INFLUENCE OF THE TYPE OF BRACKET ON MICROBIAL COLONIZATION AND GENGIVAL CLINICAL CONDITION: STUDY RANDOMIZED CLINICAL TRIAL

INFLUÊNCIA DO TIPO DE BRAQUETE SOBRE A COLONIZAÇÃO MICROBIANA E A CONDIÇÃO CLÍNICA GENGIVAL: ESTUDO CLÍNICO RANDOMIZADO

INFLUENCIA DEL TIPO DE BRACKET SOBRE LA COLONIZACIÓN MICROBIANA Y LA CONDICIÓN CLÍNICA GINGIVAL: ESTUDIO CLÍNICO ALEATORIZADO

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Abstract: It was hypothesized that patients using conventional brackets have greater bacterial colonization and worse gingival clinical condition compared to those using self-ligating brackets. Therefore, the objective of the study was to compare the influence of the type of bracket bonded on bacterial levels and gingival clinical condition. Twenty-one university students participated in the study. Each upper anterior tooth received an orthodontic bracket, one of the types conventional with elastic ligature (CL), conventional without elastic ligature (C) or self-ligating bracket (AL). After evaluation of the clinical parameters NCI (clinical attachment level), IG (gingival index), PS (pocket depth) and IP (plaque index) and professional dental prophylaxis, the brackets were bonded and the participants received oral hygiene instructions. The indices were measured again 7 and 21 days after bonding. The total bacterial load and the levels of *Aggregatibacter actinomycetemcomitans* (A.a), *Porphyromonas gingivalis* (P.g), *Tannerella forsythia* (T.f), and *Treponema denticola* (T.d) were quantified by qPCR. At 21 days, the sulcular levels of P.g and T.d were higher in the CL group. Except for P.g, the accumulation of bacteria on the brackets - total load, A.a, T.f, and T.d - increased over time for all bracket types. Although conventional brackets may have presented worse results for some microbiological parameters in relation to the clinical condition, the influence of conventional brackets with ligature was as negative as that of self-ligating brackets.

Keywords: Orthodontic Appliances; Orthodontic brackets; Bacteria; Periodontal

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Diseases; Real-Time PCR.

Resumo: Hipotetizou-se que pacientes que utilizam braquetes convencionais têm maior colonização bacteriana e pior condição clínica gengival em relação aos que usam autoligados. Com isso, o objetivo do estudo foi comparar a influência do tipo de braquete colado sobre os níveis bacterianos e a condição clínica gengival. Participaram do estudo 21 estudantes universitários. Cada dente superior anterior recebeu um braquete ortodôntico, dentre os tipos convencional com ligadura elástica (CL), convencional sem ligadura elástica (C) ou braquete autoligado (AL). Após avaliação dos parâmetros clínicos NCI (nível de inserção clínica), IG (índice gengival), PS (profundidade de bolsa) e IP (índice de placa) e profilaxia dentária profissional os braquetes foram colados e os participantes receberam instrução de higiene oral. Os índices foram novamente mensurados 7 e 21 dias após a colagem. A carga bacteriana total e os níveis de *Aggregatibacter actinomycetemcomitans* (A.a), *Porphyromonas gingivalis* (P.g), *Tannerella forsythia* (T.f), e *Treponema denticola* (T.d) foram quantificados por qPCR. Aos 21 dias os níveis sulculares de P.g e T.d foram maiores no grupo CL. Excetuando-se P.g, o acúmulo de bactérias nos braquetes - carga total, A.a, T.f e T.d – sofreu aumento ao longo do tempo para todos os tipos de braquete. Embora os braquetes convencionais possam ter apresentado piores resultados para alguns parâmetros microbiológicos em relação a condição clínica a influência do convencional com ligadura foi tão negativa quanto a do autoligado.

Palavras-chave: Aparelhos ortodônticos; Braquetes ortodônticos; Biofilme dentário; PCR em tempo real.

Resumen: Se planteó la hipótesis de que los pacientes que usan brackets convencionales tienen una mayor colonización bacteriana y una peor condición clínica gingival en comparación con aquellos que utilizan brackets autoligados. Así, el objetivo del estudio fue comparar la influencia del tipo de bracket cementado sobre los niveles bacterianos y la condición clínica gingival. Participaron en el estudio 21 estudiantes universitarios. Cada diente anterior superior recibió un bracket ortodóntico de los siguientes tipos: convencional con ligadura elástica (CL), convencional sin ligadura elástica (C) o autoligado (AL). Tras la evaluación de los parámetros clínicos NCI (nivel de inserción clínica), IG (índice gingival), PS (profundidad de bolsa) e IP (índice de placa), así como la profilaxis dental profesional, los brackets fueron cementados y los participantes recibieron instrucciones de higiene oral. Los índices se midieron nuevamente a los 7 y 21 días después de la cementación. La carga bacteriana total y los niveles de Aggregatibacter actinomycetemcomitans (A.a), Porphyromonas gingivalis (P.g), Tannerella forsythia (T.f) y Treponema denticola (T.d) se cuantificaron mediante qPCR. A los 21 días, los niveles sulculares de P.g y T.d fueron mayores en el grupo CL. Excepto por P.g, la acumulación de bacterias en los brackets (carga total, A.a, T.f y T.d) aumentó con el tiempo para todos los tipos de brackets. Aunque los brackets convencionales pudieron haber mostrado peores resultados en algunos parámetros microbiológicos, en cuanto a la condición clínica, la influencia del bracket convencional con ligadura fue tan negativa como la del autoligado.

