Comunicação Científica e Técnica em Odontologia 5

Emanuela Carla dos Santos
(Organizadora)

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contato@atenaeditora.com.br

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APRESENTAÇÃO

A inovação é o combustível do crescimento profissional em todas as áreas, mesmo na mais tradicional até a área mais tecnológica. A Odontologia é a ciência que agrega os princípios técnicos tradicionais, como por exemplo, aqueles postulados por Greene Vardiman Black, às mais avançadas tecnologias, como escâneres intraorais e impressoras 3D capazes de produzirem peças anatomicamente perfeitas, específicas para cada caso.

Pensando na propagação de conhecimento dentro das mais variadas áreas de atuação do Cirurgião Dentista, a Atena Editora disponibiliza mais um compilado de artigos, organizados em dois volumes, com a temática Comunicação Técnica e Científica em Odontologia.

Espero que a leitura do conteúdo deste E-book proporcione ampliação de conhecimentos e que também provoque curiosidade em você, leitor, pois são os novos questionamentos que impulsionam novas descobertas.

Ótima leitura.

Emanuela C. dos Santos

SUMÁRIO

CAPÍTULO 12

QUANTITATIVE EVALUATION OF BEHAVIOR AND PATTERN OF BACTERIAL ADHESION ON CERAMIC AND METAL BRACKET

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Luíza Trindade Vilela
Master Degree Student, Department of Pedodontics and Orthodontics, Universidade Federal do Rio de Janeiro, Brazil - http://lattes.cnpq.br/8994319185522709

Department of Pedodontics and Orthodontics, Universidade Federal do Rio de Janeiro, Brazil (UFRJ), Avenida Professor Rodolpho Rocco, 325, Ilha do Fundão, Rio de Janeiro, RJ, Brazil, 21941-617.

Email: luizavilela@ortodontia.ufrj.br

José Columbano Neto
Pós Doc Student, Department of Pedodontics and Orthodontics, Universidade Federal do Rio de Janeiro, Brazil - http://lattes.cnpq.br/7438849262033948

Flávio de Mendonça Copello
PhD Student, Department of Pedodontics and Orthodontics, Universidade Federal do Rio de Janeiro, Brazil - http://lattes.cnpq.br/7856184733482682

Margareth Maria Gomes de Souza
Full Professor, Department of Pedodontics and Orthodontics, Universidade Federal do Rio de Janeiro, Brazil - http://lattes.cnpq.br/1918221989593445

Ana Maria Bolognese
Full Professor, Department of Pedodontics and Orthodontics, Universidade Federal do Rio de Janeiro, Brazil - http://lattes.cnpq.br/7632528786733247

ABSTRACT: Objective: The aim of this study was to evaluate the behavior and pattern of bacterial adhesion (*S. Mutans, Lactobacillus spp and Candida Albicans*) on and around ceramic and metallic brackets. **Materials and Method:** Partial fixed orthodontic appliances were installed in 18 patients (4 metal brackets and 4 ceramic brackets with a SS 0.019 “x 0.026”). Two plaque collections were made: directly from the pre-bonded dental surface and the surface of the brackets, 21 days after bonding procedures. For the fulfillment of the macroscopic reading, plates that presented from 30 to 300 colonies were selected. Macroscopic reading, plates that presented from 30 to 300 colonies were selected. The comparisons between the microorganisms were made using the Kruskal-Wallis test, with the comparisons between pairs using the Mann-Whitney test. The Mann-Whitney test was also used to test the differences between the types of brackets. **Results:** In both types of brackets, *Streptococcus mutans* was the most present microorganisms, followed by *Lactobacillus spp.* No difference for ceramic brackets. The Scanning Electron Microscopy examination on the surface of the metal and ceramic brackets showed a heterogeneous distribution of forms suggestive of *cocci, bacilli* and *fungi* in the three

areas delimited for visualization on the surface of these accessories. **Conclusions:** There is no difference when comparing ceramic and metal brackets. The fins did not show sites of preference in colonization.
KEYWORDS: Microbiology, Dental Materials, Dental Aesthetic, Orthodontic Appliance.

1 I INTRODUCTION

The orthodontic treatment of malocclusions is briefly based on mechanical energy generated by fixed orthodontic appliance forces resulting in biological reaction to promote tooth movement [1] and because of the orthodontic devices such as brackets, they may provide additional retentive surfaces for oral bacteria and make traditional oral hygiene more difficult [2].

The buccal microbial flora is a mixture of different microorganisms and usually consist of more than 200 species [3] and some of these microorganisms are associated with enamel demineralization as *Streptococcus mutans* [4,5] and *Lactobacillus spp* [6,7]. There is also a direct relationship between gingival inflammation and dental plaque [8-11]. The frequency of *Candida* species can also be increased by the presence of these devices [12,13]. *Candida* species are present in about 50-60% of population [14] and these microorganisms are linked to an infection disease called candidiasis, especially in the immunocompromised patient population and that is the reason they are called opportunistic pathogen [15].

