

## Microbial Adhesion on Different Bracket Types in vitro

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### ABSTRACT

**Objective:** To test the hypothesis that there are differences in total bacterial counts and capacity for biofilm formation between seven different bracket types.

**Material and Methods:** By means of an in vitro experiment, seven commercially available bracket systems (Damon [A], Clarity [B], Mystique [C], Speed [D], Victory MBT [E], Micro-loc [F], and Genus [G]) were compared. A total of 25 premolar brackets of each bracket system were incubated in brain heart infusion medium containing the saliva and bacteria of two orthodontic patients. After 72 hours, the amounts of aerobe and anaerobe bacteria were determined by counting the colony-forming units (CFU). The CFU ratio (aerobe/anaerobe) also was calculated, and the black pigmented bacteria were analyzed.

**Results:** Significant differences between the different bracket types in terms of biofilm formation were found. Bracket types can be arbitrarily divided into low, intermediate, and high plaque-retaining brackets. The group with low adhesion consists of bracket types E, F, and G; the group with high adhesion of bracket types A, B, and C; and type D exhibits intermediate adhesion. The group with high microbial adhesion (A, B, and C) did present significantly lower CFU ratios (aerobe/anaerobe) than were exhibited by the other bracket systems ( $P < .05$ ).

**Conclusion:** The hypothesis is accepted. Orthodontic brackets serve as different loci for biofilm formation; in this in vitro study, significant differences were noted between the different types of brackets. (*Angle Orthod.* 2009;79:915–921.)

**KEY WORDS:** Orthodontic bracket; Orthodontic appliance design; Microbiology; Dental plaque

### INTRODUCTION

In recent decades, a great increase has occurred in patients treated orthodontically.<sup>1</sup> Most patients seek orthodontic treatment to improve their dentofacial es-

thetics, and only a minority require treatment for medical or dental reasons. Despite the widespread use of fixed orthodontic appliances, little scientific evidence is available on the microbial implications of the different bracket systems in vivo.<sup>2</sup> The origin and pathogenesis of periodontal diseases are known to be multifactorial, but dental plaque certainly is an essential precursor.<sup>3</sup>

The placement of orthodontic brackets does create new locations for plaque retention, thereby increasing plaque adhesion and the inflammatory response.<sup>4</sup> Microbiological changes after bracket placement became a topic of interest during the late 1980s, and initially, cariogenic species such as *Streptococcus mutans* and *Lactobacillus* species, as well as the subsequent decalcification of enamel, were the main topics of interest among investigators.<sup>5,6</sup> Later on, the much more complex systems of periodontopathic microbes and their changes after bracket placement came into the picture.<sup>7–9</sup>

Van Gastel et al<sup>2</sup> succeeded by means of a randomized clinical trial with split-mouth design to detect significant differences, in vivo, between two different bracket systems. Observed microbiological changes

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**Table 1.** The different commercially available brackets used in this study

	Bracket <sup>a</sup>	Manufacturer	Material	Technique	Ligation
A	Damon 3	Ormco, Orange, Calif, USA	Metal	Self-ligating	No
B	Clarity	3M Unitek, Monrovia, Calif, USA	Ceramic	Straight wire	Yes
C	Mystique	GAC International, Bohemia, New York, USA	Ceramic	Straight wire	Yes
D	Speed	Speed System Orthodontics, Cambridge, Ontario, Canada	Metal	Self-ligating	No
E	Victory MBT	3M Unitek	Metal	Straight wire	Yes
F	Micro-Loc	GAC International	Metal	Straight wire	Yes
G	Generus	GAC International	Metal	Full edgewise	Yes

<sup>a</sup> Information about the different bracket systems used in this experiment: the commercial names, manufacturers, composition, for which orthodontic technique they are used, and whether or not these brackets need to be ligated by means of an elastomeric ring.

and significant differences between the two bracket types were confirmed by the gingival crevicular fluid flow rates and the periodontal pocket depths. Because of the relation between microbiological profiles of the different bracket types and the clinical reactions, it became a matter of interest to compare biofilm formation on different bracket types by means of an in vitro study. The aim of the present investigation was to compare early microbial adhesion to different brackets in vitro, in the same circumstances as they are used clinically.

## MATERIALS AND METHODS

### Brackets

Seven commercially available orthodontic brackets as shown in Table 1 were used in this study. These 175 brackets were maxillary premolar brackets; bracket types A to F were used with the Roth prescription and a 0.022-inch slot, and G had a 0.018-inch slot with no prescription. All but the self-ligating brackets were ligated with elastomeric rings (Ormco, Glendora, Calif) by the same person.