Palabras clave: Aparatos ortodóncicos; Brackets ortodóncicos; Biofilm dental; PCR en tiempo real.

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1 INTRODUCTION

When seeking orthodontic treatment, the patient envisions aggressive aesthetic results in addition to improving chewing conditions. The retentive aspect of the brackets allows conventional cleaning, which leads to a greater accumulation of biofilm and the appearance of white lesions (Mester et al., 2022). In more severe cases, it is possible to observe gingival abnormalities and hyperplasia and, eventually, bone loss affecting the periodontal system (Corghi et al., 2014*;* Gorbunkova et al., 2016; Guo et al., 2016; Müller et al., 2021) as well as halitosis (Santonocito & Polizzi, 2022).

Conventional brackets are associated with an elastic or metallic ligature to keep the orthodontic archwire within the groove. Such ligatures almost always act as a supporting factor in the accumulation of microorganisms (Pejda et al., 2013; Folco et al., 2014; Jung et al., 2016).

Self-ligating brackets have been used in orthodontic treatments and the great promise of their use is the reduction of working and treatment time. Additionally, this system promises other advantages such as less biofilm accumulation due to the less retentive design, easier oral hygiene, and the elimination of elastic bandages (Corghi et al., 2014*;* Pejda et al., 2013; Folco et al., 2014) or metal (Jung et al., 2016), as this technique has its mechanism to keep the arc in its groove using a metal clip.

The association of *Streptococcus mutans* with different brackets has been extensively studied in the literature due to its impact on the etiology of caries (Mester et al., 2022; Müller et al., 2021; Folco et al., 2014; Jung et al., 2016; Kim et al., 2016). However, it is necessary to highlight that the biofilm is composed of a diverse microbiota (Folco et al., 2014) and there is a dynamic change in it during orthodontic treatment. The first change observed is quantitative, with a visible increase in the biofilm mass around the retentive areas, where oral hygiene is almost always poor. Qualitative changes then occur, where the proportion of bacteria already present in the periodontal health state is altered, thus initiating the periodontal disease state (Santonocito & Polizzi, 2022; Diaz et al., 2016).

Only a few studies focus on the subgingival microbiota that can migrate to the bracket region during treatment and cause gingival inflammation, bleeding, hyperplasia, destruction of periodontal support, and changes in the enamel surface (Guo et al., 2016; Jung et al., 2016).

Gram-negative anaerobic microorganisms typically subgingival, such as *Aggregatibacter actinomycetemcomitans* (A.a), *Fusobacterium nucleatum* (F.n), *Porphyromonas gingivalis* (P.g), *Prevotella intermedia* (P.i) and *Tannerella forsythia* (T.f) are strongly related to gingival inflammation and disease progression periodontal (Santonocito & Polizzi, 2022; Guo et al., 2016; Pejda et al., 2013; Jung et al., 2016; Kim et al., 2016; Bergamo et al., 2022) and its quantity may increase after bracket placement.

Although there is supposed to be less retention of microorganisms in the self-ligating bracket, there is no unanimity in the literature consulted. Since orthodontic appliances can have a strong impact on periodontal parameters, this study evaluated

the influence of the type of bracket - self-ligating and conventional with and without elastic ligature - glued after professional prophylaxis on bacterial levels and gingival clinical condition over three weeks. This work proposes to evaluate the influence of the type of bracket - self-ligating and conventional with and without elastic ligation - glued after professional prophylaxis on bacterial levels and gingival clinical condition over three weeks.

2 MATERIAL AND METHODS

2.1 PARTICIPANTS

This research was submitted to the Research Ethics Committee (CEP) and approved before the screening and inclusion of participants (CA.AE 70870017.8.0000.5501).

For this prospective randomized clinical study, 21 participants (average age of 29.2 years) were selected among students from different undergraduate courses at the University of Taubaté and they received an Informed Consent Form (TCLE) with information about the objectives and methodology that would be adopted. Individuals who agreed to participate in the study underwent anamnesis to collect personal data and verify their general health condition.

2.2 SAMPLE CALCULATION

To establish the number of individuals that should be included in the present study, a sample size calculation was carried out. To this end, a pilot study was carried out, where microbiological samples were collected from 10 individuals and processed for the pathogens to be studied. The sample calculation was conducted for all pathogens, selecting the one that determined the inclusion of a greater number of individuals. The following statistical formula was applied:

$$DMS = t5\% \sqrt{\frac{2*DMe}{N}}$$

Where DMS is the minimum significant difference that one wishes to observe (10% difference was selected), t (5%), a tabulated value of 2, and DMe the measure of observed dispersion, with the standard deviation selected.

