Original Article

Influence of Different Orthodontic Brackets on Adherence of Microorganisms In Vitro

M. I. Brusca[a]; O. Chara[b]; L. Sterin-Borda[c]; A. C. Rosa[d]

ABSTRACT

Objective: To define the capacity of different bracket materials to modify the growth and adherence of microorganisms.

Methods: Three types of brackets from the right upper central incisor were used: metallic, ceramic, and composite. *Streptococcus mutans* and *Candida albicans* were studied. The association of both species was also evaluated. The brackets were placed in flat-bottomed vials containing basal medium with 20% sucrose added; the flasks were inoculated with each of the microbial suspensions. The samples were incubated at 37°C for 48 hours, after which the brackets were removed. The supernatant was removed from the flasks, the cells adhering to the glass were counted, and the brackets were studied with electron microscopy.

Results: The adherence of *Streptococcus mutans* was not modified by the different brackets. The adherence of *Candida albicans* was increased by the composite bracket, whereas the use of metallic brackets decreased the number of colony-forming units (CFUs). By electron microscopy we demonstrated that the adherence of *Streptococcus mutans* plus *Candida albicans* together varied according to the bracket materials with composite > ceramic > metallic.

Conclusions: Orthodontic appliances serve as different impact zones and modify microbial adherence and colonization, acting as foreign reserves and possible sources of infection.

KEY WORDS: Orthodontic brackets; Microorganism adherence; *Candida albicans*; *Streptococcus mutans*

INTRODUCTION

Since the advent of increased orthodontic treatment for adult patients, the use of esthetic brackets has become increasingly popular, bringing about the need to address questions regarding microorganism adherence and biofilm development.[1,2] For a long time, the traditional orthodontic patient was considered as a low-risk patient and orthodontic procedures were considered noninvasive. Lucas et al[3] demonstrated that orthodontic treatment procedures are associated with bacteremia, as in the case of placement of a separator. Aerobic and anaerobic bacteria were isolated from blood samples of these patients.

Yeast of the *Candida* genus, *albicans* species, was analyzed in this study because it is the most frequently found microorganism in infections of the buccal mucus. This yeast has been proven to colonize on cement, enamel, and dentin, which serve as a reservoir for the spread of infection.[4] Nevertheless, the yeast's ability to survive on inert surfaces needs to be further elucidated in order to understand its virulence and dissemination routes.[5] *Streptococcus mutans* was studied in this work because of its well-documented role in the pathogenesis of caries.[6–8] Although a number of studies have demonstrated the viability of *Candida albicans* and *Streptococcus mutans* on removable orthopedic appliances, little is known about their survival on fixed orthodontic appliances.[9,10]

The aim of the present work was to evaluate the adherence of these microorganisms and to determine

[a] Assistant Professor, Microbiology Unit, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina.

[b] Assistant Professor, Physiology and Biophysics Unit, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina.

[c] Professor, Pharmacology Unit, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina.

[d] Professor and Department Chair, Microbiology Unit, School of Dentistry, University of Buenos Aires, Buenos Aires, Argentina.

Corresponding author: Dr. María Brusca, School of Dentistry, University of Buenos Aires, M.T. de Alvear 2142-2nd floor "B" (1122AAH) Buenos Aires, Argentina
(e-mail: maria_brusca@hotmail.com)

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the influence of different composition brackets on adherence. We propose that these materials serve as artificial niches, primary impact zones of the microorganisms, and serve as fomites for cross-infection.

MATERIALS AND METHODS

Brackets and Microorganisms

Three types of brackets were used: 303 metal brackets with a 0.022 slot (Ortotek, Buenos Aires, Argentina) for the straight-arch technique, ceramic brackets for the straight-arch technique (A-Company San Diego, Calif) and Morelli composite brackets (Morelli Ortodontia, Sorocaba-SP, Brazil) for the straight-arch technique. All the brackets were for the right upper central incisor.

