



Biofilm and clinical data between volunteers with self-ligating and conventional brackets: an observational study

Biofilme e dados clínicos entre voluntários com bráquetes autoligados e convencionais: um estudo observacional

Biofilm y datos clínicos entre voluntarios con brackets de autoligado y convencionales: un estudio observacional

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ABSTRACT

Introduction: Fixed orthodontic appliances can lead to enamel demineralization and gingival changes due to increased biofilm. **Aim:** To detect the presence of *Prevotella intermedia*, *Tannerella forsythia*, *Streptococcus mutans*, *Scardovia wiggsiae*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, and *Porphyromonas gingivalis* in oral biofilm and presence of white spot lesions and gingivitis in users of self-ligating and conventional brackets. **Design:** This observational controlled study was conducted from January to December 2020. The participants used fixed orthodontic therapy for 6 months and were divided into a conventional and a self-ligating bracket group. The participants underwent clinical examination, and biofilm samples were collected from their lower incisors. **Results:** No differences were found in the bacteria detection between the groups ($p>0.05$). However, white spot lesions were more common in users of self-ligating brackets ($p=0.019$). There was no association between clinical data and the detection of any microorganism ($p>0.05$). The fluorescence intensity of *A. actinomycetemcomitans* was higher in self-ligating brackets than in conventional brackets ($p<0.05$). **Implications:** The microbial diversity did not differ between the types of brackets; however, the presence of white spot lesions and the amount of *A. actinomycetemcomitans* were higher in patients with self-ligating brackets.

DESCRIPTORS

Biofilms; Orthodontic bracket; Dentistry.

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INTRODUCTION

Biofilms are microbiological derivatives of sessile communities irreversibly attached to a substrate or an interface or to each other,¹ containing a wide variety of species that interact with each other²⁻³ and are incorporated into a matrix of extracellular polymeric substances.⁴ The oral cavity is colonized by different microbial species that are naturally organized in biofilms.⁵

The orthodontic appliance can increase the areas retentive to biofilms, causing a decrease in the pH of the plaque and an increase in gingival inflammation⁶⁻⁷ due to the deepening of the gingival sulcus.⁸⁻¹⁰ Changes in oral microflora, mainly associated with the presence of food residues that increase microbial colonization,¹¹ can potentially lead to periodontal disease and demineralization of teeth, which can result in caries and/or white spots that represent a post-treatment aesthetic problem¹² in addition to reversible gingival inflammation,¹³⁻¹⁴ and periodontal damage.¹⁵

Several studies have shown that the introduction of orthodontic appliances in the mouth alters the biofilm composition and increases the prevalence of bacterial species that are known as periodontal and cariogenic pathogens.^{8,16-18} The most common periodontopathogens in orthodontic treatments are *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Tannerella forsythia* and *Fusobacterium nucleatum*.¹⁹⁻²⁰ Devices used in orthodontic appliances can also promote increased adhesion of cariogenic bacteria, such as *Streptococcus mutans*,²¹⁻²² which leads, within 6 months of appliance placement,²³ to an increased risk of enamel demineralization²⁴ and the development of white spot lesions that can progress to cavitation,²⁵ which may appear in 50% of patients who use fixed orthodontic appliances,²⁶ demonstrating the susceptibility of the appearance of white spot lesions in patients undergoing orthodontic treatment. *Streptococcus mutans*, *Porphyromonas gingivalis*, and

Aggregatibacter actinomycetemcomitans were observed in the biofilm of individuals after 48 hours of bracket installation.²⁷

The development of biofilm and white spots under orthodontic bands observed was associated with a diverse microbiota,²⁸ including *Scardovia wiggsiae*,²⁹ a new species associated with severe early childhood caries³⁰ and which were even associated with the presence of white spots and gingivitis in adolescents undergoing orthodontic treatment.³¹

Conventional brackets are associated with the use of elastomeric or stainless-steel ligatures³² and they usually lead to greater accumulation and qualitative alteration of the biofilm.³² Self-ligating brackets were introduced in clinical orthodontics with many advantages, such as the elimination of elastomer or stainless-steel ligation, reduced complexity, and fewer bacterial adhesion sites, which can facilitate better oral hygiene.³³⁻³⁴ This is due to the absence of these bandages and, also, their shape, which allows for better hygiene.²¹ Periodontopathogens *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *F. nucleatum* and *T. forsythia* have already been detected in these brackets.³⁵ There is evidence that self-ligating metal brackets accumulate less *S. mutans* than conventional ones.³⁶