After carrying out the statistical test and adopting a safety margin, it was verified the need to include approximately 24 participants to end up with at least 20 participants.

2.3 INCLUSION CRITERIA

The following inclusion criteria for participation in the study were used: satisfactory general health (absence of endocrine, hormonal, hematological, immune disorders, nutritional changes, and pathologies), gingival health, and last dental check-

up between six months and one year before the start of the search. Individuals who did not present clinical signs of periodontal inflammation on visual examination, gingival index (GI) <1, and absence of clinical attachment loss were considered healthy from a periodontal point of view (Cortelli et al., 2008).

2.4 EXCLUSION CRITERIA

In addition to individuals who did not meet the inclusion criteria, those who underwent periodontal treatment in the six months before the start of the study, and who used local or systemic antimicrobials, or anti-inflammatories in the three months before the study, were excluded from the study. individuals diagnosed with joint disorders, teeth with untreated cavities, presence of mixed or primary dentition, mouth breathers, impacted canines, and loss of permanent teeth, smokers, and ex-smokers.

2.5 DETAILING

Firstly, the patient's clinical parameters were collected (time 1). IP and GI were measured at three points on the buccal and palatal surfaces of the teeth by a single examiner previously trained and calibrated by a standard examiner. For this purpose, a North Carolina model manual periodontal probe (Hu-Friedy, Chicago, Illinois, USA) was used. For intra- and inter-examiner calibration, the Kappa (K) and intraclass correlation statistical tests were applied. The examiner was considered calibrated when he presented K results between 0.8 and 0.95.

Gingival clinical condition indicators were evaluated from Plaque Index (PI) (Loe & Silness, 1963), gingival index (GI) (Silness & Loe, 1964), pocket depth (PS), and clinical attachment level (NCI).

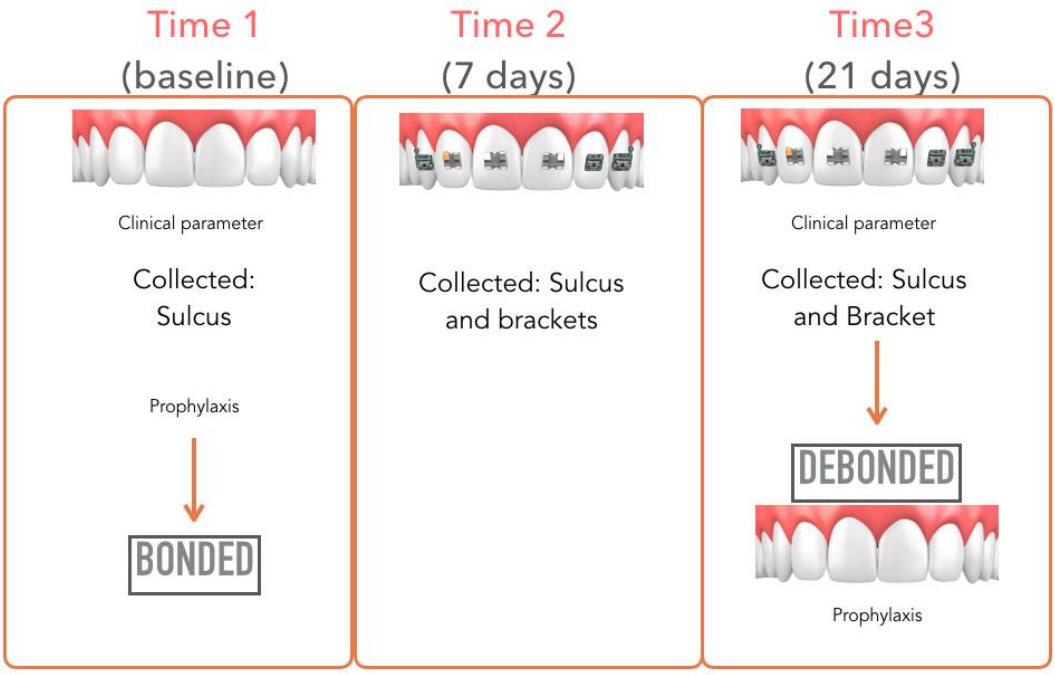
Still at time 1 (Figure 1), sulcular samples were collected. After relative isolation, removal of the supragingival biofilm, and light drying with air jets, samples from the gingival sulcus were collected from the mesiobuccal surface of the experimental teeth with sterilized absorbent paper cones (no. 30 from the Tanari brand) that were kept in position for one minute (Cortelli et al., 2008). Professional prophylaxis was then carried out to remove calculus, biofilm, and stains on all dental elements using a curette, Robinson brush, and prophylactic paste. Bonding was then carried out and the type of bracket for each tooth was chosen randomly by a simple draw among the possible types: self-ligating bracket (AL), conventional bracket (C), and conventional with ligation (CL). All brackets and elastic ligatures were from the brand Morelli (Sorocaba, São Paulo, Brazil) of the SLI (self-ligating) or Roth Max (conventional) type. The sites chosen for bonding were the center of the clinical crown of teeth 13, 12, 11, 21, 22, and 23 of each patient (Mei et al., 2017).