The brackets were placed randomly in a polyurethane box on a grid with an interbracket distance of 10 mm (Figure 1). Before the brackets were placed, the floor of the box was roughened with a diamond-coated burr at the exact places to bond the brackets, in such a manner that these areas were completely covered by the bracket bases. The brackets then were bonded with composite bonding material (Transbond Plus color change adhesive; 3M Unitek, Monrovia, Calif) that changes color from pink to white during the light-curing process. The composite was applied to the bracket base so as to cover the entire mesh, and the bracket was pressed firmly onto the box. Excessive adhesive was removed through a process that was facilitated by its pink color. Then the composite was light-cured (Dentsply QHL75 halogen curing light; Dentsply, Addlestone, Surrey, UK) for 30 seconds from both sides. The bracket placement procedure was done by one researcher (Dr van Gastel) under magnification (2.5×).

### Medium

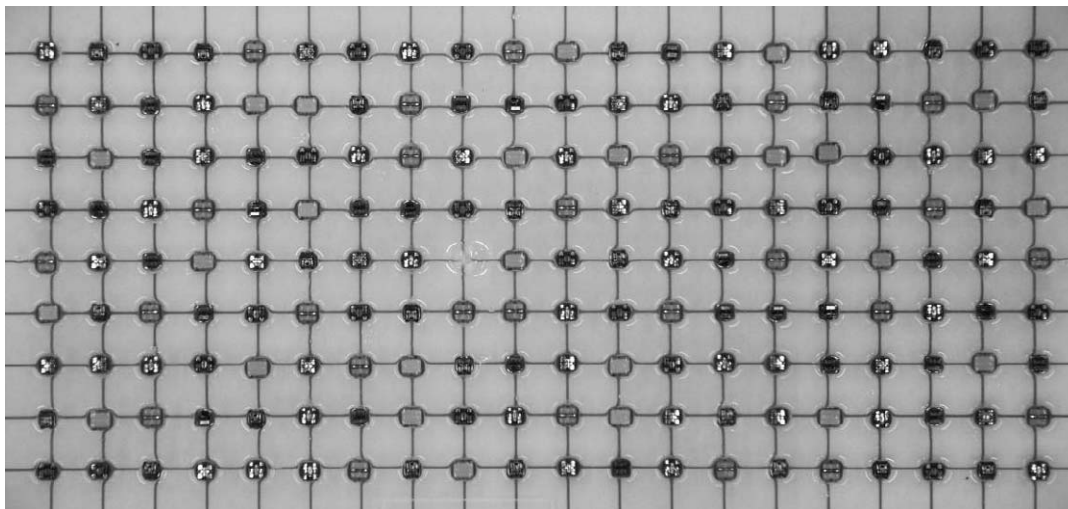
The supragingival plaque of two patients wearing fully bonded appliances was collected directly by means of sterile curettes before the start of the experiment and was transferred into a sterile flip-capped vial containing 2 mL RTF.<sup>10</sup> Saliva was collected too from these two patients, who had refrained from eating, drinking, and brushing for at least 2 hours before saliva collection. They had no acute dental caries or periodontal lesions, and they had received neither professional cleaning nor antibiotic medication in the 3 months before the study. Per patient, 5 mL unstimulated whole saliva was collected in a chilled sterile tube by the spitting method. The dental plaque and saliva were added to 1 L of Brain Heart Infusion (BHI), resulting in a concentration of  $8.0 \times 10^3$  CFU aerobe/mL and  $1.4 \times 10^4$  CFU anaerobe/mL.

The BHI medium containing bacteria and saliva of the orthodontic patients was inserted into the container in which the brackets were carried and was incubated in an aerobe oscillating incubator for 72 hours at 37°C and at 40 rpm.

### Bracket Removal

The medium was removed from the container and was analyzed microbiologically. Concentrations of bacteria in the medium at removal were  $8.3 \times 10^6$  CFU aerobe/mL;  $8.1 \times 10^6$  CFU anaerobe/mL; and  $5.3 \times 10^5$  CFU *Prevotella intermedia*/mL. The experimental setting then was washed with saline two times to remove all nonadhered bacteria.

The brackets were removed randomly with a sterile pliers and were transferred into a flip-capped vial containing 1 mL of prerduced transport medium (RTF) and coded.<sup>10</sup> The code used was not revealed, leading to a blind microbiological analysis. Just before the samples were diluted, all bacteria were removed from the brackets by sonication for 15 minutes. The samples then were homogenized by vortexing for 30 seconds and were processed in less than 15 minutes by preparing serial 10-fold dilutions in RTF.