Given the above, there is a strong suggestion that the accumulation of biofilm may be different according to the type of bracket used;³⁷ however, there is still a lack of scientific evidence on the lesser adhesion of biofilm in self-ligating brackets that justifies their use instead of conventional ones.³⁸ It is controversial whether using self-ligating systems' opening and closing mechanisms and removing the ligatures from conventional brackets can lessen the adherence of microbes and the formation of biofilm.³⁹ So, the present study aims to detect the presence of *Prevotella intermedia* (Pi), *Tannerella forsythia* (Tf), *Streptococcus mutans* (Sm), *Scardovia wiggsiae* (Sw), *Aggregatibacter actinomycetemcomitans* (Aa), *Fusobacterium nucleatum* (Fn) and *Porphyromonas*

gingivalis (Pg) in biofilm samples from self-ligating and conventional brackets and to determine the presence of white spots and gingivitis according to the type of bracket.

METHOD

This is an observational study approved by the Uberaba University Ethics Committee (CAAE 16594919.2.0000.5145). The participants were selected from adult patients who were going to be treated for their malocclusion in a dental clinic in the Department of Orthodontics of the Uberaba University from January to December 2020. A total sample of 26 patients was included. The sample size was calculated by means of BioEstat 5.3 software based on mean and standard deviation values found by a preliminary pilot study. According to this estimation, the sample size was determined with a test power of 90%, $\alpha = 5\%$, with a difference in mean and standard deviation values of 1 and 0.9, respectively. Ten volunteers had conventional metallic brackets with metal ligature and 16 had self-ligating metallic brackets (Morelli, models: Max and SLI, respectively).

These volunteers were eligible because they had been using the device for more than 6 months, were not smokers and had not used antibiotics or other medications in the last 30 days. They were also in good general health.

Bracketed teeth were examined for white spot lesions adjacent to the bonded brackets by direct visualization with 2X magnification (dental loops); and from intra-oral photographs. Gingival and plaque indices were measured in six different locations (distal-buccal, mid-buccal, mesiobuccal, disto-lingual, mid-lingual, mesio-lingual). Gingivitis was considered positive when there were 25% or more of sites with bleeding on probing and no sites with clinical attachment loss > 2 mm.⁴⁰ Biofilm samples were collected with the aid of a pre-contoured and sterilized orthodontic tool, from the buccal surface of the lower incisors and were immersed in 1X PBS

solution and transported on ice to the Microbiology laboratory of Uberaba University.

Detection of bacteria in the samples

DNA from the samples was purified using the PowerLyzer PowerSoil DNA Kit (MO-BIO, Carlsbad, CA) according to the manufacturer's instructions. The StepOne™ Real-Time PCR System (Thermo Fisher Scientific) was used to perform the samples. Primers (Invitrogen, Carlsbad, CA, USA) targeted the 16S rRNA (Table 1). Each reaction tube contained a reaction mixture, including 6.5 μ L SYBR Green Master Mix (Roche, Illinois, USA), 1 μ L of each primer, 4.5 μ L of ultrapure water, and 2 μ L of DNA extracted from samples. The cycling conditions were an initial amplification cycle of 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min.

Table 1. Oligonucleotides Used in This Study

Bacteria Primer	sequence 5'-3'
<i>Prevotella intermedia</i>	F:AATACCCGATGTTGTCCACA, R:TTAGCCGGTCCTTATTCGAA;
<i>Tannerella forsythia</i>	F:CGGGCGTGCATCTTGTCTGTCTAC, R:CTTAACCCGGCCGCTCTTTGAA;
<i>Streptococcus mutans</i>	F:TCGCGAAAAAGATAAACAACA, R:GCCCTTACAGTTGGTTAG;
<i>Scardovia wiggisiae</i>	F:GTGGACTTTATGAATAAGC, R:CTACCGTTAAGCAGTAAG;
<i>Aggregatibacter actinomycetemcomitans</i>	F:GGCGAGCCTGTATTTGATGTGCG, R:GTGCCCGGTGCTGCGTCTTTG;
<i>Porphyromonas gingivalis</i>	F:TGCAACTTGCCTTACAGAGGG, R:ACTCGTATCGCCCGTTATTC
<i>Fusobacterium nucleatum</i>	F:ACCTAAGGGAGAAACAGAACCA, R:CCTGCCTTAATTCATCTCCAT;

Source: Authors (2024).