The clinical examination was repeated, and additional samples from the gingival sulcus were collected at two time points: seven days (time 2) and 21 days (time 3) after bonding. Furthermore, samples were obtained from the gingival surface of the brackets on the seventh and 21st days of the study, which was also the final date for bracket removal. On this date, a new professional prophylaxis was performed.

Microbiological samples from the brackets were collected with a sterilized micro brush, which was kept in contact with the gingival surface of the bracket for 20 seconds. The paper cones and cut ends of the micro brushes were then placed individually in dry mini tubes and frozen at -80ºC (Pires, 2014).

Oral hygiene instruction (OH) and standardized brushing kits were distributed to all participants in the bracket bonding consultation (time 1).

Figure 1 - Flowchart of chronology and procedures performed on participants.



2.6 LABORATORY ANALYSIS

Subsequently, samples were collected and stored in empty 1.5 ml tubes. Extraction of genomic DNA from the samples was performed using the PureLink™ Genomic DNA Purification Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

The quantification of *Porphyromonas gingivalis*, *Tannerella forsythia*, *Aggregatibacter actinomycetemcomitans,* and *Treponema denticola* was performed using real-time PCR with specifically designed primers (NCBI Nucleotide Search - http://www.ncbi.nlm.nih.gov/; Primer3 software - http://frodo.wi.mit.edu/) and tested for specificity (NCBI BLAST - http://blast.ncbi.nlm.nih.gov/Blast.cgi) and Taq Man® detection system. Absolute quantification was performed by comparing the Ct [cycle in which fluorescence becomes detectable above the background fluorescence and is inversely proportional to the logarithm of the number of initial target molecules] obtained from samples with the determined Ct values of a standard curve [101 - 108 cfu/ml, from ATCC strains kindly provided by Instituto Fio Cruz – RJ (INCQS)].

2.7 STATISTICAL ANALYZES

After complete processing of the samples by q-PCR and tabulation of pathogen frequencies in all groups and times of the present study, both the microbiological data and the data on the measured clinical parameters were subjected to specific statistical treatment. To this end, a significance level of 95% (α=0,05) was adopted with the help of BioEstat 5.0 and SPSS 13.0 Software.

For all analytical situations of interest, a database was selected, and the sampling distribution characteristic was tested. As all clusters presented normal distribution, only parametric statistical tests were selected.

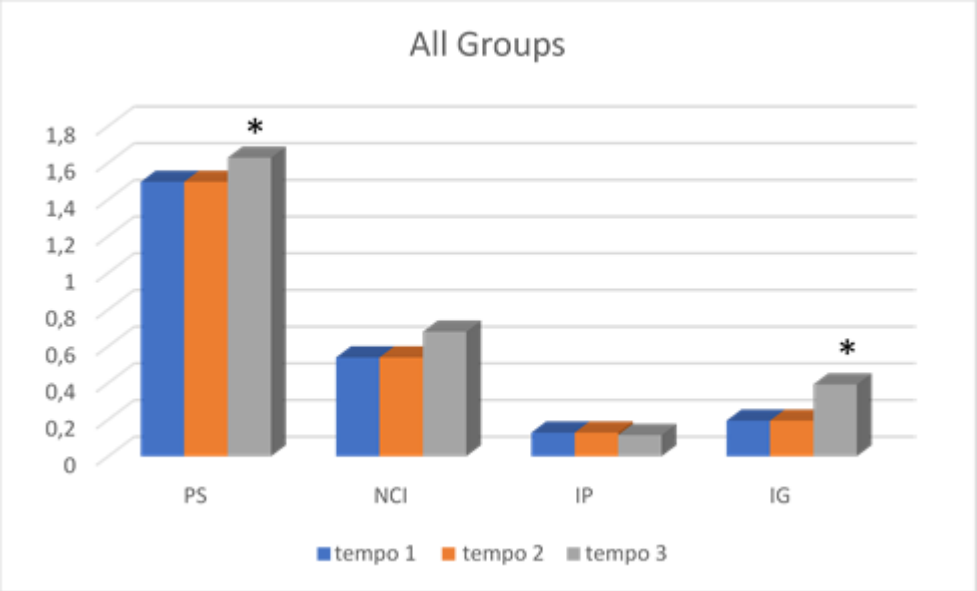
When calculating microbiological data, when comparing intergroup (level of bacteria x three bracket groups from time to time), the analysis had an independent characteristic and was performed using the student's t-test. In the intragroup comparison (bacteria level x three bracket groups between experimental times) the analysis had a dependent characteristic and was carried out with the paired t-test as well as microbiological analysis (clinical parameters x three bracket groups between times).

3 RESULTS

3.1 CLINICAL

The gingival clinical condition has changed over time. Regardless of the type of bracket, probing depth and gingival index worsened on the 21st day (figure 2).