**Figure 1.** A total of 25 premolar brackets of the seven different bracket systems were placed randomly in a polyurethane box with a grid with an interbracket distance of 10 mm.

### Culture Techniques

Dilutions of  $10^{-3}$  to  $10^{-5}$  were plated in duplicate by means of a spiral plater (Spiral Systems Inc., Cincinnati, Ohio) onto nonselective blood agar plates (Blood Agar Base II; Oxoid, Basingstoke, England), supplemented with 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  $37^\circ C$ , the total numbers of anaerobic and aerobic CFU, as well as the numbers of pigmented (black, green, brown) colonies (b.p.b. = black pigmented bacteria) on the representative nonselective anaerobic plate (containing approximately 100 colonies), were counted. From these data, the CFU ratio (CFU aerobe/CFU anaerobe) also was calculated. From the black-pigmented bacteria in the plaque samples, every third colony was subcultured on a blood agar plate. After 48 hours of anaerobic incubation, pure cultures were identified by means of DPCM, Gram staining, anaerobiosis, and a series of biochemical tests (including *N*-acetyl- $\beta$ -D-glucosaminidase,  $\alpha$ -glucosidase,  $\alpha$ -galactosidase,  $\alpha$ -fucosidase, esculine, indole, and trypsin activity) to differentiate *P. gingivalis* and *P. intermedia* from other pigmented *Porphyromonas* and *Prevotella* species.

### Statistics

All data were log 10-transformed. An ANOVA model followed by a Tukey multiple comparisons procedure was used to find significant differences between bracket types. The bracket types were divided into groups of high, intermediate, and low bacterial adhesion. Between the groups, significant differences were seen, and within one group, significant differences were not

detected. *P* values for the latter were corrected for simultaneous hypothesis testing according to Tukey-Kramer. *P* values below .05 were considered. For analysis of *Prevotella intermedia*, means and variances were estimated via a maximum likelihood model, taking into account measurements below detection limits. Residual analysis showed the presence of two outliers, which have been removed (one for bracket type B, one for type G).

## RESULTS

### CFU Aerobe (Table 2, Figure 2A)

Bracket types A, B, C, and D showed significantly higher aerobe CFU counts than did bracket types E, F, and G ( $P < .05$ ). Types A, B, C, and D did not differ significantly from each other in terms of the aerobe CFU. Within the group of bracket types with low bacterial adhesion (E, F, and G), a ranking was evident: G showed significantly lower aerobe CFU counts than did E ( $P < .05$ ), and F is positioned in between without being significantly different from type E or G.

### CFU Anaerobe (Table 2, Figure 2A)

Bracket types A, B, and C exhibited highest anaerobe CFU counts. Bracket types E, F, and G showed the least microbial adhesion. Bracket type D has an intermediate value for CFU ANAE and shows significantly higher values than are noted in types E, F, and G ( $P < .05$ ). D showed significantly lower anaerobe CFU counts than were seen in A, B, and C ( $P < .05$ ).

### CFU Ratio (Table 2, Figure 2B)

For the CFU ratio (aerobe/anaerobe), bracket types A, B, and C showed the lowest ratio and therefore

