Microbial Profile on Metallic and Ceramic Bracket Materials

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Abstract: The placement of orthodontic appliances creates a favorable environment for the accumulation of a microbiota and food residues, which, in time, may cause caries or exacerbate any pre-existing periodontal disease. The purpose of the present study was to compare the total bacterial counts present on metallic and ceramic orthodontic brackets in order to clarify which bracket type has a higher plaque retaining capacity and to determine the levels of *Streptococcus mutans* and *Lactobacillus* spp on both types of brackets. Thirty-two metallic brackets and 24 ceramic brackets were collected from orthodontic patients at the day of debonding. Two brackets were collected from each patient; one from a maxillary central incisor and another from a maxillary second premolar. Sixteen patients who used metallic brackets and 12 patients who used ceramic brackets were sampled. Bacterial populations were studied using ''checkerboard'' DNA-DNA hybridization, which uses DNA probes to identify species in complex microbial samples. The significance of differences between groups was determined using the Mann-Whitney *U*-test. Results showed no significant differences between metallic and ceramic brackets with respect to the caries-inducing *S mutans* and *L acidophilus* spp 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. This study showed higher mean counts of *Treponema denticola, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum ss vincentii, Streptococcus anginosus*, and *Eubacterium nodatum* on metallic brackets while higher counts of *Eikenella corrodens, Campylobacter showae*, and *Selenomonas noxia* were found on ceramic brackets. (*Angle Orthod* 2002;72:338–343.)

Key Words: Microbiota; Metallic brackets; Ceramic brackets; ''Checkerboard'' DNA-DNA hybridization

INTRODUCTION

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The oral environment provides the proper conditions for the colonization of a complex microbiota. In a healthy oral cavity, these microorganisms coexist in a balanced state with their host. But when changes occur in the normal oral environment, the balanced flora changes and imbalance and disease may result. Such changes can be brought about by the introduction of orthodontic appliances. In particular, metallic orthodontic brackets and bands have been found to induce specific changes in the buccal environment such as decreased pH and increased plaque accumulation,[1–3] elevated *S mutans* levels,[4–7] and increased *Lactobacillus* species.[8–11]

Although a large number of studies have shown a shift in microbial populations in the presence of orthodontic fixed appliances, limited information is available as to which bracket material would be less prone to adhesion of bacterial species and plaque accumulation. Lack of evidence regarding the plaque retaining capacity of ceramic and plastic brackets led Eliades et al[12] to study the wettability of orthodontic bracket material and the composition of salivary films absorbed onto them after 30 and 60 minutes in vivo exposure. Raw materials used for metallic, ceramic and plastic bracket manufacturing were obtained

from a manufacturer and their surface-free energy and work of adhesion were evaluated by 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. In a parallel study, Fournier et al[13] studied the affinity of *S mutans* to orthodontic brackets made from metal, plastic, and ceramic, however, their findings seemed to indicate that adherence of *S mutans* is weaker to metal than to plastic or ceramic brackets. In contrast, a recent in vitro study revealed that *Porphyromonas gingivalis* and *Escherichia coli* lipopolysaccharide adherence was greater on stainless steel brackets when compared to ceramic, plastic, and gold brackets.[14]

In light of the above information, no definite conclusion can be drawn about which bracket material has the least plaque retaining capacity. More information is needed in order to offer patients orthodontic treatment without significantly increasing their risk of developing white spots, caries, or gingival inflammation.

The objectives of the present investigation were to determine the levels of the caries-inducing *S mutans* and *L acidophilus* species on metallic and ceramic brackets and to compare the total bacterial counts and counts of species present on both bracket materials.

MATERIALS AND METHODS

Thirty-two metallic brackets and 24 ceramic brackets were collected from orthodontic patients on the day of debonding at the Boston University School of Dental Medicine Orthodontic Department and at private offices in the Boston area. Two brackets were collected from each patient: one from a maxillary central incisor and one from a maxillary second premolar. Sixteen patients were sampled from the metallic brackets group and 12 patients from the ceramic brackets group. The age range of the patients was 11 to 40 years. Brackets were transferred to 100 μL of TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH 7.6) and 100 μL of 0.5 M NaOH were added. Brackets were then stored frozen at −80°C until analysis by the "checkerboard" DNA-DNA hybridization technique.[15] This technique enables us to hybridize large numbers of DNA samples against large numbers of DNA probes on a single nylon membrane loaded with plaque samples.