Figure 2 - Average distribution of PS, NCI, IP, and IG parameters considering all brackets and comparing experimental times.
* - Statistically significant difference, p<0.05, paired t-test



3.2 MICROBIOLOGICAL

When comparing times using sulcular samples, there was no statistical difference in the levels of total bacterial load and A.a when analyzing each type of bracket (figures 3 and 4). P.g showed a reduction after prophylaxis in group C and remained stable. However, in the CL group, P.g increased over time (figure 4). T.d also reduced after prophylaxis and showed a tendency to progressively increase from time 2 to time 3 in the three types of brackets. Regarding T.F. levels, it was possible to observe an increase in both C (time2) and CL (time3).

It was then observed that after prophylaxis and oral hygiene instruction, there was a reduction in the reading of pathogens in the groove for the three bracket groups. In two and three weeks C and CL accumulated more periodontopathogens in the sulcus compared to AL.

Figure 3 - Total level of bacteria for sulcular samples from groups AL, C, and CL between experimental times.
* - Statistically significant difference, p<0.05, paired t-test

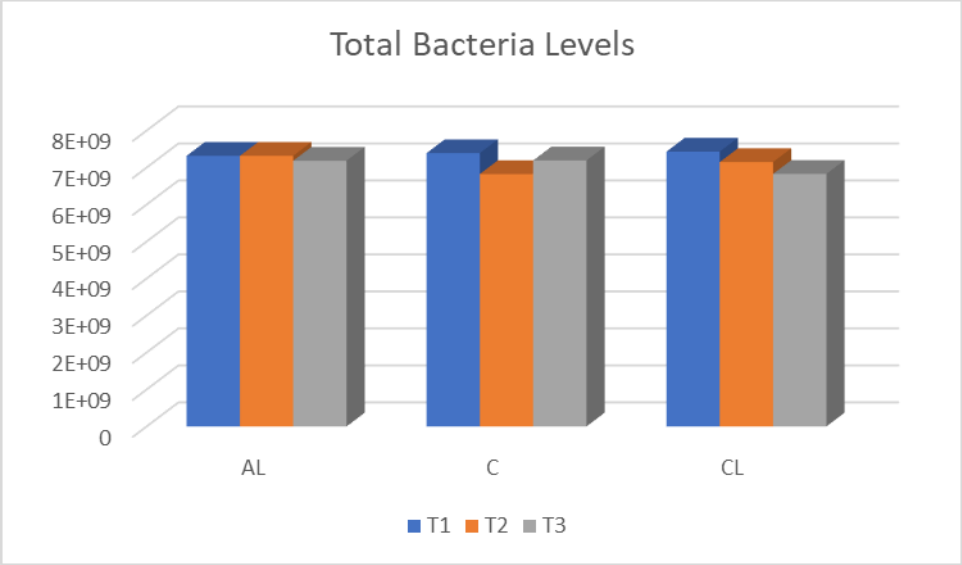
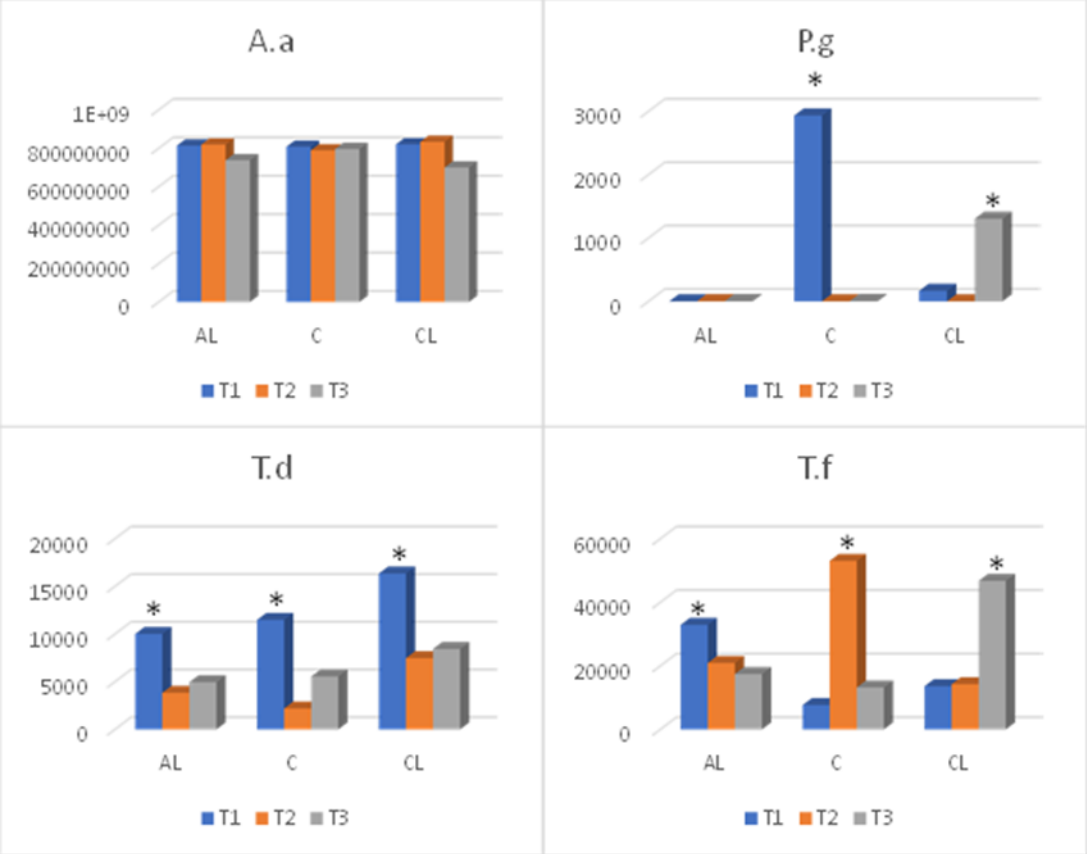


