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Influence of bracket design on microbial and periodontal parameters in vivo

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Abstract

Aim: To compare undisturbed plaque formation on teeth bonded with different types of orthodontic brackets with non-bonded control teeth, via a de novo plaque growth experiment over a 7-day period.

Material and methods: A randomized controlled trial with split-mouth design was set up enroling 16 dental students. Within each subject sites with Speed®(S) and GAC®(G), brackets and control sites were followed. Clinical periodontal parameters were recorded at baseline, on days 3 and 7. Microbiological samples were taken from the brackets and the teeth on days 3 and 7.

Results: Both anaerobe and aerobe colony-forming units (CFU) were significantly higher in S-sites than in G-sites ($p = 0.0002$, $p = 0.02$). The shift from aerobic to anaerobic species was observed earlier in S-sites than in G-sites. The aerobe/anaerobe CFU ratio was significantly lower in S-sites than in G-sites ($p = 0.01$). On day 3, the crevicular fluid flow was significantly higher in S-sites than in control sites ($p = 0.01$). On day 7, S-sites and G-sites showed a significantly higher crevicular flow than control sites (both $p < 0.0001$). More hypertrophy was seen in S- than in G- and control sites ($p = 0.05$). No significant differences for bleeding on probing were observed.

Conclusion: Bracket design can have a significant impact on bacterial load and on periodontal parameters.

Key words: bracket design; crevicular fluid; dental plaque; orthodontics; pocket depth

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Adverse reactions due to orthodontic treatment with fixed appliances have been described. Besides decalcification, leading to white spots and eventually caries (Chang et al. 1997, Glasspoole et al. 2001, Benson et al. 2003, 2005, VanMiller & Donly 2003), periodontal harm may occur (Diedrich 1989, Wehrbein & Diedrich 1992a, b, Ellis & Benson 2002). One of the adverse periodontal reactions is a hyperplastic/hypertrophic form of gingivitis, which, in our clinic, is estimated to occur in one out of 10 patients treated with fixed orthodontic appliances. This reaction might be elicited by a change in microbiological environment (Lee et al. 2005).

As the consequences for oral health are uncertain and as periodontal breakdown at young age could eventually put a burden on oral health in the long term, interruption of the orthodontic treatment is often advised when a hyperplastic/hypertrophic form of gingivitis is diagnosed.

It is well established that bacterial plaque is the primary aetiological cause of gingival inflammation and periodontitis (Loe et al. 1965). The quantity as well as the quality of plaque, which spontaneously forms after a tooth is thoroughly cleaned and pumiced, is influenced by many factors including surface characteristics (Quirynen et al. 1988, 1989, 1990). Especially surface roughness and surface free energy were found to be positively correlated with plaque growth rate (Quirynen & Bollen 1995). The presence of gingival inflammation will further increase plaque growth (Quirynen et al. 1991, Ramberg et al. 1995). Besides the total amount of bacteria, also the ratio between the aerobic and anaerobic bacteria is an important aetiological factor in the development of gingivitis and periodontitis (Socransky et al. 1991).

As the design and the material characteristics of orthodontic bracket types vary considerably (Anhoury et al. 2002), plaque adhesion and therefore also the induction of gingivitis (Loe 1965) might

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differ among currently used bracket types.

Till date no de novo plaque formation assay has been set up with orthodontic brackets in the mouth. The same holds true for randomized-controlled trials, as only case reports considering few patients have been published (Zachrisson & Zachrisson 1972, Shelley 1981, Alexander 1991).

Aim of the Study

It has been claimed by the manufacturer that due to the design, self-ligating brackets (Speed®) accumulate less dental plaque than traditional twin brackets, resulting in fewer signs of gingival inflammation. The aim of this study was to compare the microbiological environment, the clinical periodontal parameters and the crevicular fluid flow between the Speed®- and GAC®-bonded teeth with control teeth by means of a de novo plaque growth experiment with a duration of 7 days.

The hypothesis was that Speed® brackets would accumulate less plaque than traditional twin brackets, resulting in fewer signs of gingival inflammation.

Material and Methods

Subjects

Sixteen dental students from the Catholic University of Leuven (nine females and seven males, Caucasians aged between 17 and 27 years) participated (see Table 1). They were given a written explanation of the background of the study, its objectives and their involvement. After screening for their suitability and after good comprehension of the protocol, they all gave their written informed consent. During the experiment the participants could always contact the researcher for questions or remarks.

The initial placement of the brackets was performed via a randomized protocol by means of concealed envelopes.

The students were selected to fulfill the following inclusion criteria: no smoking, absence of extensive dental restorations or adhesive-fixed partial dentures, a sulcus bleeding index (Muhlemann & Son 1971) of <0.3 and no antibiotics during or up to 4 months before the study.

The students were also asked whether they already received an orthodontic treatment with fixed appliances, because this might have consequences for smoothness of the buccal enamel (Eliades et al. 2004) and as such on the microbial adhesion in the early formation of a dental plaque film (Quirynen et al. 1989, 1990).

The design of this study was approved by the Ethical Committee of the Catholic University of Leuven.

Experimental procedure

Experimental design

The study had a randomized, examiner blind, split-mouth design. In every student the mouth was divided into four quadrants, two of which served as controls. Two types of brackets, Speed® (Strite Industries, Cambridge, Ontario, Canada) and GAC® (GAC, Central Islip, NY, USA) were used (Fig. 3).

For the split-mouth comparison, 12 different sites were defined: the first and second pre-molars and the first molar of each quadrant. As one student had her first pre-molars extracted in the upper jaw, the canines were used instead.

The brackets were placed in contralateral antagonistic quadrants. The first quadrant used for bracket placement and the order in which the brackets were placed were randomly chosen by means of concealed envelopes, the second one was at the other side of the mouth in the antagonistic jaw. The GAC® (G-sites) and Speed® (S-sites) bonded teeth were alternated, giving rise to four different experimental settings (Table 2).

The teeth bonded with the different brackets were compared with each other and with the non-bonded control sites (Fig. 1).