Statistical methods

The frequencies of samples with bacterial positivity were compared to each other and to the clinical data obtained by the questionnaire using the Chi-square test or the Fisher's Exact Test. A value of $p < 0.05$ was considered statistically significant. Data were analyzed using BioStat® Software.

RESULTS

Demographic data of the sample is described in Table 2. The matching criteria were confirmed, as

no significant differences were detected in gender distribution and mean age among the participants of the two groups.

Table 2. Demographic data in the voluntaries in according to types of brackets

	self-ligating	conventional
Gender	8M and 8F	6M and 4F
Numerosity	16	10
Age (mean ±sd)	(21.5 ± 3.6)	(20.1± 2.4)

Notes: sd= standard desviation, M=Male, F=Female.

Source: Authors (2024).

The most frequently detected bacteria were Sm and Pi (57.7%) followed by Aa (53.8%) and Tf (50.0%). Pg was detected in 26.9% of samples and Fn was detected in only 11.5% of samples. Sw was detected in 26.9% of the samples. Although Sm, Pg, Tf, Aa and Fn bacteria were more frequently

detected in self-ligating brackets and Pi, Sw and Aa in conventional brackets, there were no statistically significant differences (Table 3, p>0.05) between the types of brackets. Fourteen patients had white spot lesions (53.6%) and 15 (57.7%) had gingivitis.

Table 3. Number of samples with positive and negative bacteria (*Prevotella intermedia* (Pi), *Tannerella forsythia* (Tf); *Streptococcus mutans* (Sm), *Scardovia wiggsiae* (Sw), *Aggregatibacter actinomycetemcomitans* (Aa), *Fusobacterium nucleatum* (Fn), *Porphyromonas gingivalis* (Pg)) detection according to the types of brackets

Bacteria Detection		Bracket Types:	
		Self-ligating n=16	Conventional n=10
<i>Sm</i>	Yes	10 (62.5%)	5 (50.0%)
	No	6 (37.5%)	5 (50.0%)
<i>Pg</i>	Yes	5 (31.3%)	2 (20.0%)
	No	11 (68.7%)	8 (80.0%)
<i>Pi</i>	Yes	9 (56.3%)	6 (60.0%)
	No	7 (43.7%)	4 (40.0%)
<i>Sw</i>	Yes	3 (18.7%)	4 (40.0%)
	No	13 (81.3%)	6 (60.0%)
<i>Tf</i>	Yes	9 (56.3%)	4 (40.0%)
	No	7 (43.7%)	6 (60.0%)
<i>Aa</i>	Yes	2 (12.5%)	1 (10.0%)
	No	14 (87.5%)	9 (90.0%)
<i>Fn</i>	Yes	2 (12.5%)	1 (10.0%)
	No	14 (87.5%)	9 (90.0%)

Source:Authors (2024).

Most patients with white spots (n=12/14) used self-ligating brackets, which was statistically different from patients with conventional brackets,

as only 2 out of 10 had the lesion (Table 4, p=0.019). On the other hand, there were no differences

between patients with or without gingivitis according to the type of bracket (Table 4, $p=0.82$).

Table 4. Number of samples with white spots and gingivitis according to types of brackets

Clinical data	Types of Brackets		<i>p-value</i>
	Self-ligating n=16	Conventional n=10	
<i>White spot</i>	Yes	12 (75%)	0.019
	No	2 (25%)	
<i>Gengivitis</i>	Yes	10 (62.5 %)	0.82
	No	6 (37.5 %)	

Source: Authors (2024).

Regardless of the type of bracket, 50% of the 14 samples with white spots had Sm and Pi and 35.7 % had Sw and Tf (Table 5). Among the samples with gingivitis, most had Sm (80%), Pi and Tf (73.3%). Sw, Pg, and Fn were less frequently detected (less than 34%). There was no significant association between the presence of white spots in the samples of self-ligating and conventional brackets and the

detection of most of the bacteria analyzed (Table 5, $p>0.05$), except for Fn which was detected in the 2 samples of conventional brackets and in only 1 of 11 samples obtained from self-ligating brackets (Table 5, $p=0.04$). Regarding the presence of gingivitis, there were no statistically significant differences between the types of brackets and the presence of the analyzed bacteria (Table 5, $p>0.05$).