Figure 4 - Average levels of A.a, P.g, T.d, and T.f for sulcular samples from groups AL, C, and CL between experimental times.
*- Statistically significant difference, p<0.05, paired t-test



In the readings of the samples collected with the micro brush on the three types of brackets, the levels of total bacterial load and A.a were statistically higher at time 2 and remained increased at time 3 when compared to the initial reading at the time of bonding the brackets (figure 5 and 6). T.d and T.f also increased in the three types of brackets about the day of bonding, however, the levels were even higher at 21 days when compared to 7 days (figure 6). Despite the increasing trend, there was no statistical difference in P.g levels between times for any type of bracket (figure 6).

Figure 5 - Total level of bacteria in bracket samples from groups AL, C, and CL between experimental times
* - Statistically significant difference, p<0.05, paired t-test

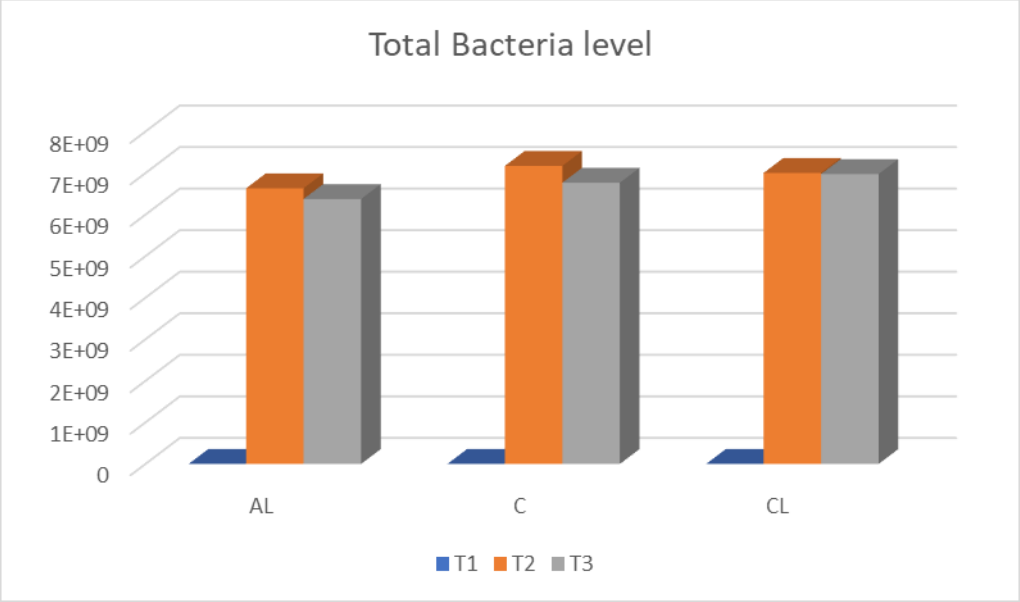
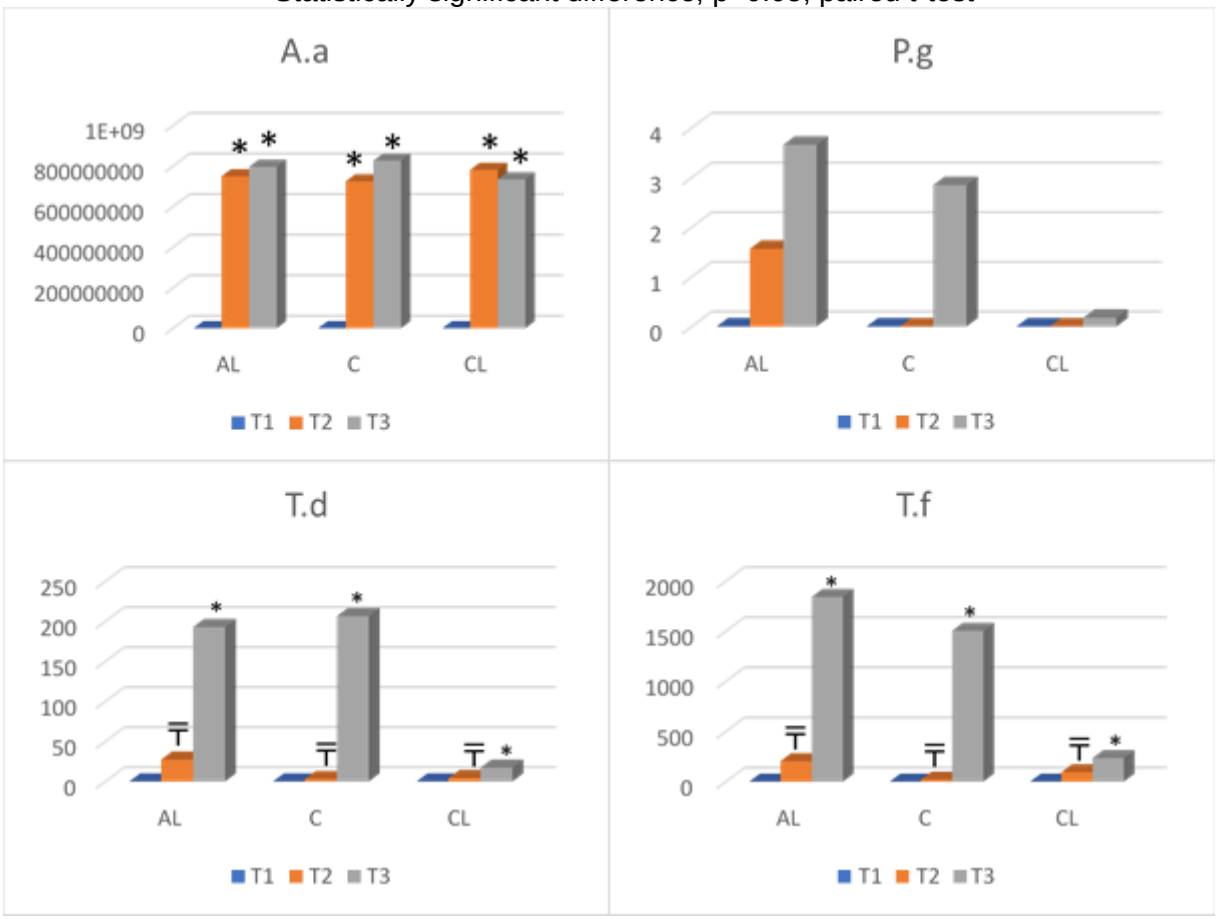