Microbial analysis and DNA-DNA hybridization

Each plaque sample was analyzed by "checkerboard" DNA-DNA hybridization as described in detail by Socransky et al.[15] Each frozen sample was dispersed twice using a sonic oscillator before discarding the brackets. Plaque samples were then boiled in a water bath for 5 minutes. The samples were neutralized using 800 μL of 5 M ammonium acetate. The released DNA of plaque samples was placed into the extended slots of a Minislot-30 apparatus

(Immunetics, Cambridge, Mass), concentrated onto a 15 × 15 cm positively charged nylon membrane (Boehringer Mannheim, Indianapolis, Ind) and fixed to the membrane by cross linking under ultraviolet light. The Minislot-30 device permitted the deposition of up to 28 plaque samples and two standards in individual lanes on a single 15 × 15 cm nylon membrane. Two lanes on each membrane had standards that consisted of a mixture at 10^5 and 10^6 cells of each bacterial species tested.

The membranes were prehybridized at 42° C for 1 hour in 50% formamide, 5 × standard saline citrate (SSC; 1 × SSC = 150 mM NaCl, 15 mM Na citrate, pH 7.0), 1% casein (Sigma, St Louis, Mo), 5 × Denhardt's solution, 25 mM sodium phosphate (pH 6.5) and 0.5 mg/mL yeast RNA (Boehringer Mannheim). The membranes with fixed-sample DNA were placed back in a Miniblotter 45 device (Immunetics) with the "sample-lanes" rotated 90° to the channels of the apparatus. This produced a 30 × 45 checkerboard pattern. Each channel was used as a hybridizing chamber for separate digoxigenin-labeled genomic DNA from each species (probe).[15] A total of 37 such probes were used for the species shown in table 1. The probes were diluted to approximately 20 ng/mL in hybridization solution (45% formamide, 5 × SSC, 1 × Denhardt's solution, 20 mM Na phosphate, pH 6.5), 0.2 mg/mL yeast RNA, 10% dextran sulfate, 1% casein), placed in individual lanes of the Miniblotter and hybridized overnight at 42°C with the device sealed inside a plastic bag. Following hybridization, membranes were washed twice at high stringency for 20 minutes each time at 68°C in phosphate buffer (0.1 × SSC, 0.1% SDS) in a Disk Wisk apparatus (Schleicher and Schuell, Keene, NH).

Detection and enumeration of the organisms

Membranes were blocked by incubation in a blocking buffer containing 1% casein in maleic acid buffer (0.1 M maleic acid, 3 M NaCl, 0.2 M NaOH, 0.3% Tween 20, pH 8.0, and 0.5% casein) for 1 hour. Hybrids were then incubated with a 1:20,000 dilution of antidigoxigenin antibody conjugated with alkaline phosphatase (Boehring Mannheim) using the method described by Engler-Blum et al.[16] After washing with maleic acid buffer 2 times for 20 minutes each, and then for 5 minutes with detection buffer (0.1 M Tris HCL, 0.1 M NaCl, 50 mM MgCl2, pH 9.5), the membranes were incubated in AttoPhos (Amersham, Arlington, Ill) overnight at room temperature. The signals were detected by scanning the membranes at 1000 volts and 200 microns using a Storm Fluorimager (Molecular Dynamics, Sunnyvale, Calif). The sensitivity of this assay was adjusted to permit detection of 10^4 cells per sample of a given species by selecting the appropriate concentration of each DNA probe. This procedure was carried out in order to provide the same sensitivity of detection for each species.[15] Signals were converted to absolute counts by com-

TABLE 1. Mean Counts ($\times$ 10^5 $\pm$ SEM) of Bacterial Data on Metallic and Ceramic Brackets