Table 5. Number of samples with white spot and gingivitis according to types of brackets and bacteria

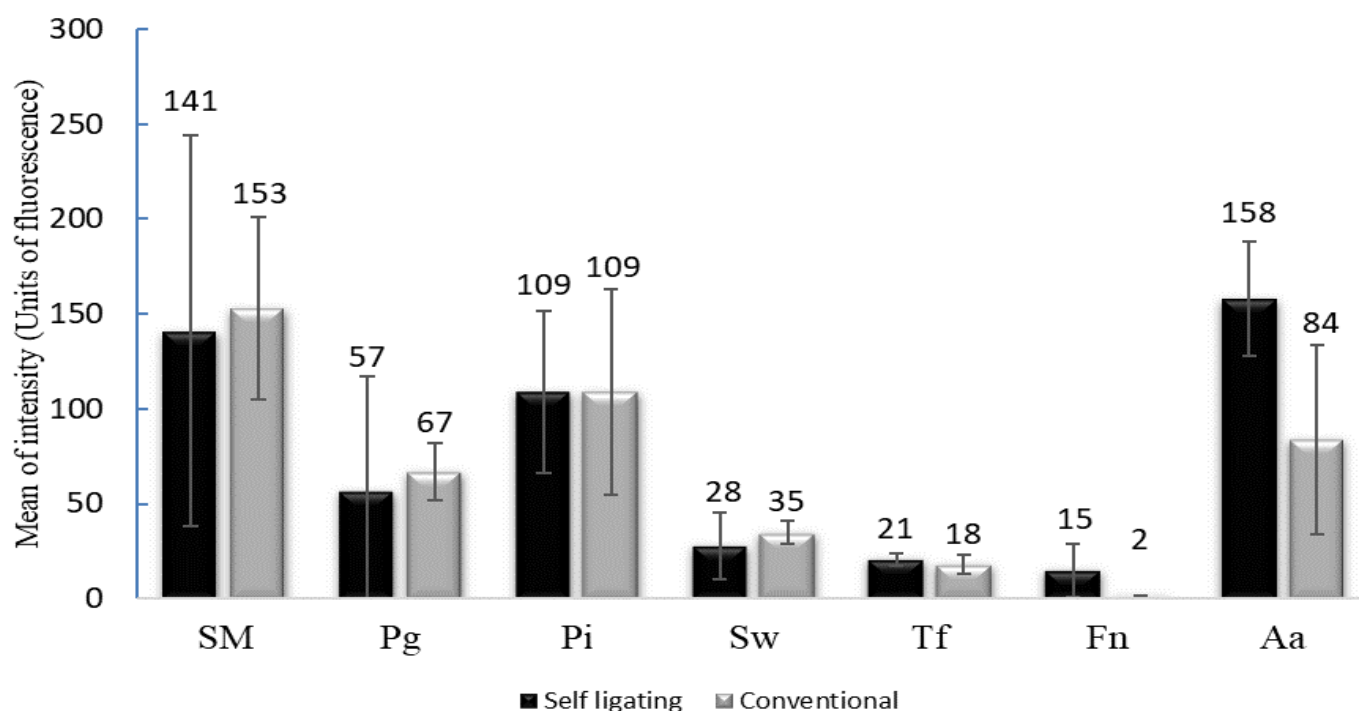
Bacteria detection	White spot detected			Gingivitis detected		
	Self-ligating (n=12)	Conventional (n=2)	<i>p-value</i>	Self-ligating (n=10)	Conventional (n=5)	<i>p-value</i>
<i>Sm</i>	Yes	7	0.44	8	4	0.49
	No	5		2	1	
<i>Pg</i>	Yes	3	0.89	4	1	0.84
	No	9		2	4	
<i>Pi</i>	Yes	5	0.44	7	4	0.83
	No	7		0	1	
<i>Sw</i>	Yes	3	0.21	3	2	0.21
	No	9		0	0	
<i>Tf</i>	Yes	6	0.58	7	4	0.83
	No	6		0	1	
<i>Fn</i>	Yes	1	0.04*	2	1	0.49
	No	11		0	4	
<i>Aa</i>	Yes	5	0.73	7	4	0.83
	No	7		0	1	

Notes: *Prevotella intermedia* (Pi), *Tannerella forsythia* (Tf); *Streptococcus mutans* (Sm), *Scardovia wiggisiae* (Sw), *Aggregatibacter actinomycetemcomitans* (Aa), *Fusobacterium nucleatum* (Fn), *Porphyromonas gingivalis* (Pg).
Source: Authors (2024).

