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ORIGINAL ARTICLE

Influence of orthodontic brackets design and surface properties on the cariogenic *Streptococcus mutans* adhesion

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KEYWORDS

Orthodontic brackets;
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Abstract *Objective:* To compare the surface properties of self-ligating metallic (SLM), ceramic esthetic, and conventional metallic (CM) brackets, and evaluate the adhesion of *Streptococcus mutans* biofilms to their surface, attempting to interpret the correlation between bracket type and enamel demineralization from a microbiological perspective.

Materials and methods: Twenty-two brackets of each group were used. The brackets' surface roughness was defined and the bacterial adhesion was performed using the strain *S. mutans* ATCC25175 with 8 h or 24 h of incubation time. The total bacterial adhesion (TBA) of biofilms was assessed using optical density (OD) methodology. To quantify bacteria viability (BV), the colony forming units (CFU) were counted. A scanning electron microscopy (SEM) observation of biofilms was also performed. **Results:** Ceramic brackets exhibited significantly higher roughness (0.304) compared to CM (0.090) and SLM (0.067) ones (C > CM = SLM). The data obtained with the TBA and BV tests showed that *S. mutans* biofilm formed on bracket groups exhibited similar results for both incubation periods. From the SEM images it is possible to observe that biofilm structure formed for 24 h was denser than that for 8 h of incubation with significantly more aggregates and cells for three groups.

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Conclusion: This *in vitro* study suggests that despite the higher surface roughness of ceramic brackets, this alone does not influence the adhesion of the *S. mutans* biofilms.

Clinical relevance: From a microbiological perspective, the bracket's design may be more relevant than its surface roughness with respect to the adhesion of cariogenic bacteria biofilm with potential risk to dental enamel integrity.

1. Introduction

Biofilm accumulation is recognized as the most common orthodontic treatment complication, occurring in about 60% of patients (Bergamo et al., 2018). In fact, the irregular surfaces of orthodontic appliances represent new retentive areas (Ong et al., 2010) and might impede effective oral hygiene, determining the increase of bacterial load and changes in oral microbiota, favouring some pathogenic species such as *Streptococcus mutans* (Lucchese et al., 2018). Furthermore, the presence of brackets can reduce the efficiency of physiological mechanism of cleaning by the tongue, cheeks and saliva (Forsberg et al., 1991).

Orthodontic accessories have evolved in terms of design and materials used (Fatani et al., 2017). Despite this evolution, accessories still create favourable conditions for microorganisms accumulation, which increases the dental demineralization process (Sharma et al., 2018).

Enamel demineralization is due to organic acids from oral bacteria (Papaioannou et al., 2007), and *S. mutans* is the most important bacteria, due to its evident activity in forming insoluble extracellular polymer and acid production (Loesche, 1986; Damle et al., 2016). Indeed, it has been recognized as the main pathogen of dental caries, with its level increasing after bonding orthodontic brackets (Scheie et al., 1984; Lundstrom and Krasse, 1987).

The oral cavity contains opportunistic bacteria living harmonically with host in an equilibrium. But changes such as placement of orthodontic brackets in this ecologic system can favour pathogenic bacteria (Lucchese et al., 2018).

Despite the evolution of brackets, the enamel demineralization by biofilms remains an issue. When the morphology was not considered and uniform size blocks of materials were used, the bracket material had a significant influence on the adhesion of *mutans streptococci* (Lim et al., 2020). However, the complex bracket design enhances biofilm formation since access for proper cleaning is hampered (van Gastel et al., 2007; Moolya et al., 2014) and the bracket drawing and its mechanism to secure the archwire can influence the increase of plaque accumulation (Bergamo et al., 2016). Despite these studies, the issue regarding conventional metallic, self-ligating, or ceramic brackets, which are very distinct in morphology, for the best maintenance of enamel integrity, from a biofilm perspective, has not been resolved.

Therefore, this study aimed to compare the surface properties of self-ligating metallic, ceramic, and conventional metallic brackets, and to evaluate the adhesion of *S. mutans* biofilms on it.