Before the study all students received oral hygiene instructions in order to ensure a healthy periodontal situation. During the study period the students visited the clinic three times (Table 3), the first time (on day 0) to record the status praesens of the periodontium [periodontal pocket depth (PPD) indicating the gingival overgrowth and or swelling, the crevicular fluid flow, bleeding on probing (BOP)] and to place the brackets. On the second visit (on day 3) the brackets were removed from the first pre-molars and the clinical measurements for these teeth were repeated. On day 7 the students came for the last clinical measurements, the removal of the last brackets and for a thorough cleaning and polishing of the teeth.

On day 3 we compared four first premolars, one bonded with a Speed® bracket, one bonded with a GAC® bracket and two non-bonded control teeth. On day 7, the other brackets were removed and then we compared the S-sites (one second pre-molar bonded with a Speed® bracket and one molar bonded with a Speed® tube) and the G-sites (one second pre-molar bonded with a Speed® bracket and one molar bonded with a Speed® tube) to the control sites (two non-bonded premolars and two non-bonded molars).

After removal of the dental plaque at baseline, all teeth were stained with erythrosine disclosing solution (4% erythrosine in water solution) to make sure there was no dental plaque remaining. From then on, the students were only

Table 1. General information on study population with data on previous orthodontic treatment, age, and gender distribution

	N	Previous treatment with fixed appliance		Age (years)	
		−	+	mean	SD
Male	7	2	5	20.8	0.8
Female	9	3	6	21.0	2.6
Total	16	5	11	20.9	1.9

Table 2. Example of the split-mouth design applied to a single patient

G-site	S-site	G-site	Control	Control	Control
16	15	14	24	25	26
46	45	44	34	35	36
Control	Control	Control	S-site	G-site	S-site

S-site, tooth bonded with a Speed® bracket; G-site, tooth bonded with a GAC® bracket; control, non-bonded tooth.

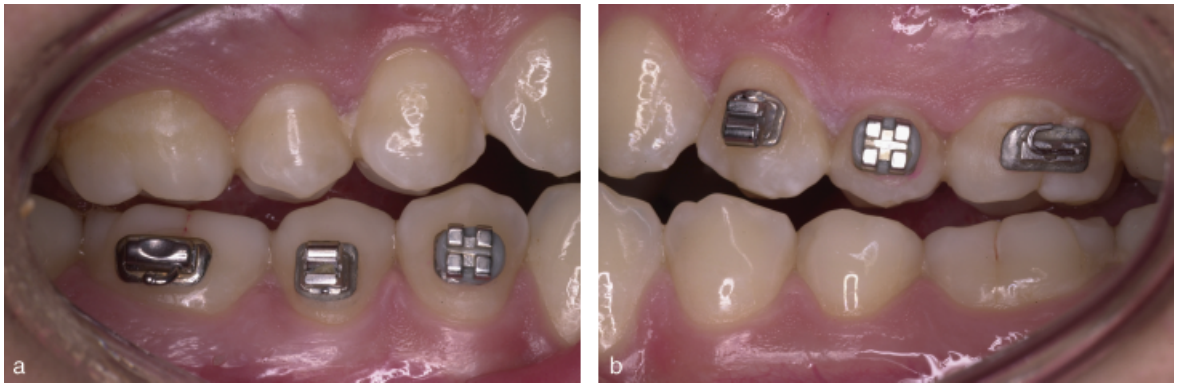

Fig. 1. Clinical example of the experimental setting on day 3 before plaque disclosure, with Speed® brackets bonded on teeth 24, 26, and 35; and GAC® brackets on teeth 25, 34, and 36.

Table 3. Flow chart of the study

	Intake	Day 0	Day 3	Day 7
Scaling and polishing		x		x
Crevicular fluid sampling		x	x	x
PPD measuring		x	x	x
BOP measuring		x	x	x
Bracket placement		x		
Removal brackets PM1			x	
Removal brackets PM2 and M2				x
Oral hygiene instruction	x	x	x	x

BOP, bleeding on probing; PPD, periodontal pocket depth.

allowed to clean the palatal and lingual surfaces of all teeth and the labial surfaces of their incisors with a single tufted brush. As such, the undisturbed plaque formation could be followed over time.

Bracket placement/removal

The quadrants to bond were isolated with cotton rolls and saliva suction. The enamel was etched locally with 37% phosphoric acid for 30 s and rinsed rigorously with water afterwards. The etching was very accurately carried out by means of disposable mini sponge applicators (3M ESPE, St Paul, USA) to avoid contact of the etching product with the gingiva. After rinsing with water excessively and drying, the sites were inspected for the characteristic dull, white, frosted appearance of adequately etched enamel. Re-etching never seemed necessary.

By means of the mini sponges, the primer (Concise™, 3M Unitek, Monrovia, CA, USA) was applied in such way that it fully covered the etched enamel. Using these sponges made it easy to etch and prime only a little surface, which was fully covered by the bracket afterwards. The two components of bonding material (Concise™, 3M Unitek) were mixed and directly applied to the bracket base. The bracket was pressed firmly onto the enamel surface and any excess of adhesive was removed. The brackets were positioned along the long axis of the teeth, above the gingival margin, and an attempt was made to place the brackets 3 mm from the gingival margin.

The involved teeth were stained with erythrosine to verify the baseline conditions as the primer might capture some pigment and give a pink shine after disclosure. Attention was paid to prevent the pink shine to be mistaken for dental plaque during the study.

After debonding, all the adhesive was removed from the teeth with a carbide bur (Komet, H282 204 010, Rock Hill, SC, USA). After drying, the teeth were analysed again for remaining adhesive. When the teeth were free of bonding material, they were cleaned with curettes, pumiced and received a fluoride application.

Periodontal parameters

At baseline, on days 3 and 7 (before and after plaque disclosing), digital colour photos were taken to follow the undisturbed plaque formation (Figs 1 and 2). At the same occasions the following parameters were scored (on day 3 only the first pre-molars and on day 7 the remaining teeth): crevicular fluid flow, probing depths and BOP.