	Metallic Brackets			Ceramic Brackets		
	Total Mean Counts	Anterior Mean Counts	Posterior Mean Counts	Total Mean Counts	Anterior Mean Counts	Posterior Mean Counts
S mutans	2.81 $\pm$ 0.44	2.47 $\pm$ 0.50	3.52 $\pm$ 0.91	2.21 $\pm$ 0.34	2.42 $\pm$ 0.35	2.98 $\pm$ 0.56
L acidophilus	3.06 $\pm$ 0.42	2.07 $\pm$ 0.36	3.78 $\pm$ 0.77	3.49 $\pm$ 0.39	3.10 $\pm$ 0.63	5.30 $\pm$ 0.83
B forsythus	0.09 $\pm$ 0.02	0.01 $\pm$ 0.01	0.13 $\pm$ 0.05	0.06 $\pm$ 0.01	0.03 $\pm$ 0.01	0.08 $\pm$ 0.03
A naeslundii1	6.56 $\pm$ 0.73	6.98 $\pm$ 1.05	8.44 $\pm$ 1.29	5.57 $\pm$ 0.52	6.49 $\pm$ 0.88	5.51 $\pm$ 0.75
T denticola**	0.14 $\pm$ 0.02	0.11 $\pm$ 0.03	0.19 $\pm$ 0.06	0.08 $\pm$ 0.02	0.07 $\pm$ 0.02	0.11 $\pm$ 0.04
P micros	1.87 $\pm$ 0.24	1.27 $\pm$ 0.17	2.99 $\pm$ 0.39	2.16 $\pm$ 0.28	1.93 $\pm$ 0.27	3.18 $\pm$ 0.78
S intermedius	0.99 $\pm$ 0.12	0.87 $\pm$ 0.13	1.49 $\pm$ 0.27	1.10 $\pm$ 0.25	0.75 $\pm$ 0.09	1.35 $\pm$ 0.31
N mucosa	4.09 $\pm$ 0.50	4.87 $\pm$ 0.96	5.24 $\pm$ 1.01	3.94 $\pm$ 0.45	3.97 $\pm$ 0.62	4.89 $\pm$ 0.97
P intermedia	1.92 $\pm$ 0.39	0.70 $\pm$ 0.13	3.80 $\pm$ 1.05	1.78 $\pm$ 0.30	1.36 $\pm$ 0.36	1.91 $\pm$ 0.65
P gingivalis	0.23 $\pm$ 0.04	0.11 $\pm$ 0.03	0.29 $\pm$ 0.06	0.16 $\pm$ 0.03	0.10 $\pm$ 0.03	0.19 $\pm$ 0.05
A actinomyce temcomitans*	0.60 $\pm$ 0.08	0.48 $\pm$ 0.16	0.88 $\pm$ 0.12	0.30 $\pm$ 0.03	0.21 $\pm$ 0.05	0.45 $\pm$ 0.05
T socranskii	0.53 $\pm$ 0.09	0.33 $\pm$ 0.08	0.85 $\pm$ 0.19	0.48 $\pm$ 0.07	0.46 $\pm$ 0.11	0.57 $\pm$ 0.14
E saburreum	3.99 $\pm$ 0.49	3.44 $\pm$ 0.60	6.90 $\pm$ 1.09	5.04 $\pm$ 0.69	5.16 $\pm$ 1.27	7.41 $\pm$ 1.31
F nucleatum ss vincentii***	3.70 $\pm$ 0.44	2.10 $\pm$ 0.29	5.84 $\pm$ 0.89	2.20 $\pm$ 0.42	1.32 $\pm$ 0.28	3.40 $\pm$ 1.09
C rectus	1.05 $\pm$ 0.17	0.38 $\pm$ 0.06	1.20 $\pm$ 0.20	0.91 $\pm$ 0.25	0.34 $\pm$ 0.09	0.77 $\pm$ 0.20
A naeslundii2	7.10 $\pm$ 0.75	8.34 $\pm$ 1.19	9.17 $\pm$ 1.73	6.37 $\pm$ 0.55	8.35 $\pm$ 0.97	6.19 $\pm$ 0.75
E corrodens***	2.83 $\pm$ 0.42	2.00 $\pm$ 0.46	4.23 $\pm$ 1.05	5.26 $\pm$ 0.58	4.37 $\pm$ 0.74	7.18 $\pm$ 1.06
S anginosus**	1.16 $\pm$ 0.11	1.21 $\pm$ 0.17	1.73 $\pm$ 0.25	0.75 $\pm$ 0.09	0.84 $\pm$ 0.12	0.70 $\pm$ 0.07