2. Materials and methods

2.1. Sample

The sample consisted of 66 brackets:22 conventional metallic (3 M Gemini®) - CM group, 22 self-ligating metallic (3 M Portia®) - SLM group, and 22 ceramic (3 M Gemini Crystal Clear®) - group C, all corresponding to the MBT prescription upper central incisor.

Brackets designed for the microbial analysis (18/group) had their bases covered with Filtek Resin Z350 XT Flow (3 M, Moronvia–CA, USA) for the retention mesh to not influence the result. Then, they were cleaned by soaking in enzymatic detergent (20 min), washed in distilled water, and sterilized (121 °C, 15 min).

2.2. Surface measurements

The surface roughness was determined for three brackets from each group using a digital roughness meter (SJ-310 Mitutoyo, Japan), adjusted with a cut-off of 0.25 mm and 0.3 mm range of motion of the tip reading. The brackets were stabilized and a 5 μm radius and 90° angle tip was moved over in a horizontal direction on the vertical center of the clip (SLM bracket) or the distocervical, mesioincisal and distoincisal wings (CM and C brackets). The tip was moved at a speed of 0,5 mm/s under constant pressure of 4 mN (Rani et al., 2021). The roughness of each bracket was determined by the arithmetic mean of the two measurements.

To evaluate morphology, one specimen of each group was cleaned and then gold coated in a 50 mTorr, 20 mAmp during 120 s using a gold target witch obtaining a layer of gold of 12 nm (Denton Vacuum, model Desk IV). Each material was evaluated using scanning electron microscopy (SEM) with a 15KV JEOL-JSM–6390LV (JEOL BRASIL, SP, Brazil) microscope with magnification set at 1000x and 5000x.

2.3. Bacterial strains and growth conditions

Previously to the assays, an overnight culture of the strain S. mutans ATCC 25175 in Brain Heart Infusion agar (BHIA) (Kasvi, Paraná - Brazil), pH 7.4 ± 0.2, was used to prepare the test bacterial inoculum. To perform this, one up to five colonies were inoculated in fresh Brain Heart Infusion broth (BHI) (Kasvi, Paraná - Brazil) supplemented with 1% (w/v) sucrose (BHIS) until the turbidity match 10^8 CFU/ml (0.5 McFarland).

The strain was grown in BHIS and incubated in microaerophilic atmosphere using a candle jar at 36 °C without agitation.

2.4. Total bacterial adhesion (TBA) of biofilms

TBA was assessed using optical density (OD) methodology. The bracket specimens were individually and aseptically placed into a 96-well microplate (Kasvi, Paraná – Brazil), with the base facing down. Then the prepared planktonic bacterial inoclulum (200 µL) was carefully added to each well. Two plates were incubated at 36 °C in microaerophilic atmosphere, one for 8 h and another for 24 h. The assay was performed in duplicate and repeated at three different times.

After incubation, each bracket was carefully transferred to a new, clean, dry well. It was then gently washed twice with phosphate buffered saline (PBS), pH 7, 0.1 M (Kasvi, Paraná - Brazil), to eliminate planktonic bacteria, and dried in a laminar flow environment. The adhered cells were stained with crystal violet solution (10 µg/mL in sterile water) (Kasvi, Paraná - Brazil) for 15 min at 37 °C. The samples were again washed twice with PBS and dried. The dye was then solubilized in an 80:20 mixture of alcohol:acetone by stirring at room temperature for 20 min using a shaking platform, and OD measured in the supernatant at 595 nm using a Zenyth 3100 spectrophotometer (Alfagene, Wals-Siezenheim, Austria) proceeding to three reading cycles (Pereira et al., 2011).