After isolation of the teeth from saliva with cotton rolls and gently drying to prevent contamination, the brackets were removed with a sterile debonding plier and the supra-gingival dental plaque was taken away with sterile curettes. This was carried out without traumatizing the gingiva and without disturbing the plaque film on the remaining sites.

The supra-gingival plaque and the bracket were transferred into flip-capped vials containing 2.0 ml of pre-reduced transport medium (RTF) (Syed & Loesche 1972). The dental plaque and the bracket(s) removed from teeth with the same bracket type were pooled. Also the plaque from all control teeth was pooled at days 3 and 7, respectively.

Microbial samples were not taken at baseline for two reasons: first of all, there was only very little dental plaque available because of the good oral hygiene, and secondly, the possible inter-individual differences at the start of the experiment are erased by comparing different sites in one subject (the split-mouth protocol).

Each sample was homogenized by vortexing for 30 s and coded. The formulation used was not revealed until all analyses were completed, leading to a blind microbiological analysis.

The Periopaper® (#593525, Ora Flow Inc., Amityville, NY, USA) absorbent strips were used to collect crevicular fluid (Griffiths 2003). The crevicular fluid was sampled at the buccal crevice of the test and control teeth (baseline, day 3 and 7, respectively) as depicted in Table 3. Dental plaque was carefully removed without traumatizing the

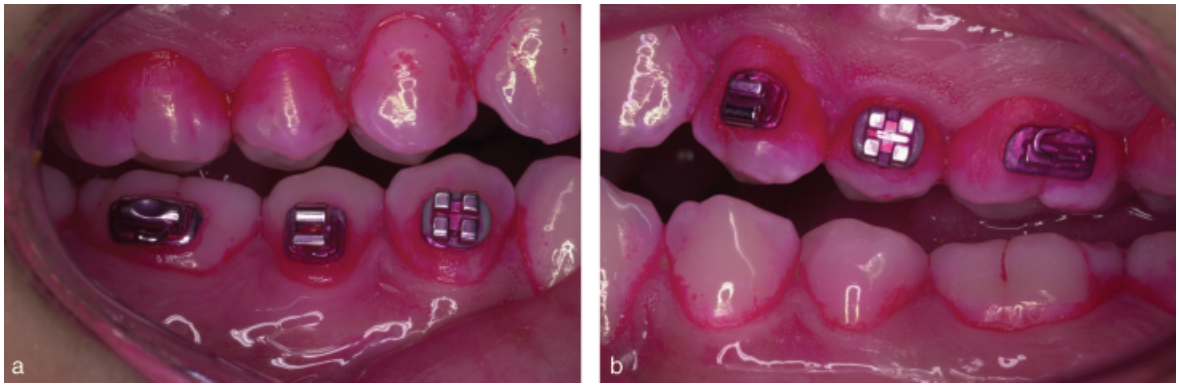

Fig. 2. Clinical example of the experimental setting on day 3 after plaque disclosure, with Speed® brackets bonded on teeth 24, 26, and 35; and GAC® brackets on teeth 25, 34, and 36.

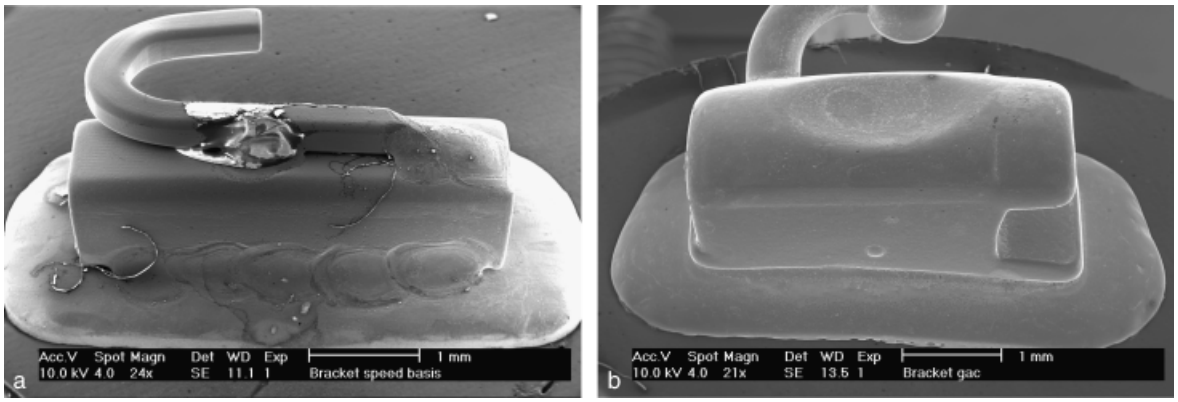

Fig. 3. Scanning electron microscopic images of the different brackets, the Speed® bracket on the left and the GAC® bracket on the right.

Table 4. Amount of colony-forming units (CFU) and the CFU ratio (aerobe/anaerobe) per site, days combined

	CFUaerobe	*p*-value	CFUanaerobe	*p*-value	CFU ae/anae	*p*-value
Averages per site						
S-sites	1.20E+06		2.20E+06		0.57	
G-sites	6.40E+05		8.70E+05		0.73	
Control	2.70E+05		4.10E+05		0.62	
Differences between sites						
S-G	6.00E+05	0.02	1.30E+06	0.0002	−0.16	0.01
S-Control	9.60E+05	<.0001	1.70E+06	<.0001	−0.05	0.54
G-Control	3.70E+05	0.001	4.60E+05	0.002	0.12	0.05

The first part displays the averages per site, the second part the differences between the sites with the corresponding *p*-values.
ae, aerobe; anae, anaerobe.

gingiva as this would increase the production of crevicular fluid (Tanaka et al. 1998). The presence of dental plaque has also been shown to have a marked effect on the recorded volume of crevicular fluid in the strip (Stoller et al. 1990, Griffiths et al. 1992, Griffiths 2003). The samples were taken after isolating the sites from saliva with cotton rolls and gently drying to prevent contamination. Periopaper® strips were placed into the sulcus until light resistance was experienced (Griffiths 2003).