The following strains were studied: a wild strain of *Streptococcus mutans* provided by the Malbran Institute (Buenos Aires, Argentina), an ATCC 35668 strain of *Streptococcus mutans*, oral strains of *Candida albicans* isolated by our laboratory, and an ATCC 10231 strain of *Candida albicans*. The association of both species was also evaluated. *Candida albicans* was seeded in Saboreau dextrose agar and incubated under aerobic conditions at 37°C for 18 hours. *Streptococcus mutans* was seeded in Todd Hewitt broth under microanaerobiosis at 37°C for 24 hours. Microbial suspensions were prepared with each species on reaching the exponential growth phase.

Adhesion Tests

First, the studies were performed in test tubes. The brackets were transferred to basal medium with 20% sucrose added. This medium was used given that mutans streptococci produce glucans from sucrose, which allows adherence to the glass surface of the test tube. Ten tubes of each species were analyzed. Nine tubes of each of the groups were inoculated with 0.5 ml of the microbial suspension in concentrations of 1×10^5 and 1×10^7 colony-forming units (CFUs)/mL bacteria and yeast, respectively. The remaining tube served as a control. The tubes were tilted at an angle of 25° and incubated under microanaerobiosis at 37°C for 2 days. Macroscopic observation of the samples was performed.

Adherence was scored as low, medium, or high. Adherence was considered low when a fine biofilm was observed on the wall of the test tube and found to detach completely on setting the tube upright. Partial detachment of the biofilm was considered medium adherence, and no detachment was considered high adherence. Establishing a standardized score using the test tubes was difficult because of their curved shape, which rendered it impossible to make a grid that could be used repeatedly. Removing the brackets was also complicated because of the height of the tubes. The use of a wire to place the brackets in a fixed position was found unsuitable because many of the microorganisms adhered to the wire and thus biased the results. For this reason, we developed the adhesion tests. The test was repeated using small flat-bottomed vials so that the brackets could be placed on the bottom of the vial, avoiding the use of the wire, and could be easily removed using straight-point tweezers.

In addition, the shape of the vials also allowed placement of a grid on the flat bottom in order to measure adherence. The grid was made by drawing the circumference of the vial on a sheet of celluloid film. The diameter was measured and divided drawing two vertical lines and two horizontal lines, thus obtaining a 9-square grid. Adherence was measured on the squares. The points where the grid was to be placed were marked on the base of the vial using a glass-labeling pen. A bracket was placed at the bottom of each of the vials containing culture medium, and the vials were inoculated with one of the studied microorganisms.

Experimental Groups

Experimental groups were created as follows: Group I: metal brackets (n = 24), Group II: composite brackets (n = 24), Group III: ceramic brackets (n = 24) and Group IV: control (n = 24) vials without brackets. Seventy-two samples were studied: for experimental vials containing brackets, there were eight brackets of each type for each of the three groups of microorganisms. The procedure was performed close to a culture oven, and the vials were placed in a crate made ad hoc to secure them in a fixed position. The samples were incubated at 37°C for 48 hours.

The brackets were removed using sterile straight-point tweezers, placed in petri dishes, and processed for analysis using scanning electron microscopy. The supernatant in the vials was removed, the vials were turned upside down to perform macroscopic examination of the cells adhered to the glass, and the specially-designed score grid was placed on the bottom of the vials. After removal of the brackets, each of the experimental vials was compared to the corresponding control vial (containing culture medium inoculated with the corresponding microorganism) in order to evaluate adherence to the brackets. The brackets were processed for electron microscopy. They were dehydrated in alcohol, dried, fixed in 10% formaldehyde, treated with gold palladium, and observed using a Phillips XL 30 MP microscope, 28.9 contrast, 52× magnification.

The biological controls included small vials contain-

Table 1. Colony-Forming Units (CFUs) Adhering to the Glass Surface of the Flat-Bottomed Vials Without Brackets[a]

Microorganism	CFU/ml	No. of Experiments
Candida albicans	6 ± 0.2	8
Streptococcus mutans	59 ± 1.0	8
Candida albicans + *Streptococcus mutans*	45 ± 2.1	8

[a] Values are mean ± SE.

ing earth with spores (alternative method) in keeping with a technique previously developed by our group and commercial strips with bacterial spores containing 1.6×10^4 *Bacillus stearothermophilus* and 2.3×10^6 *Bacillus subtilis* var *niger*. The viability of the spores in the earth and strips was tested prior to the study, using staining techniques that allow for morphological evaluation.