2.5. Bacteria viability (BV) of biofilms

To quantify BV, colony forming units (CFU) were counted. The microplates were prepared as described in TBA. After incubation, the brackets were gently washed twice with PBS, to remove planktonic cells, and aseptically transferred to a sterile microcentrifuge tube containing 1 mL PBS and 2 glass beads, to be vortexed (K40-1010, Kasvi, Paraná - Brazil) for 30 s at 3,300 RPM to release adhered cells, thus forming a suspension. For each suspension, different saline dilutions (up to 10^{-6}) were prepared and seeded (100 µL) in mitis salivarius sucrose agar supplemented with potassium tellurite. For each dilution, three plates were seeded. The plates were incubated in microaerophilia (36 °C/48 h). CFU per bracket were quantified on the plates showing from 30 to 300 colonies. The final CFU value in the brackets was calculated as the number of CFU counted X dilution factor X 10. The results were converted to their corresponding logarithm ($\log_{10}$ CFU/mL).

The TBA and BV tests were performed in duplicate considering each bracket group, and repeated at three different times, thus obtaining three repetitions for each incubation time studied. As negative control of bacterial growth, one bracket from each group was processed in the same way using culture medium without inoculum.

2.6. SEM observation of biofilms

Evaluation of biofilm formation and adhesion was performed using SEM with magnification set at 1000x and 5000x. For this, after incubation, *S. mutans* biofilms on brackets were gently washed (PBS), fixed (2.5% glutaraldehyde, 30 min, 25 °C), washed (0.1 M cacodylate buffer, pH 7.2), and dehydrated (series of ethanol solutions 30, 50, 70, 90% and twice in 100%, 15 min at each concentration). The critical point was obtained in carbon dioxide (Tousimis, model Autosamdri-815). Brackets were transported to aluminum holders and coated with 5 nm gold. The test used one bracket from each group for each time period and scanned the total surface of the bracket.

2.7. Statistical analysis

To evaluate the surface roughness, initially the distribution of data was analysed with Kolmogorov-Smirnov test. After verification, One Way ANOVA was applied to determine the mean values and Tukey's post hoc for multiple comparisons of the different groups. For TBA and BV, the non-parametric Kruskal-Wallis test was used to analyse the difference between the groups followed by the Mann-Whitney test. The significance level used was $\alpha = 0.05$. Data were analyzed using SPSS 21.0 (Chicago, USA).

3. Results

Ceramic brackets exhibited significantly higher roughness compared to CM and SLM ones (Table 1).

The groups C (Fig. 1B) and CM (Fig. 1C) showed more irregular surfaces than SLM (Fig. 1A). Although SLM has some cracks, surface defects were more visualized in the C and CM groups (Fig. 1A, B, and C). SLM at a 1000x magnification was observed without obvious pores, but the pores were evident at higher magnification (Fig. 2A). In group C a geometric pattern (Figure2B, 1000x) was observed that resembles honeycombs at 5000x (Fig. 1B). The images obtained from group C demonstrated crystal deposition that contributes to an irregular surface with the view of many bacterial colonization sites (Fig. 1B). For the CM group, the surface defects were clearly visible (Fig. 1C).

It is possible to observe that *S. mutans* biofilm structure formed on bracket surfaces for 24 h of incubation (Fig. 2D, 2E, 2F) were denser than that for eight hours (Fig. 2A, 2B, 2C), with significantly more aggregates and cells, indicating that adhesion depends on *S. mutans* contact time with the brackets.

The OD exhibited similar results (Fig. 3A) for both incubation periods. In the same way, the data obtained with the CFU count test presented no significant differences (Fig. 3B).

4. Discussion

Every bacterial retention site in the mouth contributes to biofilm increase which can lead to dental decay. Brackets are one of these retention sites. If the material was associated with greater accumulation of biofilm (Lim et al., 2020), the bracket morphology was not considered, although bracket design enhances biofilm formation and periodontal parameters (van Gastel et al., 2007; Moolya et al., 2014).

There are different bracket materials and designs available in the market, and knowledge about which of them is associated with lower bacterial adhesion, and enamel demineralization, can influence the choice for treatment, especially considering poor hygiene (Sharma et al., 2018). The most widely used brackets are the CM ones, because of their favourable characteristics (Agarwal et al., 2016). SLM brackets were introduced to create a system with less friction, providing a reduction in treatment time (Ong et al., 2010). These have advantages compared to CM, such as eliminating ligature, which may facilitate oral hygiene (Jung et al., 2016). However,

Table 1 Values of the variables analysed and the comparison between groups of brackets.