After keeping the strip in place for 30 s, the absorbed volume was measured with the Periotron® 6000 (Ora Flow Inc.). As environmental factors can affect the rate of fluid evaporation from paper strips (Tozum et al. 2004), the measurements were carried out within 5 s after removal of the strip from the crevice to minimize evaporation. Four strips were used per tooth.

The pocket depths were measured at the buccal sides of the teeth: both proximal and strictly buccal for the premolars, and proximal, mesio-buccal, mid-buccal and disto-buccal for the molars. The pocket depths were measured with a Merrit B® Probe (Hufriedy, Chicago, IL, USA) and rounded off to the nearest 0.5 mm. The BOP for each of the above-mentioned sites per tooth was also registered, 20 s after probing the depth of the pocket (absent = 0, present = 1). These parameters were scored at days 0, 3 and 7 and the examiner was blinded to the previous scores.

Culture techniques

All samples were transferred to the laboratory and processed in <12 h. Serial 10-fold dilutions were prepared in RTF.

Dilutions of 10^{-3}–10^{-5} were plated in duplicate by means of a spiral plater (Spiral Systems® Inc., Cincinnati, OH, USA) onto non-selective blood agar plates (Blood Agar Base II®, Oxoid, Basingstoke, UK), supplemented with

Table 5. Amount of colony-forming units (CFU) and the CFU ratio (aerobe/anaerobe) per site displayed per day

Day	Materials	CFU aerobe		CFU anaerobe		CFU ae/anae	
		value	SD	value	SD	value	SD
Averages per site							
3	S-sites	1.23E+06	2.51E+06	1.93E+06	2.04E+06	0.64	0.38
	G-sites	4.62E+05	5.36E+05	5.77E+05	6.64E+05	0.80	0.27
	Control	1.84E+05	5.83E+05	2.26E+05	7.38E+05	0.70	0.46
7	S-sites	1.23E+06	1.01E+06	2.40E+06	1.82E+06	0.51	0.41
	G-sites	8.80E+05	8.58E+05	1.31E+06	1.12E+06	0.67	0.36
	Control	3.94E+05	3.70E+05	7.28E+05	5.16E+05	0.54	0.37

Day	Materials	Difference	*p*	Difference	*p*	Difference	*p*
Differences between sites							
3	S-G	7.64E+05	0.12	1.35E+06	0.009	− 0.17	0.58
	S-Control	1.04E+06	<0.0001	1.70E+06	<0.0001	− 0.07	0.96
	G-Control	2.78E+05	0.16	3.51E+05	0.08	0.10	0.90
7	S-G	3.53E+05	0.8	1.09E+06	0.25	− 0.16	0.05
	S-Control	8.39E+05	0.0007	1.68E+06	0.0005	− 0.03	0.99
	G-Control	4.86E+05	0.04	5.81E+05	0.28	0.13	0.08

The first part displays the averages per site with the corresponding standard deviations, the second part the differences between the sites with the corresponding *p*-values.
ae, aerobe; anae, anaerobe.

Table 6. Periotron® read-outs per site displayed per day

	Crevicular fluid volume		
	day	materials	value	SD
Absolute values				
	3	S-sites	31.58	16.00
		G-sites	22.85	18.23
		Control	19.36	9.22
	7	S-sites	38.10	15.28
		G-sites	38.51	16.16
		Control	25.27	10.89

Day	Materials	Difference	*p*
Difference between sites			
3	S-G	8.73	0.30
	S-Control	12.23	0.01
	G-Control	3.49	0.93
7	S-G	− 0.41	1.000
	S-Control	12.83	< 0.0001
	G-Control	13.24	<0.0001

The first part displays the averages per site with the corresponding standard deviations, the second part the differences between the sites with the corresponding *p*-values.

haemine (5 mg/ml), menadione (1 mg/ml) and 5% sterile horse blood.

After 7 days of anaerobic (80% N_2, 10% CO_2 and 10% H_2) and 3 days of aerobic incubation at 37° C, the total number of anaerobic and aerobic colony-forming units (CFU) were counted. From these data, the CFU ratio [CFUaerobe/CFUanaerobe (CFUae and CFUanae)] was also calculated.

Statistics

A linear mixed model was fit to the data. Teeth nested within jaws and students were used as the experimental unit. This allowed to control for both inter-subject and intra-mouth variability. To ensure a correct interpretation of the *p*-values, residual values were tested for normality by means of a normal quantile plot. For those variables where the residuals were not normally distributed, a log transformation was applied to the data.

ANOVA tables were made to see whether there was an interaction effect between time and treatment and whether the separate material and day effects were significant. When the separate effects were significant without a significant interaction, group means were taken over all groups of the other factor for comparison. As such the averages were taken over more data to give more power to the significance tests. When a significant interaction effect was found, all the groups were analysed separately. Whether the groups were taken together or separately, their comparison was always corrected with the Tukey–Kramer correction for simultaneous hypothesis testing. The provided *p*-values are rounded off up to the first significant digit.

Moreover, a distinction was made between the subjects with more and less than average formation of crevicular fluid on day 0. The statistical analyses were repeated on these two groups separately to see whether there was a significant difference in the reaction to the placement of brackets between both subgroups.

Results

Periodontal parameters

Crevicular fluid

The crevicular fluid volume showed a significant different material effect over time (Table 6). Because of this significant interaction effect, the data of days 3 and 7 could not be combined. An overall increased crevicular fluid flow from day 3 to 7 was obvious . On day 3 there was only a significantly higher flow in S-sites compared with control sites ($p = 0.01$), but on day 7 the flow was significantly higher in S- and G-sites *versus* control sites (both $p < 0.0001$). The difference between S- and G-sites never reached a level of significance (day 3, $p = 0.30$, day 7, $p = 1.00$).