S sanguis	1.69 $\pm$ 0.19	1.66 $\pm$ 0.25	2.31 $\pm$ 0.34	2.08 $\pm$ 0.27	3.15 $\pm$ 0.53	2.11 $\pm$ 0.37
A gerencseriae	3.51 $\pm$ 0.35	3.07 $\pm$ 0.35	4.85 $\pm$ 0.64	4.53 $\pm$ 0.49	4.94 $\pm$ 0.60	3.86 $\pm$ 0.70
C ochracea	0.75 $\pm$ 0.16	0.27 $\pm$ 0.07	1.27 $\pm$ 0.36	0.98 $\pm$ 0.19	0.44 $\pm$ 0.17	1.16 $\pm$ 0.32
A israelii	6.59 $\pm$ 0.68	6.01 $\pm$ 0.86	9.46 $\pm$ 1.34	6.52 $\pm$ 0.53	7.00 $\pm$ 0.78	6.71 $\pm$ 0.90
E nodatum***	3.49 $\pm$ 0.58	2.09 $\pm$ 0.33	4.42 $\pm$ 0.95	1.64 $\pm$ 0.27	1.43 $\pm$ 0.42	1.99 $\pm$ 0.41
P nigrescens	1.31 $\pm$ 0.23	0.64 $\pm$ 0.13	2.47 $\pm$ 0.62	1.28 $\pm$ 0.37	0.39 $\pm$ 0.10	2.41 $\pm$ 1.00
A odontolyticus	4.51 $\pm$ 0.48	4.88 $\pm$ 0.57	7.01 $\pm$ 1.19	3.93 $\pm$ 0.52	5.77 $\pm$ 1.16	4.15 $\pm$ 0.85
F nucleatum ss polymorphum	3.05 $\pm$ 0.44	1.65 $\pm$ 0.27	5.15 $\pm$ 1.13	4.01 $\pm$ 0.68	3.11 $\pm$ 0.70	4.82 $\pm$ 1.38
C showae**	1.44 $\pm$ 0.29	0.49 $\pm$ 0.10	2.25 $\pm$ 0.78	2.98 $\pm$ 1.00	1.06 $\pm$ 0.22	1.96 $\pm$ 0.43
F periodonticum	2.37 $\pm$ 0.33	1.64 $\pm$ 0.42	3.38 $\pm$ 0.70	2.82 $\pm$ 0.46	2.21 $\pm$ 0.55	4.14 $\pm$ 1.08
S constellatus	0.53 $\pm$ 0.06	0.42 $\pm$ 0.02	0.64 $\pm$ 0.09	0.51 $\pm$ 0.04	0.50 $\pm$ 0.04	0.61 $\pm$ 0.05
F nucleatum ss nucleatum	3.03 $\pm$ 0.37	1.59 $\pm$ 0.27	4.96 $\pm$ 0.79	3.58 $\pm$ 0.76	2.44 $\pm$ 0.53	3.94 $\pm$ 1.15
C gingivalis	0.97 $\pm$ 0.21	0.40 $\pm$ 0.11	1.74 $\pm$ 0.51	1.35 $\pm$ 0.30	0.64 $\pm$ 0.18	1.68 $\pm$ 0.38
S gordonii	1.85 $\pm$ 0.23	1.79 $\pm$ 0.22	3.34 $\pm$ 0.55	1.66 $\pm$ 0.24	1.82 $\pm$ 0.41	2.72 $\pm$ 0.51
S noxia***	1.14 $\pm$ 0.21	0.50 $\pm$ 0.11	2.36 $\pm$ 0.56	2.80 $\pm$ 0.39	1.73 $\pm$ 0.62	3.27 $\pm$ 0.73
P melanino genica	2.53 $\pm$ 0.41	2.07 $\pm$ 0.44	5.13 $\pm$ 1.06	3.43 $\pm$ 0.53	3.31 $\pm$ 0.69	5.10 $\pm$ 1.22
S mitis	2.22 $\pm$ 0.26	2.59 $\pm$ 0.40	3.35 $\pm$ 0.56	2.92 $\pm$ 0.41	3.56 $\pm$ 0.82	3.31 $\pm$ 0.66
C sputigena	1.23 $\pm$ 0.20	0.73 $\pm$ 0.17	1.44 $\pm$ 0.33	1.92 $\pm$ 0.36	1.02 $\pm$ 0.28	1.93 $\pm$ 0.42
L buccalis	3.03 $\pm$ 0.50	1.54 $\pm$ 0.42	5.48 $\pm$ 1.26	3.50 $\pm$ 0.50	4.07 $\pm$ 1.10	3.56 $\pm$ 0.86

