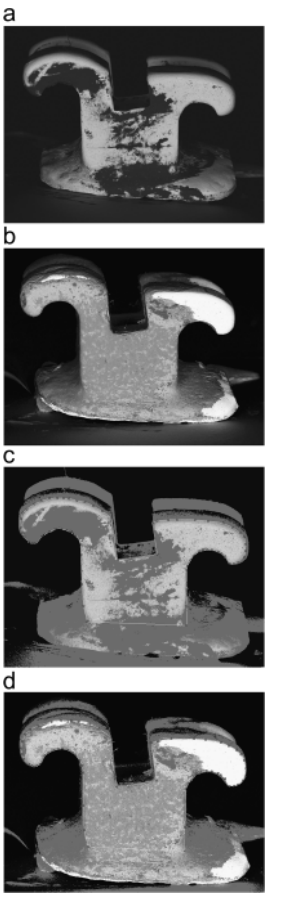

Figure 3 Converted Rutherford backscattering detection method photomicrographs of an uncoated (a) and a polytetrafluoroethylene-coated (b) bracket viewed from the approximal aspect. Biofilm coverage was calculated based on grey levels (c and d).

Discussion

In the present study, mid-term analysis of biofilm formation on orthodontic brackets was performed in adolescent patients. This age cohort was selected because it is the major treatment group in orthodontics, and these patients possess primary teeth, which can be used for study purposes without inducing lesions on permanent teeth. Primary teeth were selected for bonding, as cleaning and finishing of bonded tooth surfaces induces enamel loss of about 50 μm (Al Shamsi *et al.*, 2007). Furthermore, mechanical bracket debonding entails the risk of enamel fractures (Stratmann *et al.*, 1996). As clinical studies involving adolescents are associated with ethical concerns, sample size was reduced to the minimum with respect to statistical requirements.

Biofilm formation on alloplastic materials endangers the integration of medical devices and the integrity of human tissues. Consequently, the clinical implementation of anti-adhesive and antibacterial surfaces for medical purposes are a major goal in current research (Fu *et al.*, 2006; Coughlan *et al.*, 2008). PTFE was selected as the coating in the present study since clinical research has shown that the use of PTFE coatings reduces bacterial adhesion on medical devices (Berry *et al.*,

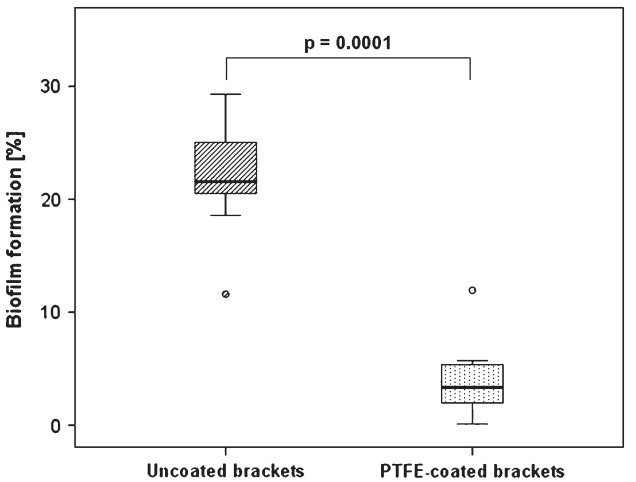

Figure 4 Total biofilm formation of uncoated and polytetrafluoroethylene-coated brackets.

Table 1 Mean values and comparison of biofilm formation on uncoated and polytetrafluoroethylene (PTFE)-coated surfaces with respect to location (per cent).

	Uncoated	PTFE coated	*P*-value	*P*-value (Bonferroni)
Buccal	17.7±4.4	2.7±3.2	0.0001	0.0005
Occlusal	16.0±7.2	1.2±1.2	0.0001	0.0005
Cervical	21.8±11.7	6.4±9.5	0.043	0.215
Mesial	28.1±9.9	6.8±4.4	0.0001	0.0005
Distal	29.7±17.0	3.8±3.1	0.001	0.005

2000). So far, PTFE has been used in the field of orthodontics to reduce the frictional resistance between the archwire and the bracket slot, but not as an anti-adhesive coating to reduce biofilm formation (De Franco *et al.*, 1995; Husmann *et al.*, 2002; Bortoly *et al.*, 2008). The PTFE was white coloured to demonstrate that an anti-adhesive coating also fulfils aesthetic demands of patients during fixed orthodontic treatment.