Pocket probing

On day 1 no significant inter-material differences in PPD were present ($p = 0.9997$, 0.9999 1.00) (Table 7). The increase in probing depth (Table 8) was significantly higher for S-sites compared to G- and control sites ($p = 0.05$). The latter was due to changes at the proximal sites ($p = 0.03$) as the mid-buccal sites showed no significant changes. The portion of sites BOP clearly increased over time for all sites ($p < 0.0001$), but inter-site differences were not detected ($p = 0.44$).

Microbiological parameters

The lack of a significant interaction effect for CFUae, CFUanae and CFU ratio CFUae/CFUanae legitimated combination of the data on days 3 and 7 (Table 4). The numbers of aerobic and anaerobic CFU in supragingival plaque samples from the different sites showed significant material differences (Table 4). S-sites in general allowed more plaque formation than G-sites. In Table 5 the results are separately depicted per day and per material. S-sites showed significantly higher CFUae than G-sites ($p = 0.02$) and both G- and S-sites had higher values than control sites ($p = 0.001$ and $p < 0.0001$, respectively) (Table 4). The CFUae were significantly higher for all sites on day 7 when

compared with day 3 ($p = 0.01$). Also for the CFUanae, significant differences between the materials were seen (Table 4). The interaction effect between day and site was borderline significant ($p = 0.1$). In Table 5 the data are depicted per day. On day 3, the CFUanae were only significantly higher in the S-sites when compared with control sites ($p < 0.0001$). On day 7, more anaerobic species were seen and both S- and G-sites showed significantly higher values than the control sites ($p = 0.0007$ and 0.04, respectively). Between S- and G-sites no significant differences for CFUanae were seen either on day 3 or on day 7.

The ratio CFUae/CFUanae was significantly lower for both S- and control sites, than for G-sites ($p = 0.01$ and 0.05, respectively) (Table 4 and Fig. 4). When the data per day were separated, there was only one significant difference for the S-sites compared to the G-sites (Table 5). In this case, on day 7 the CFU ratio (CFUae/CFUanae) was significantly lower in the S-sites compared with G-sites ($p = 0.05$).

When a distinction was made between the subjects with more and less than average formation of crevicular fluid on day 1, no significant differences in the reaction on bracket placement for both the microbiological and clinical periodontal parameters were recorded. The same can be said about the subjects that received previous orthodontic treatment. No differences in undisturbed plaque growth were seen compared with the non-treated students.

Table 7. The mean pocket depth measurements (PPD) in millimeters, displayed per day and per site with the corresponding standard deviations (SD)

Day	Materials	PPD	SD
1	S-sites	2.02	0.40
	G-sites	2.06	0.35
	Control	2.05	0.38
3	S-sites	2.40	0.34
	G-sites	2.22	0.27
	Control	2.30	0.36
7	S-sites	2.30	0.41
	G-sites	2.27	0.39
	Control	2.23	0.37

PPD, periodontal pocket depth.

Discussion

This de novo plaque growth experiment with split-mouth design succeeded in detecting some significant differences between the bonded teeth and the non-bonded control teeth and also between the Speed®- and GAC®-bonded teeth. We could observe more significant differences between the different sites when all the means were combined and averaged over all days, not taking in account whether they were recorded on days 3 or 7. Of course, this is only statistically valid if no interaction effect is present. Depicting the data per day reduced the total number of measurements per category and therefore also the power of the statistical analysis.

The study was designed to make a valid comparison on days 3 and 7. On day 3 the four first pre-molars, one bonded with a Speed® bracket, one with a GAC® bracket and two non-bonded control teeth, were compared. On day 7 the other brackets were removed and then the Speed®-bonded teeth (one second pre-molar bonded with a Speed® bracket and one molar-bonded with a Speed® tube) and the GAC®-bonded teeth (one second pre-molar bonded with a GAC® bracket and one molar-bonded with a GAC® tube) were compared with the control teeth (two non-bonded pre-molars and two non-bonded molars). By combining a bracket and a tube of one system, we compensated the fact that a molar tube has a different design and a bigger surface than a pre-molar bracket.

The use of dental students with good oral health as subjects for this study is important as several studies indicated an increased plaque accumulation in the presence of gingival inflammation (Quirynen et al. 1991, Rowshani et al. 2004). This increased plaque formation has also been shown in experimental gingivitis studies (Quirynen et al. 1991, Daly & Highfield 1996). As we were most interested in the early dental plaque formation, the duration of the study was set at 7 days. This was also positive for the compliance of the dental students as longer periods would have resulted in a decrease in compliance of non-brushing. It was rather easy to verify the presence of undisturbed dental plaque formation; first of all the regularity of the biofilm on the bonded teeth could be easily observed. Secondly, the tooth mesial of the teeth under investigation served as control (in most cases the canine); when the dental plaque on the buccal surface of this tooth was not disturbed, the more distal teeth were supposed to be untouched.

To ensure blind evaluation, the measurements on day 1 were carried out before the randomization (before opening the concealed envelopes). On days 3 and 7 the measurements were carried out after removal of the brackets so that blinded measuring was ensured again. The researcher did not know which bracket was bonded on which tooth. All laboratory analyses were performed with an unknown coding system, which was only revealed after the completion of the study.

The increased probing depth recorded during this study is most likely caused by gingival enlargement or by deeper penetration of the probe into the weakened junctional epithelium. As these two processes could simultaneously contribute to the increase in probing depth, a distinction between these processes cannot be made with the instruments used in this study. During the relatively short period of this study, gingivitis was induced, but attachment loss probably did not occur (Zachrisson & Zachrisson 1972, Kloehn & Pfeifer 1974).

The increase in crevicular fluid, described before, is unlikely to be induced by the procedure of bracket

Table 8. Increase in periodontal pocket depth (PPD) compared to day 1 in millimeters between the different sites, calculated over all the measurements (PPD-tot) and proximal (PPD-prox) sites

	Difference in PPD with day 1				
	materials	average	materials	difference	*p*-value
PPD-tot	S-sites	0.35	S-G	0.16	0.05
	G-sites	0.19	S-control	0.14	0.05
	Control	0.21	G-control	− 0.02	0.93
PPD-prox	S-sites	0.44	S-G	0.23	0.03
	G-sites	0.21	S-control	0.17	0.09
	Control	0.27	G-control	0.06	0.69

PPD, periodontal pocket depth.