$*$ Total mean counts significantly different at $P <$.05, Mann-Whitney test
$**$ Total mean counts significantly different at $P <$.01, Mann-Whitney test
$***$ Total mean counts significantly different at $P <$.001, Mann-Whitney test

parison with the standard lanes on the membrane. The Storm Fluorimager improved the ability to quantify species in plaque samples by avoiding the need for film and extending the linear range of detection over 4 orders of magnitude. Thus, counts at 10^4 and 10^8 could be evaluated on the same membrane.

Statistical analysis

Statistical analysis was performed using nonparametric techniques because the data were not normally distributed.

Means and standard errors of the mean were calculated for each bracket group. Between group comparisons were made with the Mann-Whitney U-test. Differences were considered significant at $P < $ .05.

RESULTS

The total counts of 37 species tested on metallic and ceramic brackets were determined (Table 1). Results showed statistically significant differences between metallic and ceramic brackets for 8 species. Specifically, the mean
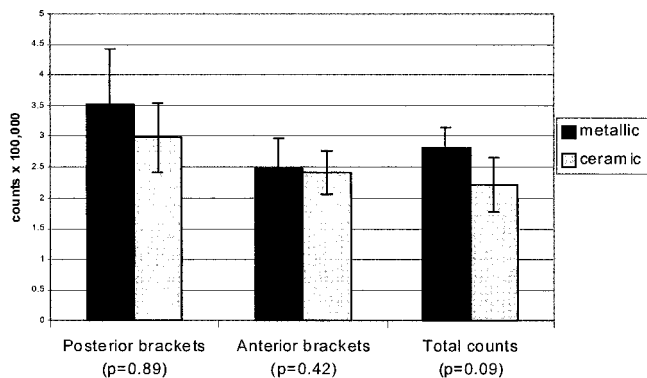
FIGURE 1. Mean counts of S. mutans ($\times 10^5$, $\pm$ SEM) on metallic and ceramic brackets.
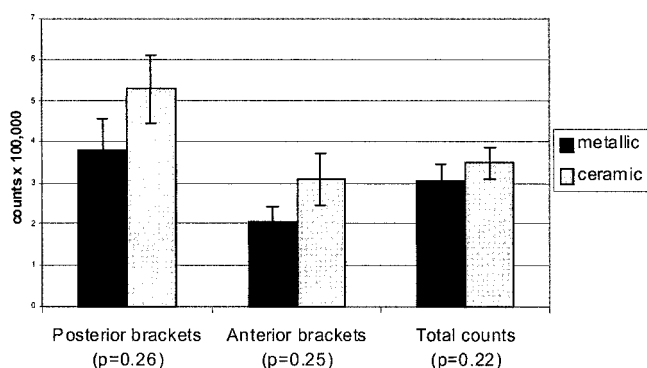

FIGURE 2. Mean counts of L. acidophilus ($\times 10^5$, $\pm$ SEM) on metallic and ceramic brackets.

counts of the caries-inducing species, *S mutans* and *L acidophilus*, were not found to differ between metallic and ceramic brackets (Figure 1 and Figure 2). Even when anterior brackets and posterior brackets were considered separately, no differences were detected in *S mutans* levels between the two bracket materials. Again, no differences were found when anterior or posterior brackets were compared separately.

Five species were found to be significantly higher on the metallic than on the ceramic brackets ($P < .05$). *T denticola* counts were significantly higher on metallic brackets (0.14×10^5 on metallic and 0.08×10^5 on ceramic). Similarly, *A actinomycetemcomitans* and *S anginosus* were higher on metallic brackets ($P < .05$). These differences were only found on brackets from posterior teeth. *F nucleatum ss vincentii* and *E nodatum* counts were also higher on metallic brackets ($P < .05$) and these differences were detected in brackets from both anterior and posterior teeth ($P < .05$).

Conversely, a higher mean count was found on ceramic brackets for *E corrodens*, *C showae*, and *S noxia* (Table 1). Higher counts of *E corrodens* were found on ceramic brackets from both anterior and posterior teeth ($P < .05$). In addition, mean *C showae* counts were significantly higher on ceramic brackets ($P < .01$). These differences were limited to anterior brackets. Similarly, *S noxia* was, on av-

erage, higher on ceramic brackets (2.80×10^5 on ceramic and 1.14×10^5 on metallic) with the differences limited to anterior brackets ($P < .05$).

Streptococcus sanguis, *Actinomyces gerencseriae* and *Streptococcus constellatus* counts were not significantly different between metallic and ceramic brackets ($P > .05$). However, some significant differences were found for all three species when the counts on anterior metallic and anterior ceramic brackets were considered separately with higher counts on ceramic brackets ($P < .05$). In contrast, *Campylobacter rectus* counts were higher on posterior metallic brackets ($P < .05$). Again, when both anterior and posterior counts were combined, no significant difference in *C rectus* levels was found between the two types of brackets.