Figure 6 - Average levels of A.a, P.g, T.d, and T.f in bracket samples from groups AL, C, and CL between experimental times
*- Statistically significant difference, p<0.05, paired t-test



4 DISCUSSION

Bracket bonding is considered the second major moment of change in the oral microbiota after tooth eruption, promoting a significant impact on the patient's oral health (Müller et al., 2021; Anhoury et al., 2002; Cardoso et al., 2015). There is some unanimity regarding the change in periodontal clinical parameters after this impact, but such changes are also accompanied by a new grouping of the microbial complex in the host's gingival sulcus, favoring the accumulation of putative microorganisms (Mester et al., 2022; Jung et al., 2016).

Although there are more than 300 species of periodontopathogens identified in different individuals, only a few are strongly associated with periodontal disease (Bergamo et al., 2022). In the present study, we quantified those most strongly associated with aggressive diseases: *A.a*, *P.g*, *T.f*, and *T.d*, and, to read specific levels of these bacteria more sensitively and accurately, we used real-time PCR (Pejda et al., 2013).

Although bracket retentivity, even if undesirable, is an inherent characteristic of the piece, there is evidence that different designs and associated ligatures exert different responses in clinical and microbiological parameters (Santonocito & Polizzi, 2022; Jung et al., 2016; Bergamo et al., 2022), our study aimed to elucidate the impact

that conventional, conventional ligated and self-ligated brackets have on these parameters and eliminate possible biases observed in other studies.

In the intergroup comparison, we observed that the increase in the colonization of periodontopathogens was predominant in the sulcus in groups C and CL when comparing the three brackets from time to time, showing that the design of the bracket had a greater influence on the colonization of the sulcus than on the colonization of the bracket itself.

In the intragroup analysis (between times) we observed that the pre-existing microbiota in the patients' groove had a reduction in levels one week after bonding (time2). This fact is possibly due to the prophylaxis and hygiene instructions that all participants received before bracket bonding. Although our results are consistent with most studies (Hartenbach et al., 2018), some authors (Jung et al., 2016) did not find a relationship between the oral hygiene index and bacterial adhesion.

When we compared the sulcular reading one and three weeks after bonding (times 2 and 3), we observed that C and CL accumulated more periodontopathogens than AL, with C having an increase in T.f in one week and CL with an increase in *P.g* and *T.f* in three weeks. Our study is in agreement with those who observed the same increase after 3 months of study in group C and an increase in *T.f* levels in CL over a period of 6 months (Guo et al., 2016; Guo et al., 2017). Pejda et al. (2013) did not find a statistical difference in sulcus readings for *T.f, P.g*, and *T.d* levels at times longer than four months, but they used a methodology different from ours in a cross-sectional study.