	Conventional metallic (CM)	Self-ligating metallic (SLM)	Ceramic (C)	Significance*	p-value
Surface roughness (μm)	0.09 ± 0.03	0.067 ± 0.01	0.30 ± 0.07	C > CM = SLM	0.001
Optical density 8 h	2.04 ± 1.15	2.20 ± 0.01	2.87 ± 0.34	C = CM = SLM	0.174
Optical density 24 h	3.21 ± 0.54	2.96 ± 0.88	3.79 ± 0.14	C = CM = SLM	0.550
Log CFU/bracket 8 h	4.84 ± 0.23	5.88 ± 0.81	5.56 ± 2.87	C = CM = SLM	0.482
Log CFU/bracket 24 h	6.29 ± 1.75	5.86 ± 1.49	5.45 ± 1.74	C = CM = SLM	0.554

* ANOVA was performed to compare surface roughness, Kruskal Wallis test was performed to compare differences in amounts of bacteria among bracket type.

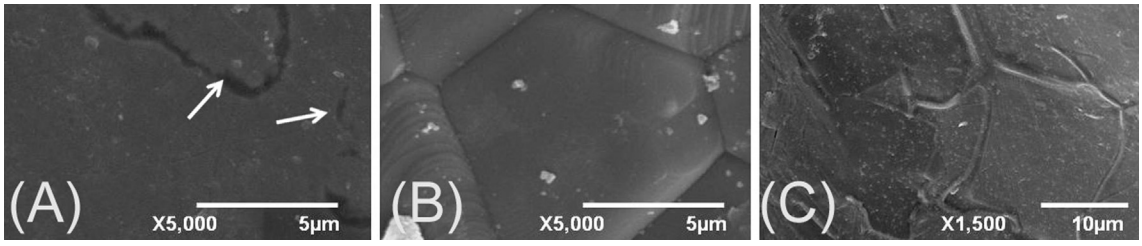

Fig. 1 SEM images of self-ligating metallic (A), ceramic (B) and conventional metallic (C) brackets surface. White arrows show the cracks.