Statistical analysis of the remaining species yielded no significant difference with respect to their presence on metallic and ceramic brackets. This was also true when comparing anterior metallic brackets to anterior ceramic brackets as well as posterior metallic brackets to posterior ceramic brackets.

DISCUSSION

A number of studies have investigated the influence of orthodontic therapy and appliances on the oral microbial flora. These changes could potentially have a significant impact on patient oral health, including gingival inflammation and demineralization of teeth. New genetic techniques to identify and enumerate the bacterial composition of microbial populations have been applied to a number of different clinical problems, including periodontal diseases and endodontic lesions. Studies of this nature have been made easier with the advent of rapid methods for the enumeration of species using DNA probes. The ''checkerboard'' technique employed in this investigation was developed by Socransky et al[15] to rapidly process large numbers of plaque samples for detection of multiple species. ''Checkerboard'' analysis does not require bacterial viability and offers speed and accuracy and has the advantage over cultural techniques in that it is less time-consuming, less labor intensive and less expensive.[17] Comparison of the findings obtained by checkerboard hybridizations and traditional cultural techniques suggested no difference in species found, although DNA probes are superior to culture for the detection of periodontal pathogenic species.[18–20] In the current study, the levels of 37 bacterial species on metallic and ceramic orthodontic brackets were examined by ''checkerboard'' analysis. Our data suggest that differences in the bacterial composition of dental plaque formed on each bracket type exist; however, the composition is, for the most part, very similar between the two bracket types and may be of limited clinical significance. The differences detected certainly do not favor one bracket type over another with respect to bacterial accumulation.

Demineralization and dental caries are occasional sequelae of orthodontic therapy. Levels of *S mutans* and *L acidophilus*, two species often associated with dental caries, were similar between plaques isolated from the two bracket types. Results of an in vitro study by Fournier et al[13] demonstrated that adhesion of *S mutans* was weaker on metallic than on plastic and ceramic brackets, indicating that metallic brackets had a lower potential for bacterial accumulation than plastic and ceramic brackets. However, despite these differences in in vitro adhesion, the present study suggests that this may have little effect on the microbial populations that colonize orthodontic brackets in vivo. They do demonstrate the presence of these cariogenic organisms on orthodontic brackets and reinforce the need for meticulous oral hygiene and fluoride therapy for orthodontic patients in order to maintain health.

Gingival changes also often accompany orthodontic therapy. The present study suggests that the levels of most subgingival organisms were similar in plaques isolated from the two bracket types. For example *P gingivalis* mean counts were very similar on the metallic and ceramic brackets isolated from both posterior and anterior teeth. *P gingivalis* is a species present in the normal gingival sulcus, but increases significantly with adult periodontitis and maintains elevated levels after recovery.[22] Although Knoernschild and coworkers[14] reported that *P gingivalis* adhesion was greatest on stainless steel brackets, this greater in vitro affinity for stainless steel brackets does not seem to lead to higher *P gingivalis* levels in dental plaque formed on this bracket type.

Despite the relative similarity of most microbiota between the two bracket materials, the differences in levels of certain species on the two bracket types suggest that the microbial flora differs somewhat between them. For example, *A actinomycetemcomitans* were significantly higher on metallic brackets than on ceramic brackets. Large numbers of *A actinomycetemcomitans* have been isolated from the periodontal pockets of patients with juvenile periodontitis.[23] Additional bacteriological studies using a split mouth design would help delineate any possible relationships between bracket composition and the microbial flora that colonize them. Moreover, whether these differences have any clinical significance is unclear. Clinical studies of dental and gingival health between patients with each bracket type would help determine any possible clinical significance of these subtle differences in plaque composition between the two bracket types.

CONCLUSIONS

No significant difference was found in the accumulation of the caries-inducing *S mutans* and *L acidophilus* between metallic and ceramic brackets. Metallic brackets showed significantly higher mean counts than ceramic brackets for the periodontal organisms *T denticola*, *A actinomycetem-*

comitans, *F nucleatum ss vincentii*, *S anginosus*, and *E nodatum*. Ceramic brackets showed significantly higher mean counts than metallic brackets for the periodontal organisms *E corrodens*, *C showae*, and *S noxia*. No obvious pattern of bacterial colonization favoring one bracket material over the other was found.

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