Orthodontic treatment has been increasingly requested by adult patients and young adults mainly due to the constant increase of aesthetic requirements and the search for an apleasant appearance [16]. Treatment with discrete and aesthetic devices is highly requested by these patients [17]. Dental materials manufacturers have been sought alternatives for discrete treatment by developing technologies and techniques for specific treatments such as aesthetic brackets.

The rough surface of the brackets provides a highly favorable ecologic niche for the adherence of microorganisms and the continuous development of biofilm [18-19]. Several studies have analyzed the bacterial adhesion on different types of metal and ceramic brackets [1, 20-22], but few studies evaluate the colonization of these devices in detail according to the design of the brackets.

Therefore, the aim of this study was to evaluate the presence of *Streptococcus mutans, Lactobacillus spp* and *Candida albicans* of the buccal microbiota on the surface of teeth before and after the devices bonding and analyze how the colonization of these microorganisms is distributed over aesthetic and metallic brackets in different zones of these accessories.

2 I MATERIAL AND METHOD

Prior to the start of the study, the project was approved by the Human Ethics

Committee of the University Hospital Clementino Fraga Filho of the Federal University of Rio de Janeiro by the number 2.796.767.

2.1 Brackets and Microorganisms

Two types of brackets were used: metal and ceramic brackets (3M UNITEK, St Paul, Minnesota, USA) both with a 0.022 slot for edgewise-arch technique. All the brackets were for the canines and bicuspid tooth.

Three microorganisms were investigated: *Streptococcus mutans, Lactobacillus spp* and *Candida albicans*. To analyze *Streptococcus mutans* it was used a selective *Mitis Salivarius* agar modified by the addition of 20% sucrose and 0.2 ul bacitracin per ml. The plates were incubated under anaerobic conditions at 37 °C for 48 hours. The *Lactobacillus* spp were assessed by the culture of these in *Rogosa* agar at 37 °C for 72 hours. Finally, to analyze *Candida* albicans it was used a selective CHROM agar at 37 °C for 48 hours.

For all the experiments were selected plaques that presented from 30 to 300 visible colonies macroscopically. Then, the colonies were counted and the inoculated amount was converted by the 10-1, 10-2, 10-3 and 10-4 dilution factor (PATTERSON, REVANKAR, KIRKPATRICK et al., 1996).

2.2 Brackets bonding, and biofilm collection

All patients received the same oral hygiene instructions one week before bonding brackets and they were instructed not to eat food and not to brush their teeth for a minimum of 12 hours before the first collection of the dental biofilm to determine the microbiological profile of each patient. The brackets were positioned following the protocol illustrated in Figure 1. Immediately after the brackets bonding, a passive segmented arch was inserted and after 21 days the second collection of biofilm was performed using a sterile probe for each bracket.

14 Metal	13 Ceramic	23 Metal	24 Ceramic
44 Ceramic	43 Metal	33 Ceramic	34 Metal

Figure 1 Distribution of metal and ceramic brackets in the buccal surfasse of the upper and lower canines and first bicuspids for each patient

2.3 Scanning Electron Microscopy

The brackets were processed for electron microscopy. They were dehydrated in alcohol, dried, fixed in 10% formaldehyde, then they were treated with gold palladium, and observed using a Scanning Microscope JEOL-JSM 530 with a magnification of 35x, 1,000x, 2,000x, 5,000x and 7,500x to verify the microorganisms arrangement in the composition of the colonies.

2.4 Statistical analysis

Descriptive statistics procedures were used to express the results as median and interquartile range (IQR). The normality of the data was tested using the Shapiro-Wilk test. The comparisons between the microorganisms were made using the Kruskal-Wallis test, with the comparisons between pairs using the Mann-Whitney test. The Mann-Whitney test was also used to test the differences between the types of brackets. Correlations of Pearson and Spearman were used to test the associations between the different between microorganisms counts in the baseline (before the brackets bonding) and after 21 days of the procedure. The significance level adopted was 5% ($\alpha = 0.05$) and the analyses were performed in IBM SPSS Statistics for Windows (IBM SPSS, 21.0, 2012, Armonk, NY: IBM Corp.).

3 I RESULTS

It was found that in both types of brackets, *Streptococcus mutans* was the most present microorganisms, followed by *Lactobacillus spp*. No statistical difference was found in the counts of Streptococcus mutans, *Lactobacillus spp*. and *Candida Albicans*, according to the type of bracket (Table 1).

Microorganism	Bracket Type		*p-value
	Metalic	Ceramic	
Streptococcus mutans (CFU/mL)	13,06 ± 1,58[a]	12,32 ± 2,93[a]	0,393
Lactobacillus spp. (CFU/mL)	7,88 ± 3,33[b]	7,23 ± 3,88[b]	0,862
Candida Albicans (CFU/mL)	2,30 ± 4,22[c]	0,00 ± 4,63[c]	0,342
†p-value	< 0,001	< 0,001	

Table 1. Colony forming units (CFU) of the different microorganisms obtained from the patients brackets surface after 21 days of the brackets bonding.