**Table 2.** Comparisons between the different bracket types

Comparison <sup>a</sup>	CFU AE	P Value	CFU ANAE	P Value	CFU Pi	P Value	CFU Ratio (AE/ANAE)	P Value
B/A	0.9667	1	1.8122	.13	1.2820	.9989	0.5506	.001
C/A	0.8082	.98	1.3418	.86	0.8704	1	0.6794	.27
D/A	0.5401	.15	0.4515	.01	0.2664	.1513	1.6963	.04
E/A	0.1159	<.0001	0.1151	<.0001	0.0928	.0006	3.4072	<.0001
F/A	0.0685	<.0001	0.0618	<.0001	0.0073	<.0001	5.0548	<.0001
G/A	0.0376	<.0001	0.0318	<.0001	0.0532	<.0001	7.0974	<.0001
C/B	0.8362	.99	0.7406	.8427	0.6789	.9868	1.2340	.8888
D/B	0.5596	.22	0.2492	<.0001	0.2078	.0455	3.0811	<.0001
E/B	0.1199	<.0001	0.0635	<.0001	0.0724	.0001	6.1887	<.0001
F/B	0.0709	<.0001	0.0341	<.0001	0.0057	<.0001	9.1812	<.0001
G/B	0.0389	<.0001	0.0175	<.0001	0.0415	<.0001	12.8914	<.0001
D/C	0.6694	.65	0.3364	.0001	0.3061	.2613	2.4963	<.0001
E/C	0.1434	<.0001	0.0858	<.0001	0.1066	.0017	5.0153	<.0001
F/C	0.0848	<.0001	0.0460	<.0001	0.0084	<.0001	7.4405	<.0001
G/C	0.0465	<.0001	0.0237	<.0001	0.0611	.0001	10.4448	<.0001
E/D	0.2142	<.0001	0.2550	<.0001	0.3484	.5011	2.0091	.001
F/D	0.1267	<.0001	0.1368	<.0001	0.0275	.0028	2.9806	<.0001
G/D	0.0695	<.0001	0.0704	<.0001	0.1996	.0999	4.1841	<.0001
F/E	0.5913	.32	0.5365	.1	0.0788	.0952	1.4835	.2519
G/E	0.3243	.0002	0.2761	<.0001	0.5729	.9706	2.0826	.001
G/F	0.5484	.2	0.5146	.06	7.2661	.3498	1.4038	.45

<sup>a</sup> Comparisons between the different bracket types for the aerobic colony-forming units (CFU), the anaerobe CFU, the *Prevotella intermedia* CFU, and the CFU ratio (aerobe/anaerobe). Values are depicted as the ratio between two bracket types and corresponding *P* values. Damon (A), Clarity (B), Mystique (C), Speed (D), Victory MBT (E), Micro-loc (F), and Genus (G).

probably the most unfavorable biofilm. Within this group, a significant difference was noted only between A and B ( $P < .05$ ), with lower values for B. Bracket types E, F, and G formed the group with significantly higher CFU ratios than the rest ( $P < .05$ ), with G showing a significantly higher ratio than E ( $P < .05$ ). Type D was situated between the group with the higher and the group with the lower ratio and differed significantly from both groups ( $P < .05$ ).

#### CFU *Prevotella intermedia* (Table 2, Figure 2A)

Bracket types A, B, and C showed a high detection frequency of *P intermedia*. CFU *P intermedia* counts of bracket types A, B, and C were significantly higher than in the other types ( $P < .05$ ), but no significant differences were noted between types A, B, and C. Bracket types E, F, and G showed the lowest CFU *P intermedia* counts and showed no significant intra-group differences. Bracket type D exhibited significantly lower *P intermedia* counts than did A, B, and C and significantly higher *P intermedia* counts than did E, F, and G ( $P < .05$ ).

#### DISCUSSION

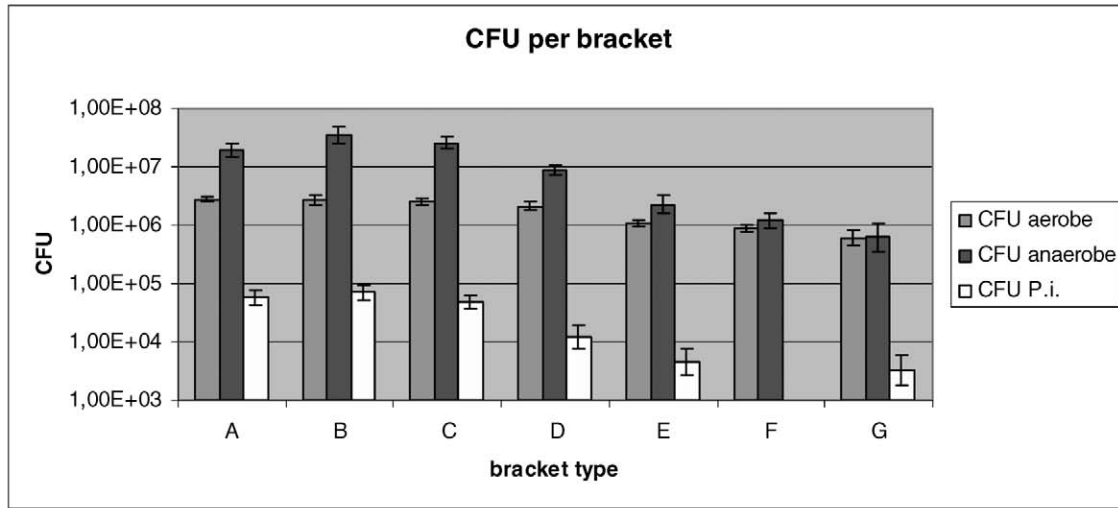
A pilot study indicated that the presence of saliva in the medium was necessary to give adhesion of the oral bacteria to the bracket surfaces. Saliva of the patient donating the dental plaque was used so an optimal interaction would occur between the salivary com-