An archwire segment was engaged using elastic ligatures in all brackets to provide an environment with plaque-retentive sites that can be compared with treatment conditions. Elastic ligatures are known to induce an increase in biofilm accumulation (Turkkahraman *et al.*, 2005). Iatrogenic biofilm dislodgement during the removal process was reduced by minimizing the contact surface when placing the pliers on the bracket wings. To ensure biofilm formation that can be compared with long-term orthodontic treatment, the investigation was performed over the specific period of 8 weeks (Ristic *et al.*, 2007).

In the present study, quantitative analysis of biofilm formation on brackets was performed using the RBSD technique and surface analysis software. This method has been previously used to detect biofilm on supra- and subgingival surfaces of orthodontic bands and dental implant abutments (Elter *et al.*, 2008; Demling *et al.*, 2009). With the RBSD technique, element-contrast photomicrographs are obtained by showing surfaces with low atomic weight (such as oral biofilms) as darker grey values than surfaces with higher atomic weight (such as stainless steel). The representation of three-dimensional structures such as brackets in two-dimensional photographs results in distortion. In this study, the problem of distortion was overcome, as the photomicrographs were obtained from identical views, data were compared pairwise, and relative biofilm formation was compared.

On uncoated brackets, biofilm was found on 22.2±5.4 per cent of the surfaces. This amount of biofilm is comparable with that on other intraoral alloplastic materials, such as titanium (Heuer *et al.*, 2007). On uncoated brackets, the biofilm was mostly located on surfaces that are not accessible with a toothbrush or the tongue. These findings can be explained by reduced shear forces in these regions, where bacteria are protected from mechanical removal and hydrodynamic effects such as saliva flow or movement of oral soft tissues (Quirynen and Bollen, 1995; Hannig, 1999).

In contrast, only 4.0±3.6 per cent of the coated surfaces were covered with biofilm. Even on occult and covered surfaces, hardly any biofilm was found on coated brackets. These results can be explained by the material characteristics of PTFE, which consists of carbon and fluorine, and can be chemically described as a fluoropolymer. These fluorocarbons exhibit a high electronegativity and, as a consequence, are not susceptible to dispersive factors, such as Van der Waals forces. The exploitation of dispersive forces is considered to be the predominant attachment mechanism for microorganisms on hard intraoral surfaces (Eliades *et al.*, 1995).

Results of biofilm analysis showed a relatively high standard deviation, representing interindividual differences in the amount of biofilm-covered surfaces. This can be explained by covariables, such as nutrition, tongue activity, and oral hygiene.

Pairwise comparison of data showed a significantly lower biofilm formation for all bracket locations. These results demonstrate that the anti-adhesive effect of a biofilm-reducing PTFE coating can be found on the complete bracket. However, after Bonferroni correction, the differences were not significant for the cervical surfaces. The values for biofilm formation on cervical surfaces indicate that in a larger cohort, significant differences between uncoated and PTFE-coated brackets would also have been found for this location.

On surfaces with high shear forces, the PTFE coating was partially abraded. The coated brackets used in this research must be considered prototypes. Long-term stability of the PTFE coating might be improved by application of other coating techniques or selective coating of surfaces with low shear forces (Luo *et al.*, 2008).

Conclusions

Uncoated orthodontic brackets are highly susceptible to biofilm formation that endangers the integrity of oral hard and soft tissues by means of decalcification and periodontal disease. A PTFE coating on brackets reduced the biofilm

formation to a minimum. Although the coating was partially abraded on surfaces exposed to high shear forces, the results of the study are encouraging. In the future, coatings with long-term stability could contribute to reduced biofilm accumulation on fixed orthodontic appliances.

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