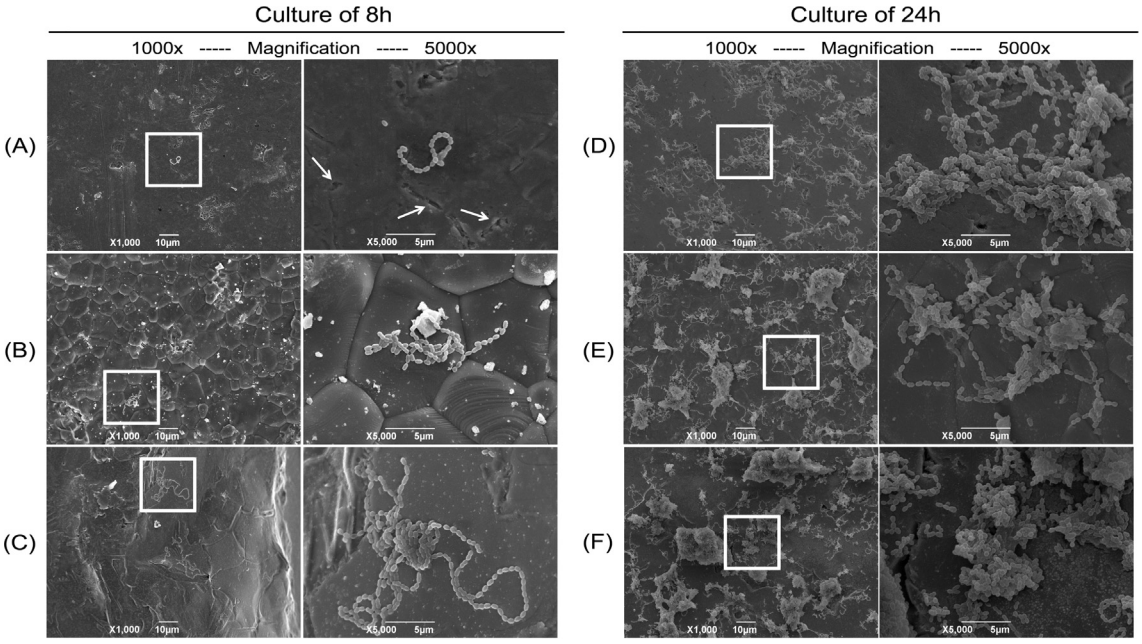

Fig. 2 SEM images of self-ligating metallic (A and D), ceramic (B and E) and conventional metallic (C and F) brackets surface at 1000x and 5000x with S. mutans biofilms formed during 8 h and 24 h. White squares represent the visible area at 5000x magnification. White arrows show the cracks.

metal brackets have esthetic disadvantages, so the demand for ceramic esthetic appliances has become a growing requirement (Maltagliati et al., 2006). The ceramic brackets have a satisfactory esthetic, but are more fragile and present a greater risk of fracture (Ren et al., 2014).

Despite these several characteristics, the cariogenic potential of different brackets remains uncertain. This study demonstrated that the surface roughness of ceramic brackets is significantly greater than other brackets, as well as Lim et al. (2020) work. Surface roughness is the dominant property in relation to bacterial adhesion (Ren et al., 2014) and when the roughness values are equal to or greater than 0.2 μm this can lead to greater bacterial plaque retention (Agarwal et al., 2016).

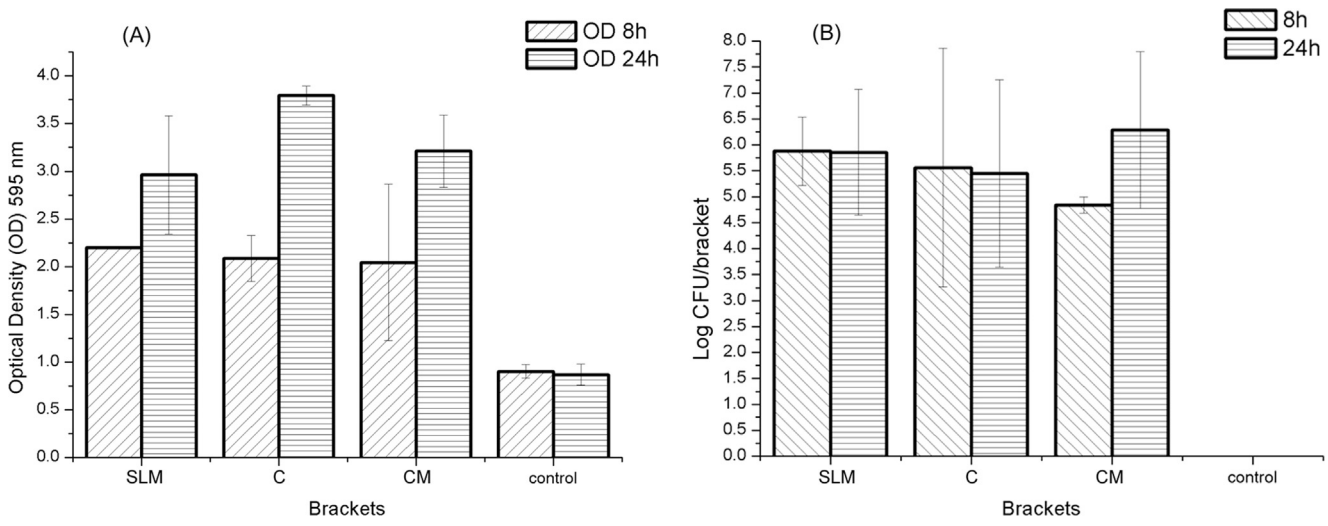

Fig. 3 S. mutans biofilm formed on the surfaces of brackets Self-ligating metallic (SLM), Ceramic (C) and Conventional metallic (CM) group during 8 h and 24 h of incubation. (A) optical density; (B) Colony Forming Units counts. Data were shown as mean ± standard deviation.