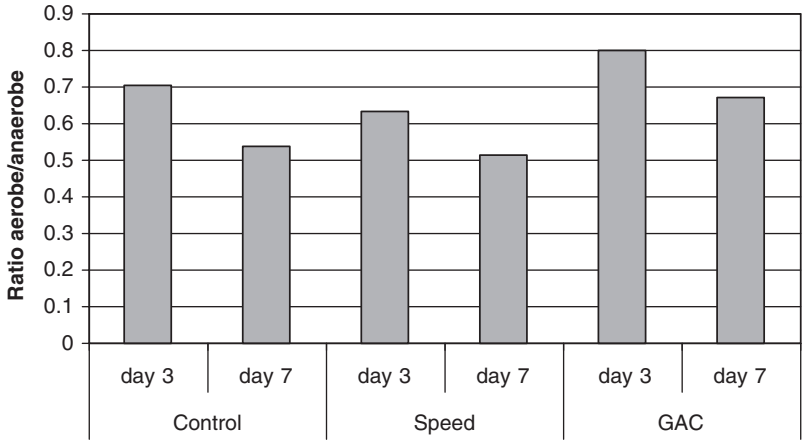

Fig. 4. Mean colony forming units ratio (*y*-axis) on the different sites depicted per day.

placement alone. Phosphoric acid is widely used as an etching product in both adhesive dentistry and in orthodontics but necrotizing effects on periodontal soft tissues have been reported (Forsberg 1982). White ulcerative lesions were described in cases where phosphoric acid came accidentally in contact with the mucosa for several minutes (Akman et al. 2005). To avoid these adverse effects in this study, phosphoric acid was applied locally at a safe distance from the gingival margin with a little non-soaked mini-sponge. After 30 s the tooth was excessively rinsed with water.

Dental adhesives can have adverse effects on the gingiva too. They are toxic to the gingival fibroblasts in vitro (Huang et al. 2002). Particularly the residual monomers may cause gingival inflammation and irritation (Gioka et al. 2005). The same gingival contact prevention protocol as used for phosphoric acid was applied for the bonding material. The bonding material was directly polymerized and thus contact with the gingival margin was prevented. These were the reasons why we assumed that these two products did not contribute to the inflammatory response seen in this de novo plaque growth experiment. Etching and bonding of the control teeth were not considered because of this prevention protocol and the fact that we were interested in the effects of bracket placement, compared with non-treated (non-bonded) teeth.

Besides the crevicular flow, the BOP tendency is an other parameter to quantify gingival inflammation and was significantly higher on day 7 compared with baseline for all sites. There were however no significant inter-site differences detected on the different days. A possible explanation for the lack of significant inter-material differences was possibly due to the lower discriminative value of this parameter.

Comparison of the bonded teeth to the non-bonded control teeth showed a faster undisturbed plaque formation in the bonded sites, two to eight times more CFU were counted (Tables 4 and 5). These results are consistent with some changes in crevicular fluid flow and the PPD measurements (Tables 7 and 8). Concerning the crevicular flow, neither on day 3 nor on day 7, significant differences between the two bracket types were seen. But the higher bacterial load and the lower CFU ratio (CFUae/CFUanae) at the S-sites might have resulted in a faster increase in crevicular fluid flow there. The crevicular flow at the S-sites was already significantly higher than at the control sites on day 3, whereas the G-sites did not show higher crevicular flows compared with the control sites earlier than day 7.

A significantly higher crevicular flow in the S-sites *versus* control sites on day 3 ($p = 0.01$) was seen without a significant difference in CFU ratios (CFUae/CFUanae) between these two groups (Tables 5 and 6). Nevertheless there was a significant increase in absolute values of both aerobe and anaerobe CFU, which could be the explanation. On day 7, the G-sites showed significantly higher crevicular flows than the control sites, without a significantly lower CFU ratio (CFUae/CFUanae) and without significantly higher anaerobe CFU.

The fact that the CFU ratio (CFUae/CFUanae) was lower in the control sites than in the G-sites was another unexpected outcome. A possible explanation is that the dental plaque retrieved from the control sites is mostly formed in strict contact with the gingival margin as well as the inter-dental space (Fig. 4). These two niches are predominantly colonized by anaerobe bacteria. The total amount of bacterial plaque however was higher on G-sites than on the control teeth. This was expected as the total surface available for plaque adhesion is significantly larger and more irregular at the G-sites. The natural automatic cleaning of the teeth by food mastication is not or less present with brackets in place. The lower CFU ratio (CFUae/CFUanae) as mentioned above was not supported by the crevicular fluid flow, which was lower in the control sites. This implies that in this experiment, the CFU ratio (CFUae/CFUanae) alone is not responsible for the development of gingivitis, but also the total amount (absolute values) of bacteria.

Significant differences were seen for the increase in pocket depth (Table 8) and CFUae, CFU anae and the CFU ratio (CFUae/anae) (Table 4). The increased CFU counts in the S-sites compared with the G-sites were not expected because of the limited dimensions of the Speed® bracket and the presence of a smooth clip instead of an elastomeric ligature. To find differences in surface characteristics between the two bracket types, scanning electron microscopic (SEM) images with several enlargement factors were taken. These qualitative SEM images revealed remarkable irregularities on the interfaces between the different parts of the Speed® attachments (both of the bracket and the tube). These parts seem to be welded together causing an irregular surface, which might have lead to the increased plaque adhesion in the S-sites.