ponents and the microorganisms; this is recognized as the primary step in biofilm formation and associated diseases.<sup>11</sup> All brackets were placed in one recipient to ensure the same environmental circumstances for each bracket. Because of random placement of the brackets, differences in shear forces due to the medium could not contribute to differences in microbial adhesion between bracket types. The experimental setting as used in this study is an uncommon way to grow anaerobe biofilms. The oscillating incubator and the polyurethane box do not provide an anaerobic environment, and BHI certainly is not the first-choice medium to grow anaerobes. We wanted to simulate the aerobic oral environment, and this probably underestimates the number of anaerobe species, but it will be true for all bracket types. The fact that we did find anaerobe species (*P intermedia*) most likely will be due to the mutual protective environment between different species in the multispecies biofilm. The same process makes it possible to find anaerobic species in supra-gingival dental plaque.

To ensure blind evaluation, all laboratory analyses were done with an unknown coding system, which was revealed only after completion of the study. Elastomeric rings are said to promote bacterial adhesion compared with metal ligatures.<sup>12</sup> To compare the brackets in a fair way, the non-self-ligating brackets were ligated with an elastomeric ligature because this was most comparable to the clinical situation. Results

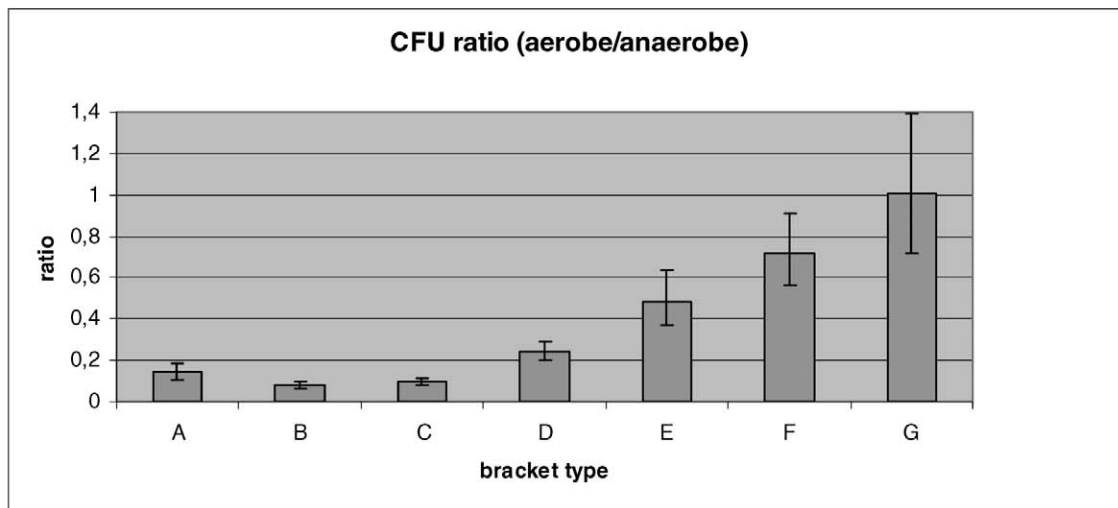


A.



Colony-forming units (CFU)/bracket for the different bracket types, with a logarithmic scale. Values are displayed as the mean and the 95% confidence interval. Damon (A), Clarity (B), Mystique (C), Speed (D), Victory MBT (E), Micro-loc (F) and Generus (G).

B.



CFU ratio (aerobe/anaerobe) for the different bracket types. Values are displayed as the mean and the 95% confidence interval. The lower the CFU ratio (aerobe/anaerobe) the more pathogenic the microfilm. Damon (A), Clarity (B), Mystique (C), Speed (D), Victory MBT (E), Micro-loc (F) and Generus (G).