Statistical Analysis

The results were expressed as number of CFUs/mL. Statistical analysis were performed and the results were expressed as mean values ± SE. One-way analysis of variance with a Scheffé ad hoc test was used for multiple comparisons. A value of $P < .05$ was considered significant.

RESULTS

The results obtained under these experimental conditions showed that the capacity of each of the studied microorganisms to adhere to glass and brackets was determined.

Table 1 shows the number of CFUs adhering to the glass surface of the flat-bottomed vials without brackets. It can be seen that *Streptococcus mutans* exhibited the highest CFU number whereas *Candida albicans* exhibited the lowest ones. Both species (*Candida albicans* and *Streptococcus mutans*) added together in the flat-bottomed vial resulted in a significant decrease in CFU/mL compared with *Streptococcus mutans* alone ($P < .001$). These results are in accordance with those performed in test tubes (data not shown).

Figure 1A shows quantitatively that the number of CFUs of *Streptococcus mutans* was not modified in the presence of different bracket types, compared with the control without brackets. The weights of the brackets were: metallic, 0.00015 g, composite, 0.00035 g, and ceramic, 0.00020 g; differences in bracket weight did not influence the results. Figure 1B shows the original *Streptococcus mutans* CFUs/mL after the different types of brackets were taken off.

Figure 2A shows comparatively the adherence of *Candida albicans* among the bracket types. It can be seen that the number of CFUs differs according to the bracket composition. The adherence of *Candida albi-*

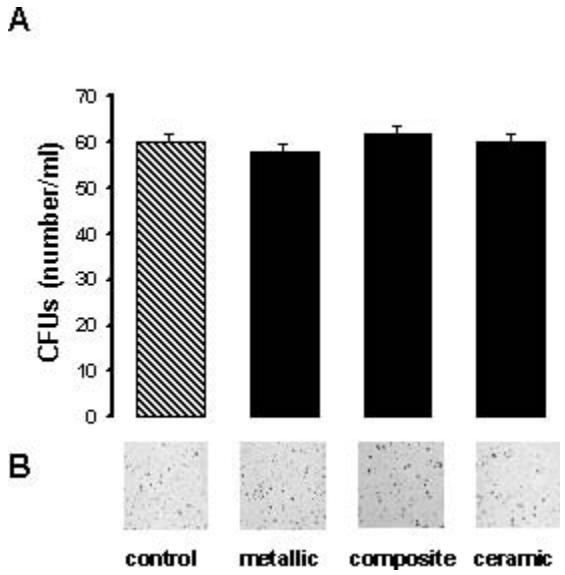

Figure 1. (A) Colony-forming units (CFUs)/mL of *Streptococcus mutans* adhering to glass surface, after removal of the different bracket types, compared with the control without brackets. Values are mean ± SE of 8 experiments in each group. (B) Original *Streptococcus mutans* CFUs/mL after brackets were taken off, representative of 8 experiments in each group. Values are mean ± SE of 8 experiments in each group.

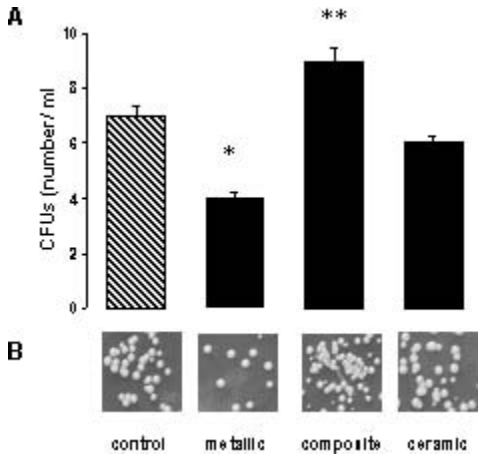

Figure 2. (A) Comparison of the adherence of *Candida albicans* among bracket types. Values are mean ± SE of 8 experiments in each group. *$P < .001$ vs control; **$P < .001$ vs control. (B) Original *Streptococcus mutans* CFUs/mL after brackets were taken off, representative of 8 experiments in each group. Values are mean ± SEM.