Because randomized-controlled trials of this type have not been performed so far, it is not possible to compare our findings to those of other authors. Different orthodontic bracket types have not been compared microbiologically and clinically in vivo yet. Nevertheless there are indications that placement of orthodontic fixed appliances has an impact on the microbiological characteristics of the dental plaque. Lee et al. (2005) found significant differences in the prevalence of putative periodontal pathogens in subgingival dental plaque from gingivitis lesions in orthodontic patients. Their study succeeded in detecting significant differences

between the subgingival dental plaque of gingivitis lesions in patients with and without orthodontic fixed appliances. *T. forsythia, T. denticola and P. nigrescens* were significantly more common in the samples obtained from the orthodontic patients than in the samples obtained from the non-orthodontic control patients (Lee et al. 2005). Their results as well as ours indicate that the local changes associated with the wearing of orthodontic brackets may affect the prevalence of periodontal pathogens in dental plaque. However, no differences in PPD were seen between their groups and no different bracket types were evaluated. Also the orthodontic group might have received more hygiene instructions resulting in a better oral hygiene, making the two groups difficult to compare.

Huser et al. (1990) performed clinical and bacterial examinations before the beginning of treatment and after placement of the orthodontic appliances up to 90 days. Plaque index and bleeding scores increased significantly on banded teeth as compared with control sites, the probing depth however remained within normal values for both test and control groups. The composition of dental plaque was only determined by dark-field microscopy and showed significant shifts in the test sites after banding with an increase in the percentage of spirochetes, motile rods, filaments, and fusiforms. During the same period no significant changes in the bacterial distribution were observed in the control group (Huser et al. 1990). Petti et al. (1997) performed a similar study with a duration of 6 months. They compared the influence of fixed appliances with removable orthodontic appliances on supra and subgingival microflora. Periodontal parameters were not examined. Their data suggest that in well-motivated patients keeping up with the oral hygiene, gingivitis and periodontitis did not occur during the first 6 months of treatment. The significant modification of oral microbiota, shown by subjects with fixed appliances however, is in line with our findings and suggests that the risk for gingivitis in the following months of therapy is still high and the risk for periodontitis cannot be excluded (Petti et al. 1997).

Regardless of the level of plaque control, many subjects undergoing fixed orthodontic treatment develop generalized gingivitis within a short time (Zachrisson & Zachrisson 1972, Kloehn & Pfeifer 1974, Alexander 1991, Sallum et al. 2004). The qualitative change in the microbiota, which involves the growth of periodontopathogenic bacteria, could be associated with the gingival inflammation around the orthodontic brackets. Different orthodontic bracket materials could therefore have dissimilar clinical manifestations.

Further research should be performed to visualize the potentially different periodontal complications of different orthodontic bracket systems used in treatment with fixed appliances in such way that brackets can be designed to reduce plaque adhesion.

References

Akman, A. C., Demiralp, B., Guncu, G. N., Kiremitci, A. & Sengun, D. (2005) Necrosis of gingiva and alveolar bone caused by acid etching and its treatment with subepithelial connective tissue graft. *Journal of Canadian Dental Association* **71**, 477–479.

Alexander, S. A. (1991) Effects of orthodontic attachments on the gingival health of permanent second molars. *American Journal of Orthodontics and Dentofacial Orthopedics* **100**, 337–340.

Anhoury, P., Nathanson, D., Hughes, C. V., Socransky, S., Feres, M. & Chou, L. L. (2002) Microbial profile on metallic and ceramic bracket materials. *Angle Orthodontics* **72**, 338–343.

Benson, P. E., Pender, N. & Higham, S. M. (2003) Quantifying enamel demineralization from teeth with orthodontic brackets – a comparison of two methods. Part 2: validity. *European Journal of Orthodontics* **25**, 159–165.

Benson, P. E., Shah, A. A. & Willmot, D. R. (2005) Measurement of white lesions surrounding orthodontic brackets: captured slides vs digital camera images. *Angle Orthodontics* **75**, 226–230.

Chang, H. S., Walsh, L. J. & Freer, T. J. (1997) Enamel demineralization during orthodontic treatment. Aetiology and prevention. *Australian Dental Journal* **42**, 322–327.

Daly, C. G. & Highfield, J. E. (1996) Effect of localized experimental gingivitis on early supragingival plaque accumulation. *Journal of Clinical Periodontology* **23**, 160–164.

Diedrich, P. (1989) [Correlations of orthodontics and periodontics]. *Fortschritte der Kieferorthopadie* **50**, 347–364.

Eliades, T., Gioka, C., Eliades, G. & Makou, M. (2004) Enamel surface roughness following debonding using two resin grinding methods. *European Journal of Orthodontics* **26**, 333–338.

Ellis, P. E. & Benson, P. E. (2002) Potential hazards of orthodontic treatment – what your patient should know. *Dental Update* **29**, 492–496.

Forsberg, C. M. (1982) The effect of 50 per cent phosphoric acid on oral soft tissues. *Swedish Dental Journal* **15** (Suppl.), 37–40.

Gioka, C., Bourauel, C., Hiskia, A., Kletsas, D., Eliades, T. & Eliades, G. (2005) Light-cured or chemically cured orthodontic adhesive resins? A selection based on the degree of cure, monomer leaching, and cytotoxicity. *American Journal of Orthodontics and Dentofacial Orthopedics* **127**, 413–419.

Glasspoole, E. A., Erickson, R. L. & Davidson, C. L. (2001) Demineralization of enamel in relation to the fluoride release of materials. *American Journal of Dentistry* **14**, 8–12.

Griffiths, G. S. (2003) Formation, collection and significance of gingival crevice fluid. *Periodontology 2000* **31**, 32–42.

Griffiths, G. S., Wilton, J. M. & Curtis, M. A. (1992) Contamination of human gingival crevicular fluid by plaque and saliva. *Archives of Oral Biology* **37**, 559–564.

Huang, T. H., Tsai, C. Y., Chen, S. L. & Kao, C. T. (2002) An evaluation of the cytotoxic effects of orthodontic bonding adhesives upon a primary human oral gingival fibroblast culture and a permanent, human oral cancer-cell line. *Journal of Biomedical Materials Research* **63**, 814–821.