Clarifying the role of these two microorganisms *T.f* is a Gram-negative anaerobic bacterium that needs to coexist with other pathogens so that it can appropriately express its virulence and reach its growth potential (Oettinger-Barak et al., 2014). However, *P.g* is a strict anaerobic Gram-negative and the need for conditions anaerobic bacteria leads to a delay in its colonization until certain microorganisms have reduced the oxygen concentration in the area, therefore the increase in the *P.g* level tends to be late (in our work observed only after three weeks), but dangerously progressive.

When observing the micro brush sample of the brackets between times, we observed that AL, C, and CL behaved similarly in all readings. There was an increase in the levels of total bacterial load and *A.a* from time 2 and this remained without increase about time 3 despite the final reading being only after three weeks. Our results are consistent with Pejda et al. (2013) and Guo et al. (2017) who observed an increase in *A.a* after six weeks. However, when reading *T.d* and *T.f* levels, there was a significant progressive increase when comparing times 1 with 2 and 2 with 3 in all bracket groups. In our samples we only used metal brackets, the increase in *T.d* in the three groups can be explained by the affinity that this pathogen has with metallic surfaces, the high surface tension of this material favors the initial colonization of *T.d* in the pieces (Anhoury et al., 2002).

It is necessary to remember that the “red complex” pathogens (*P.g, T.d, and T.f*) combine strategies for their individual and joint growth, causing this pathogenic consortium to significantly increase the host’s clinical parameters (Holt & Ebersole, 2005). Hartenbach et al., (2018) observed, in just one week, a clinical picture of gingivitis in healthy patients with compromised oral hygiene. Our patients also rely on the retentive aspect of the brackets and the difficulty this creates in oral hygiene. The change in periodontal parameters proved to be progressive and with solid coherence compared to our study's microbiological readings.

When measuring periodontal parameters for all brackets between time points, NCI and IG increased their levels on the 21st day. When the same levels were

observed group by group between experimental times, IG levels also increased regularly in the three groups of brackets at time 3, as in the study by Nalçaci et al. (2014) who observed the same result in AL and CL when reading IG.

Several studies associate negative changes in periodontal parameters with orthodontic therapy (Corghi et al., 2014*;* Müller et al., 2021; Santonocito & Polizzi, 2022; Diaz et al., 2016). In our study, the most alarming finding was the loss of the clinical level of insertion in a short space of time in the AL and CL groups. The increase in NCI is concomitant with the orthodontic intervention and there is a consequent loss of support. It is important to highlight that our samples were collected from anterior teeth and that this decrease in support is accompanied by gingival recession, which can have a significant impact on the patient's aesthetics (Gorbunkova et al., 2016).

Analyzing previous studies, we found that to demonstrate any statistical difference in IP, a period longer than one month would be necessary (Guo et al., 2016; Mummolo et al., 2013; Yang et al., 2017; Liu et al., 2011), in our work, clarification on oral hygiene care, the short time spent studying, more frequent visits for collections and the intrinsic motivation of each patient were limiting factors for observing a statistical difference in PI readings between periods. and brackets. Pithon et al. (2011) observed higher PI values in individuals with self-ligating brackets when compared to conventional brackets over a period of 21 days. The authors attribute this to microscopic irregularities in the fins. Using the same line of reasoning, the PS reading also did not demonstrate a statistical difference due to the short study time, Corghi et al. (2014) found changes related to PS, however, when comparing distant collections in a cross-sectional study.

There is no consensus regarding the reversibility of the impact of orthodontic appliances upon removal. Guo et al. (2017) observed that periodontopathogen levels return to normal, obviously depending on the patient's oral hygiene. However, some authors (Kim et al., 2016; Liu et al., 2021) stated that despite the reduction of the subgingival microbiota, the levels of the remaining most aggressive periodonto pathogens (P.g, A.a, and T.f) may still be critical for the periodontium. Clinical parameters can also undergo irreversible changes and the gums become more fibrotic, requiring surgical recontouring to return to their natural appearance (Pan et al., 2017).

Therefore, knowledge about the influence that orthodontic appliances and different types of brackets have on the periodontium, and more specifically on periodontal pathogens, is essential for the selection of material and technique to be appropriate for each patient. Monthly reinforcements regarding oral hygiene and joint work with a periodontist, especially in higher-risk cases, are essential to ensure that the dental support element is not compromised.

5 CONCLUSION

Although conventional brackets may have presented worse results for some microbiological parameters about clinical conditions, the influence of conventional brackets with ligation was as negative as that of self-ligation. Therefore, the hypothesis that patients using conventional brackets have greater bacterial colonization and worse clinical gingival conditions compared to those using self-ligating brackets was rejected.

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INTEREST CONFLICTS

The authors declare that there is no conflict of interest related to this study.

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