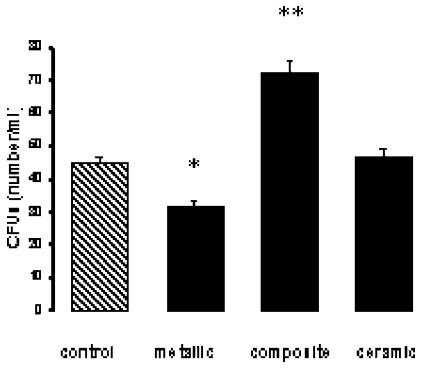

Figure 3. Colony-forming units (CFUs)/mL of *Streptococcus mutans* plus *Candida albicans* added together after removal of the different bracket types compared with control without brackets. Values are mean ± SE of 8 experiments in each group. $*P < .001$ vs control, $**P < .001$ vs control.

cans was significantly increased ($P < .001$) when the composite brackets were present, but with the ceramic brackets the adherence was similar to that of the control. On the contrary, the CFU number decreased significantly ($P < .001$) when metal brackets were present. Figure 2B shows the original CFUs/mL of *Candida albicans* in the culture medium after removal of different bracket materials.

When *Streptococcus mutans* and *Candida albicans* were added together, a decrease in CFUs/mL in the presence of metallic brackets was observed (Figure 3). To the contrary, the composite brackets triggered an increase in CFU/mL, whereas with the ceramic brackets, the number of CFU/mL was similar to the control.

Figure 4A shows the electron microscopy image of the different brackets (metallic, ceramic, and composite) removed from the vials containing *Streptococcus mutans* and *Candida albicans* together. It can be seen that the adherence of microorganisms to the brackets was great on the slot zone (Figure 4B) and varied according to bracket composition. It was higher on the composite and lower on the metallic brackets.

DISCUSSION

Several authors have studied the viability of *Candida albicans* and *Streptococcus mutans* on the acrylic material used to make removable dentures.[11–13] It is well documented that in addition to other factors such as carbohydrate intake, oral hygiene, and genic acid of oral microorganisms, orthodontic appliances promote changes in the oral microbiota.[5] The present study demonstrates that fixed appliances exert the same effects. The microorganisms exhibited highest adherence to the esthetic brackets because they find a highly favorable ecological niche in the more porous and less smooth structure of the bracket material.

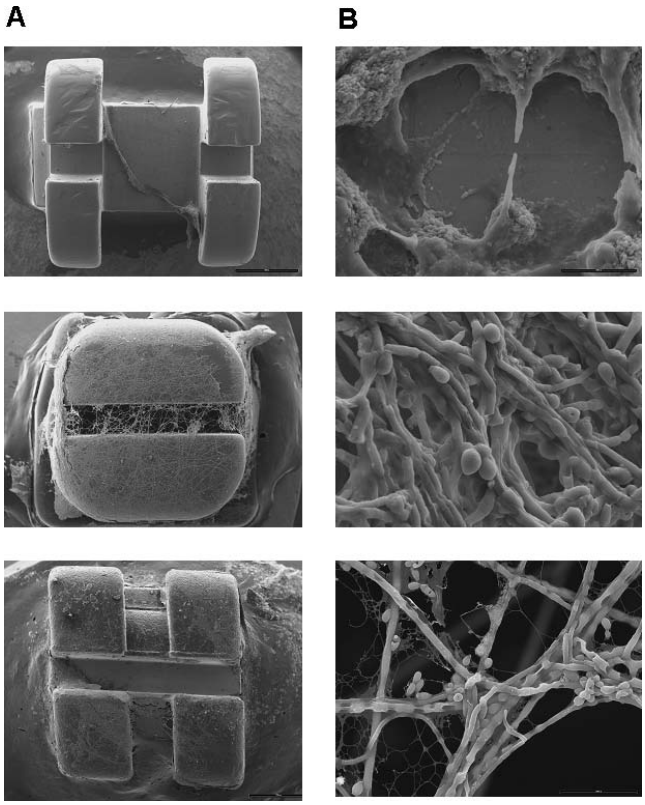

Figure 4. (A) The electron microscopy image of the different brackets (metallic, ceramic, and composite) removed from the vials containing *Streptococcus mutans* and *Candida albicans* is shown. (B) It can be seen that the adherence of microorganisms to the brackets was major on the slot zone.