The mean fluorescence intensity emitted by PCR, which represents the amount of material detected, for each bacterium and type of device is shown in Figure 1. There were no statistically significant differences for most bacteria ($p>0.05$)

except for Aa, which had a higher mean fluorescence in the self-ligating bracket samples (Figure 1, $p=0.005$).

Figure 1. The mean intensity of fluorescence emitted by positive detection of bacteria in the samples collected in self-ligating and conventional brackets



Notes: *Prevotella intermedia* (Pi), *Tannerella forsythia* (Tf); *Streptococcus mutans* (Sm), *Scardovia wiggisiae* (Sw), *Aggregatibacter actinomycetemcomitans* (Aa), *Fusobacterium nucleatum* (Fn), *Porphyromonas gingivalis* (Pg).

Source: Authors (2024).

DISCUSSION

The results showed that *S. mutans* and *P. intermedia* were the most detected bacteria, followed by *T. forsythia*, *A. actinomycetemcomitans*, *S. wiggisiae* and *P. gingivalis*. No differences were found in the detection of these bacteria in the different types of brackets. White spot lesions were more recurrent in users of self-ligating devices. There was no association between the presence of gingivitis or white spot and the detection of any specific microorganism.

Although there are several preventive measures to control the formation of biofilm during orthodontic treatment, there is still no concrete solution to prevent its development.⁴¹ It must be

considered that, although it is indisputable that the components of the orthodontic appliance provide retentive areas for the accumulation of microorganisms, as the results showed, there is a lack of evidence regarding the microbial diversity of biofilm associated with the types of brackets.

All samples presented at least one type of bacteria analyzed (especially *S. mutans* and *P. intermedia*, which were found in approximately 58% of samples), providing a suitable environment for the development of an oral infection.⁴² This can lead to the appearance of white spots and gingivitis, which were detected in 55% and 58% of teeth respectively. Fixed orthodontic appliances in general have surface roughness, which facilitates the accumulation of

biofilm and consequently causes pathological changes in the gingival tissues.⁴³ There is evidence that *Prevotella intermedia* increases at the start of treatment but returns to pre-treatment levels several months after appliance removal.^{19,44-45}

Some studies report that self-ligating brackets are less susceptible to bacterial colonization due to their shape and the lack of metallic or elastomeric ligatures.^{21,46} The accumulation of *S. mutans* in conventional ligatures seemed to be 4.9 times higher than in self-ligating brackets,⁴⁷ although it has been demonstrated that these ligatures represent a bio-hostile material for microbial survival.^{21,48} On the other hand, other studies were not unanimous in reporting a possible influence of bracket design on the adhesion of *S. mutans*³⁸ as our results show. The quantitative analysis of *S. mutans*, *Streptococcus sobrinus*, and *Lactobacillus acidophilus* also did not find statistically significant differences between self-ligating and conventional brackets.⁴⁹

Pedja et al.³⁸ also did not find differences in the detection of periodontopathogens in different types of stainless steel brackets (conventional and self-ligating). Garcez et al.⁵⁰ and Pithon et al.⁵¹ showed a lower formation of periodontopathogen biofilms in conventional stainless steel brackets connected with ligatures than in self-ligated brackets. In this study, we collected samples from conventional brackets without ligatures and we did not find differences in the diversity of colonization between types of brackets. Additionally, the amount of *Aggregatibacter actinomycetemcomitans* was higher in self-ligating brackets than in conventional brackets.

The discovery of *Scardovia wiggsiae* and its involvement with caries in a quarter of the population has led many researchers to reassess and reexamine the saliva of patients in various populations.^{30,51-52} *S. wiggsiae* was detected in 26,9%

of samples, similar to the percentage (24%) found by Row et al.,⁵¹ unlike the results of Whiteley and Kingsley⁵² who found it in 14% of adult patients undergoing orthodontic treatment. The present result corroborates this research, especially when it is observed that more than 70% of the patients with the detected bacteria had white spots associated with the brackets.

Although Polat et al.⁵³ did not find differences in white spot lesion development between conventional straight wire and self-ligating brackets, other studies³⁶ showed that the incidence of white spot was lower in the self-ligation than in the conventional ligation. In contrast, here, we found that the majority of white spots detected were in patients with self-ligating brackets.