**Figure 2.** (A) Colony-forming units (CFU)/bracket for the different bracket types, with a logarithmic scale. Values are displayed as the mean and the 95% confidence interval. Damon (A), Clarity (B), Mystique (C), Speed (D), Victory MBT (E), Micro-loc (F), and Generus (G). (B) CFU ratio (aerobe/anaerobe) for different bracket types. Values are displayed as the mean and the 95% confidence interval. The lower the CFU ratio (aerobe/anaerobe), the more pathogenic are the microbiota. Damon (A), Clarity (B), Mystique (C), Speed (D), Victory MBT (E), Micro-loc (F), and Generus (G).

of this study indicate major differences between the different brackets in terms of adhesion of supragingival oral microbes. The bracket types can be arbitrarily divided into groups with low, intermediate, and high microbial adherence. Bracket types E, F, and G showed

low microbial adhesion; bracket types A, B, and C high adherence; and type D is intermediate. Bracket types that presented high microbial adhesion also showed lower CFU ratios (aerobe/anaerobe) and higher CFU *P. intermedia* counts, probably because of a thicker

and more mature biofilm, leading to a more anaerobic environment at the base of this biofilm. *P. intermedia* is known to be one of the species that are elevated in orthodontic patients compared with nonorthodontic controls.<sup>13</sup>

In our study, both ceramic brackets (E and F) were in the high microbial adhesion group and displayed the lowest CFU ratios (aerobe/anaerobe); this is congruent with the findings of some researchers.<sup>14,15</sup> Others did not find significant differences between brackets manufactured from different materials.<sup>16</sup> Eliades et al<sup>17</sup> studied the basic principles of bacterial adhesion. They compared the wettability and the composition of salivary films on orthodontic raw bracket materials through contact angle measurements. Stainless steel presented the highest critical surface tension and total work of adhesion, indicating an increased potential for microorganism attachment on metallic brackets. These findings are not in line with ours as we saw significantly higher adhesion on the ceramic brackets compared with the metal ones.

Anhoury et al<sup>16</sup> studied 32 metallic brackets and 24 ceramic brackets from orthodontic patients at the day of debonding and detected no significant differences between metallic and ceramic brackets with respect to caries-inducing *S. mutans* and *L. acidophilus* counts. Mean counts of 8 of 35 additional species differed significantly between metallic and ceramic brackets, with no obvious pattern favoring one bracket type over the other.<sup>16</sup>

Until now, only a few reports have described microbial alterations after placement of different bracket systems in vivo. Türkahraman et al<sup>12</sup> conducted a study to investigate the differences in microbial flora and periodontal status between two different arch wire ligation techniques. These authors observed that although teeth ligated with elastomeric rings exhibited slightly higher numbers of microorganisms than teeth ligated with steel ligature wires, the differences were not statistically significant and thus can be ignored. Moreover, the two arch wire ligation techniques showed no significant differences in terms of gingival index, plaque index, or pocket depth of bonded teeth. However, teeth ligated with elastomeric rings were more prone to bleeding.<sup>12</sup>

In our study, brackets ligated with an elastomeric ring did not per se show higher bacterial counts compared with self-ligating brackets. For the metallic brackets (A, D, E, F, and G), we can even conclude that the self-ligating brackets showed significantly higher ( $P < .05$ ) bacterial counts and a lower CFU ratio (aerobe/anaerobe) than were seen with classic brackets with elastomeric rings in this in vitro study. Scanning electron microscopic (SEM) images with several enlargement factors revealed remarkable ir-

regularities on the interfaces between different parts of the self-ligating brackets.<sup>2</sup> These parts seem to be welded together, causing an irregular surface, which might have led to increased biofilm formation on the self-ligating brackets.

Results of this present study are congruent with those of our previous study.<sup>2</sup> Van Gastel et al<sup>2</sup> compared plaque formation on teeth bonded with bracket types D and G by means of an RCT with a split-mouth design with nonbonded control teeth. In that study, both anaerobic and aerobic colony-forming units (CFU) were significantly higher in teeth bonded with bracket D than in teeth bonded with bracket G ( $P = .0002$  and  $P = .02$ , respectively). The shift from aerobic to anaerobic species was observed earlier in teeth bonded with bracket D than in teeth bonded with bracket G. The aerobe/anaerobe CFU ratio was also significantly lower in teeth bonded with bracket D than in teeth bonded with bracket G ( $P = .01$ ). These differences were also visible in the clinical periodontal parameters. In the present study, the D brackets did show significantly higher CFU counts (except for CFU *P. intermedia*) and lower CFU ratios than were seen in the G brackets.

Results from this in vitro study cannot be extrapolated to an in vivo setting. Additional comparisons by means of an RCT with several different bracket types could fill the lacuna in this field of clinical orthodontics.

## CONCLUSION

- Orthodontic brackets serve as different loci for biofilm formation; in this in vitro study, significant differences were noted between the different types of brackets.

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