The highest roughness of ceramic brackets was confirmed, since a honeycomb pattern was visible, albeit CM and SLM brackets also showed irregular surfaces. Polycrystalline ceramic brackets are composed of aluminum oxide particles and binders, so it are molded cut and burned (Maltagliati et al., 2006). The manufacturing method of ceramic brackets can produce pores, imperfections, cracks, and microfractures (van Gastel et al., 2009; Vidor et al., 2015), justifying the findings of this study.

The higher roughness and microstructure pattern of the C group was not expressed in *S. mutans'* higher adhesion. *S. mutans* is involved in beginning the adhesion process required for oral biofilms installation (Dunne Jr., 2002), which is influenced by surface characteristics (Teughels et al., 2006). The *mutans streptococci* adhesion is positively correlated with the surface roughness favouring the binding sites and inhibiting bacterial detachment (Lee et al., 2009; Pereira et al., 2011; Park et al., 2019). The data obtained in this study do not reinforce the study by Lim et al. (2020), since no significant difference was detected between bracket groups for both assay TBA and BV. Lim et al. (2020) did not consider the bracket area and morphology, and showed that bracket materials significantly influence the adhesion of *mutans streptococci*. Our results may be explained by the presence of aluminum oxide on the ceramic bracket surface, acting as antibacterial surface treatment (Iijima et al., 2013).

On the other hand, the lower roughness presented by SLM brackets may have been compensated by the presence of the NiTi clip as a binding element that resulting in an additional site for bacterial adhesion and proliferation (Tupinambá et al., 2017). It may have contributed to similar adherence data between these brackets and the others. Nonetheless, it has been reported that the self-ligating brackets contribute to low adhesion for periodontal pathogenic bacteria because it eliminate the need for ligatures (Pejda et al., 2013).

Interestingly, the amount of *S. mutans* adhered in CM brackets was similar to other groups, since the surface roughness was significantly lower than the C group. The TBA analysis performed shows that extended incubation time increased *S. mutans* adhesion on brackets regardless of group. This fact can be clearly observed comparing the SEM images for 8 h with 24 h of culture incubation time.

Therefore, the total viable bacterial cells remains rather stable from 8 h to 24 h of culture incubation, except for the CM group, where an increase greater than one log can be observed. This might suggest that a larger sample may be necessary to obtain conclusive results considering the CM group with others. Although only one bacterial species was considered, the adhesion pattern of total bacteria recovered from human saliva is similar to *S. mutans* onto bracket material surfaces (Lim et al., 2020). Assuming that saliva is considered a reservoir for oral bacteria (Salli and Ouwehand, 2015), higher levels of *S. mutans* in the mouth may represent an extra risk for enamel demineralization during orthodontic treatment.

Although one of the limitations of this research is that it did not measure the total area of the brackets, this study suggests that bracket surface roughness does not play an important role in the *S. mutans* adhesion and the bracket design may be more relevant to potential risk to dental enamel integrity. Therefore, extrapolating the results for clinical application, although this is an *in vitro* study, for patients with poor oral hygiene and/or high caries activity and/or periodontal disease history, brackets with less complex design should be considered, in order to minimize adverse effects during orthodontic treatment. Additional comparisons through a randomized clinical trial with different types of brackets and for longer times could bridge the gap in this field of clinical orthodontics.

5. Conclusion

Ceramic brackets presents the higher surface roughness, however, this fact alone does not influence the *in vitro* adhesion of *S. mutans* biofilms. So, from a microbiological perspective, based on cariogenic bacteria biofilm profile, the bracket design may overlap the roughness property for the enamel integrity maintenance.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

For this type of study, formal consent is not required.

CRediT authorship contribution statement

Raphaella Barcellos Fernandes: Methodology, Investigation, Data curation, Writing – original draft. **Ana Bárbara Polo:** Methodology, Investigation. **Vinicius Novaes Rocha:** Methodology, Investigation. **Robert Willer Farinazzo Vitral:** Methodology, Writing – review & editing, Visualization. **Ana Carolina Morais Apolônio:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision. **Marcio José da Silva Campos:** Conceptualization, Writing – review & editing, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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