Choosing the right type of orthodontic appliance is crucial for the success of the treatment, taking into account the specific needs of each patient. Additionally, maintaining and controlling the biofilm, the bacterial film, which is formed on teeth, is fundamental to prevent complications such as cavities and periodontal diseases during orthodontic treatment. Regular oral hygiene, coupled with periodic visits to the orthodontist for adjustments and assessments, not only optimizes the effectiveness of the treatment but also contributes to long-term oral health, ensuring an aligned and healthy smile.

CONCLUSION

In conclusion, *Prevotella intermedia* and *Streptococcus mutans* were the most frequently detected bacteria in the bracket biofilm. There were no differences in the colonization of bacteria in biofilm samples from self-ligating and conventional brackets. The clinical data showed that white spot lesions were more recurrent in users of self-ligating brackets.

Introdução: Aparelhos ortodônticos fixos podem levar à desmineralização do esmalte e alterações gengivais devido ao aumento do biofilme. **Objetivo:** Detectar a presença de *Prevotella intermedia*, *Tannerella forsythia*, *Streptococcus mutans*, *Scardovia wiggisiae*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum* e *Porphyromonas gingivalis* em biofilme oral e presença de lesões de mancha branca e gengivite em usuários de bráquetes autoligados e convencionais. **Delineamento** Este estudo observacional controlado foi realizado de janeiro a dezembro de 2020. Os participantes utilizaram terapia ortodôntica fixa por 6 meses e foram divididos em grupo de bráquetes convencionais e autoligáveis. Os participantes foram submetidos a exame clínico e amostras de biofilme foram coletadas de seus incisivos inferiores. **Resultados:** Não foram encontradas diferenças na detecção de bactérias entre os grupos ($p>0,05$). Entretanto, lesões de mancha branca foram mais comuns em usuários de bráquetes autoligados ($p=0,019$). Não houve associação entre os dados clínicos e a detecção de qualquer microrganismo ($p>0,05$). A intensidade de fluorescência de *A. actinomycetemcomitans* foi maior nos bráquetes autoligados do que nos bráquetes convencionais ($p<0,05$). **Implicações:** A diversidade microbiana não diferiu entre os tipos de bráquetes; entretanto, a presença de lesões de mancha branca e a quantidade de *A. actinomycetemcomitans* foram maiores nos pacientes com bráquetes autoligáveis.

DESCRITORES

Biofilmes; Bráquetes ortodônticos; Odontologia.

RESUMEN

Introducción: Los aparatos de ortodoncia fijos pueden provocar desmineralización del esmalte y cambios gingivales debido al aumento de biopelícula. **Objetivo:** Detectar la presencia de *Prevotella intermedia*, *Tannerella forsythia*, *Streptococcus mutans*, *Scardovia wiggisiae*, *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum* y *Porphyromonas gingivalis* en biofilm oral y la presencia de lesiones de mancha blanca y gingivitis en usuarios de brackets de autoligado y reales. **Delineación:** Este estudio observacional controlado se llevó a cabo de enero a diciembre de 2020. Los participantes utilizaron terapia de ortodoncia fija durante 6 meses y se dividieron en un grupo de brackets convencional y de autoligado. Los participantes se sometieron a un examen clínico y se recogieron muestras de biopelículas de sus incisivos inferiores. **Resultados:** No se encontraron diferencias en la detección de bacterias entre los grupos ($p>0,05$). Sin embargo, las lesiones de manchas blancas fueron más comunes en los usuarios de brackets de autoligado ($p=0,019$). No hubo asociación entre los datos clínicos y la detección de algún microorganismo ($p>0,05$). La intensidad de fluorescencia de *A. actinomycetemcomitans* fue mayor en brackets de autoligado que en brackets ocasionales ($p<0,05$). **Implicaciones:** La diversidad microbiana no difiere entre los tipos de brackets; sin embargo, la presencia de lesiones de mancha blanca y la cantidad de *A. actinomycetemcomitans* fueron mayores en pacientes con brackets de autoligado.

DESCRIPTORES

Biopelículas; brackets de ortodoncia; Odontologie.

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COLLABORATIONS

RDN, VRG-M, MSG and MAN: Contributions to the study design. CGG and LLG: contributions to data collection. RDN, VRG-M: contributions to data analysis and interpretation. RDN, VRG-M and MSG: contributions to the discussion of results and writing

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AVAILABILITY OF DATA

Files from the research.

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CONFLICTS OF INTEREST

There are no conflicts of interest to declare.