This observation is in agreement with reports by Fournier et al,[14] who studied adherence of *Streptococcus mutans* alone, unlike our study, which evaluated *Streptococcus mutans* in association with *Candida albicans*, and concluded that affinity of the microorganism for metal brackets was significantly lower than that for brackets made of plastic or porcelain. Tronchin et al[15] demonstrated that yeasts adhere directly to plastic, forming a fine layer or biofilm on the surface of the synthetic device. This is comparable to our observation regarding the plastic brackets. The adhered cells are more resistant to the effect of antifungal drugs, and serve as infectious foci, which can result in the spread of infection in the host.[16] In addition, adherence of lipopolysaccharides (LPS) from gram-negative bacteria to different bracket types (steel, porcelain, plastic, and gold) was evaluated,[17] showing that LPS has a great affinity for all the materials. Contrary to our results, which correspond to gram-positive prokaryote and eukaryote, the authors found that adherence to steel brackets was higher.[17]

In our study, the yeasts exhibited numerous cell elongations in the presence of composite, which could be interpreted as potential intercellular bridges in-

volved in the adhesion mechanism that allows the formation of pseudohyphae. The formation of pseudohyphae is noteworthy, although the culture medium used does not induce filamentation of *Candida albicans*. Hyphae formation has been considered a virulence factor, associated with greater invasive capacity, tissue invasion, and greater resistance to phagocytes.[18,19] In vitro plaque formation was greater in the presence of composite when associating with *Candida albicans* and *Streptococcus mutans* than in the control group. These results suggest that although the adherence mechanisms involved in fungus and *Streptococcus mutans* colonization may differ, once the microorganisms are established they do not inhibit each other, but rather seem to exert a synergistic effect.

In 1986, Bagg and Silverwood[20] evaluated the mechanisms involved in intermicrobial adherence studying the association of *Candida albicans* with several oral bacteria such as strains of *Streptococcus sanguis*, *Streptococcus salivarius*, *Streptococcus mutans*, *Streptococcus mitis*, *Fusobacterium nucleatum*, and *Actinomyces viscosus*. The authors suggested the existence of different mechanisms that allow association of microorganisms, one of which involves lectins, which favor the association of *Fusobacterium nucleatum* and *Actinomyces viscosus* with *Candida albicans*.

It must be kept in mind that other in vivo factors also affect film retention. These include the presence of elastomers, metal ligatures, or nickel, titanium, or steel arches and adhesives, which form a critical interface because they facilitate microbial adherence. Sukontapatipark et al[21] found no differences in microbial adherence when comparing brackets legated with rubber bands and those legated with metal ligatures, possibly because the difference in microorganism adherence is related to the bracket material and not the device used to hold the brackets to the arches.

The inhibitory effect of the materials was also analyzed. Manufacturers usually provide information about the physical properties of the materials, but often fail to include information about their antimicrobial properties. None of the studied materials exhibited bacteriostatic properties. There are reports in the literature demonstrating copper and dental amalgams to exhibit antimicrobial properties,[22–24] whereas titanium has been found to exert a weaker antimicrobial effect.[25] Applied to the clinical setting, our results with composite brackets could explain the increase in microbial plaque and inflammation of tissues adjacent to orthodontic appliances and the subsequent increase in caries risk and periodontal disease.

Dale et al[26] pointed out that orthodontists have to use protection barriers and sterilize instruments, hand pieces, and pliers. These recommendations have been confirmed by the American Dental Association and the Centers for Disease Control because this is the only way to guarantee a safe environment for patients, professionals, and coworkers. Biological controls are used to evaluate the effectiveness of the process and of the equipment, and they serve to indicate that all forms of microbial life have been destroyed. These tests have been performed in compliance with (International Organization for Standardization) standards 1138/1995 and local Instituto Argentino de Normatización (Argentine Institute of Normative) standards 37102-3/1999.

Considering the limitations of this study, allow us to propose that the use of metallic brackets may be useful in a particular group of patients with a risk of *Candida albicans* infection, such as patients with diabetes, immunosuppressive states, and periodontal disease.

CONCLUSIONS

- The capacity of microorganisms to adhere and grow is dependent on bracket composition.
- Metallic brackets decreased yeast adherence, whereas composite brackets facilitated it.

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