Huser, M. C., Baehni, P. C. & Lang, R. (1990) Effects of orthodontic bands on microbiologic and clinical parameters. *American Journal of Orthodontics and Dentofacial Orthopedics* **97**, 213–218.

Kloehn, J. S. & Pfeifer, J. S. (1974) The effect of orthodontic treatment on the periodontium. *Angle Orthodontics* **44**, 127–134.

Lee, S. M., Yoo, S. Y., Kim, H. S., Kim, K. W., Yoon, Y. J., Lim, S. H., Shin, H. Y. & Kook, J. K. (2005) Prevalence of putative periodontopathogens in subgingival dental plaques from gingivitis lesions in Korean orthodontic patients. *Journal of Microbiology* **43**, 260–265.

Loe, H. (1965) Physiology of the gingival pocket. *Academy review of the California Academy of Periodontology* **13**, 6–14.

Loe, H., Theilade, E. & Jensen, S. B. (1965) Experimental gingivitis in man. *Journal of Periodontology* **36**, 177–187.

Muhlemann, H. R. & Son, S. (1971) Gingival sulcus bleeding – a leading symptom in initial gingivitis. *Helvetica Odontologica Acta* **15**, 107–113.

Petti, S., Barbato, E. & Simonetti, D. A. (1997) Effect of orthodontic therapy with fixed and removable appliances on oral microbiota: a six-month longitudinal study. *New Microbiologica* **20**, 55–62.

Quirynen, M. & Bollen, C. M. (1995) The influence of surface roughness and surface-free energy on supra- and subgingival plaque formation in man. A review of the literature. *Journal of Clinical Periodontology* **22**, 1–14.

Quirynen, M., Dekeyser, C. & van, S. D. (1991) The influence of gingival inflammation, tooth type, and timing on the rate of plaque formation. *Journal of Periodontology* **62**, 219–222.

Quirynen, M., Marechal, M., Busscher, H., el-Abiad, M., Arends, J. & van, S. D. (1988) The influence of surface characteris-

tics on the early bacterial colonization of intra-oral hard surfaces. *Journal of Clinical Dentistry* **1** (Suppl. A), A14–A19.

Quirynen, M., Marechal, M., Busscher, H. J., Weerkamp, A. H., Arends, J., Darius, P. L. & van, S. D. (1989) The influence of surface free-energy on planimetric plaque growth in man. *Journal of Dental Research* **68**, 796–799.

Quirynen, M., Marechal, M., Busscher, H. J., Weerkamp, A. H., Darius, P. L. & van, S. D. (1990) The influence of surface free energy and surface roughness on early plaque formation. An in vivo study in man. *Journal of Clinical Periodontology* **17**, 138–144.

Ramberg, P., Axelsson, P. & Lindhe, J. (1995) Plaque formation at healthy and inflamed gingival sites in young individuals. *Journal of Clinical Periodontology* **22**, 85–88.

Rowshani, B., Timmerman, M. F. & Van der velden, U. (2004) Plaque development in relation to the periodontal condition and bacterial load of the saliva. *Journal of Clinical Periodontology* **31**, 214–218.

Sallum, E. J., Nouer, D. F., Klein, M. I., Goncalves, R. B., Machion, L., Wilson, S. A. & Sallum, E. A. (2004) Clinical and microbiologic changes after removal of orthodontic appliances. *American Journal of Orthodontics and Dentofacial Orthopedics* **126**, 363–366.

Shelley, W. B. (1981) Gingival hyperplasia from dental braces. *Cutis* **28**, 149–150.

Socransky, S. S., Haffajee, A. D., Smith, C. & Dibart, S. (1991) Relation of counts of microbial species to clinical status at the sampled site. *Journal of Clinical Periodontology* **18**, 766–775.

Stoller, N. H., Karras, D. C. & Johnson, L. R. (1990) Reliability of crevicular fluid measurements taken in the presence of supragingival plaque. *Journal of Periodontology* **61**, 670–673.

Syed, S. A. & Loesche, W. J. (1972) Survival of human dental plaque flora in various transport media. *Applied Microbiology* **24**, 638–644.

Tanaka, M., Hanioka, T., Kishimoto, M. & Shizukuishi, S. (1998) Effect of mechanical toothbrush stimulation on gingival microcirculatory functions in inflamed gingiva of dogs. *Journal of Clinical Periodontology* **25**, 561–565.

Tozum, T. F., Hatipoglu, H., Yamalik, N., Gursel, M., Alptekin, N. O., Ataoglu, T., Marakoglu, I., Gursoy, U. K. & Eratalay, K. (2004) Critical steps in electronic volume quantification of gingival crevicular fluid: the potential impact of evaporation, fluid retention, local conditions and repeated measurements. *Journal of Periodontal Research* **39**, 344–357.

VanMiller, E. J. & Donly, K. J. (2003) Enamel demineralization inhibition by cements at orthodontic band margins. *American Journal of Dentistry* **16**, 356–358.

Wehrbein, H. & Diedrich, P. (1992a) [The periodontal changes following orthodontic tooth movement – a retrospective histological study in humans. 1.]. *Fortschritte der Kieferorthopadie* **53**, 167–178.

Wehrbein, H. & Diedrich, P. (1992b) [The periodontal changes following orthodontic tooth movement – a retrospective histological study in man. 2.]. *Fortschritte der Kieferorthopadie* **53**, 203–210.

Zachrisson, S. & Zachrisson, B. U. (1972) Gingival condition associated with orthodontic treatment. *Angle Orthodontics* **42**, 26–34.

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Clinical Relevance

Scientific rationale for the study: So far, no de novo plaque growth study has been set up to monitor microbial as well as periodontal changes around bonded teeth. We compared these changes around non-etched and non-bonded teeth *versus* teeth bonded with two different bracket types by means of an RCT.

Principal findings: Some significant microbial and clinical differences were recorded between the teeth bonded with different bracket types and the control teeth.

Practical implications: The placement of brackets with different design can present different risks for periodontal disease at short time and the long-term results are not elucidated so far.