The results are expressed as median ± interquartile range.

* Mann-Whitney test; † Kruskal-Wallis test: [a,b,c] distinct letters (column) indicate statistical difference between the microorganisms by the Mann-Whitney test.

Table 2 shows the correlations between the counting of the different microorganisms obtained from dental enamel in the baseline and the brackets surface of the patients after 21 days of the bonding of the fittings. Positive and moderate

correlation was observed between the presence of *Candida Albicans* in the initial condition and in the final condition. No correlation was found for *Streptococcus mutans* and *Lactobacillus* spp.

Microorganism	Bracket Type	
	Metalic	Ceramic
Streptococcus mutans	$r_{Pearson}$ = 0,18 (p = 0,476)	$r_{Pearson}$ = 0,18 (p = 0,476)
Lactobacillus spp.	$r_{Spearman}$ = 0,03 (p = 0,904)	$r_{Spearman}$ = 0,24 (p = 0,336)
Candida Albicans	$r_{Spearman}$ = 0,62 (p = 0,006)	$r_{Spearma}$n = 0,52 (p = 0,026)

Table 2. Correlations between the counting of the different microorganisms obtained from dental enamel in the baseline (before the brackets bonding) and the patients brackets surface after 21 days.

The Scanning Electron Microscopy examination on the surface of the metal and ceramic brackets showed a heterogeneous distribution of forms suggestive of *cocci*, *bacilli* and *fungi* in the three areas delimited for visualization on the surface of these accessories (Figures 2 e 3).

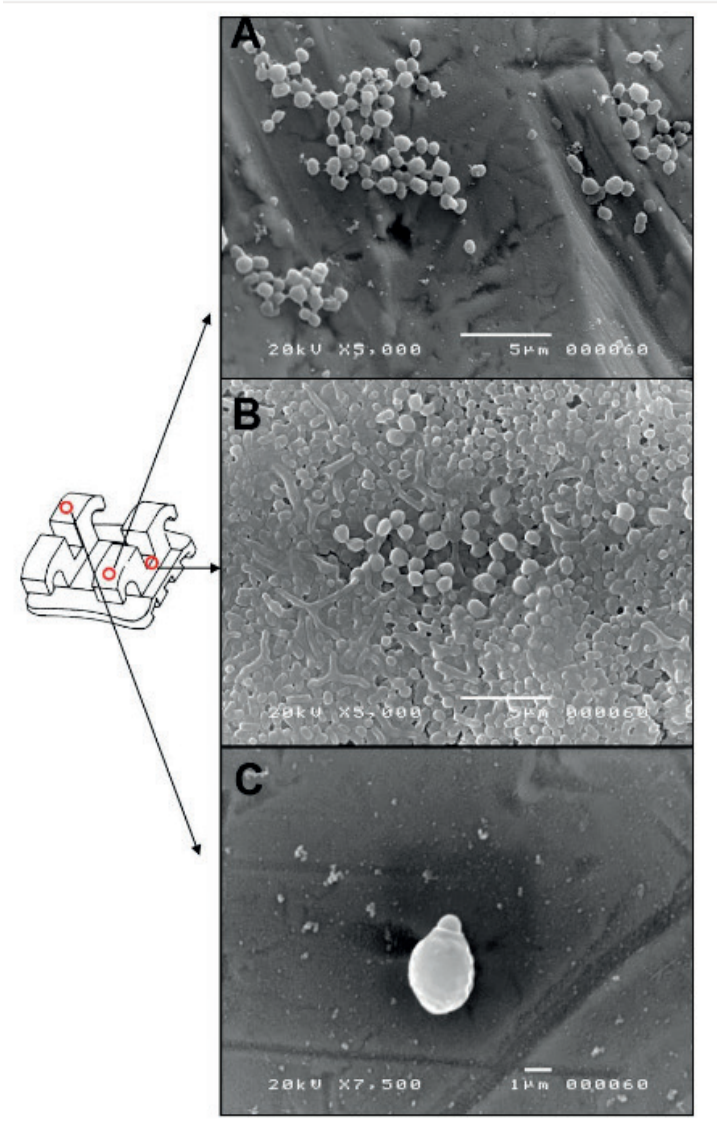

Figure 2 SEM images of the pre-delineated areas of the metal bracket surface (patient 15, tooth 14). A) predominant colonization of fungal forms; B) central area of the slot, with cluster

of microorganisms suggesting spherical, fungal and rods forms; C) microorganism in the form suggestive of fungus (yeast/blastospore) at the moment it performs reproduction by budding. Magnification: A) 5,000x, B) 5,000x and C) 7,500x.

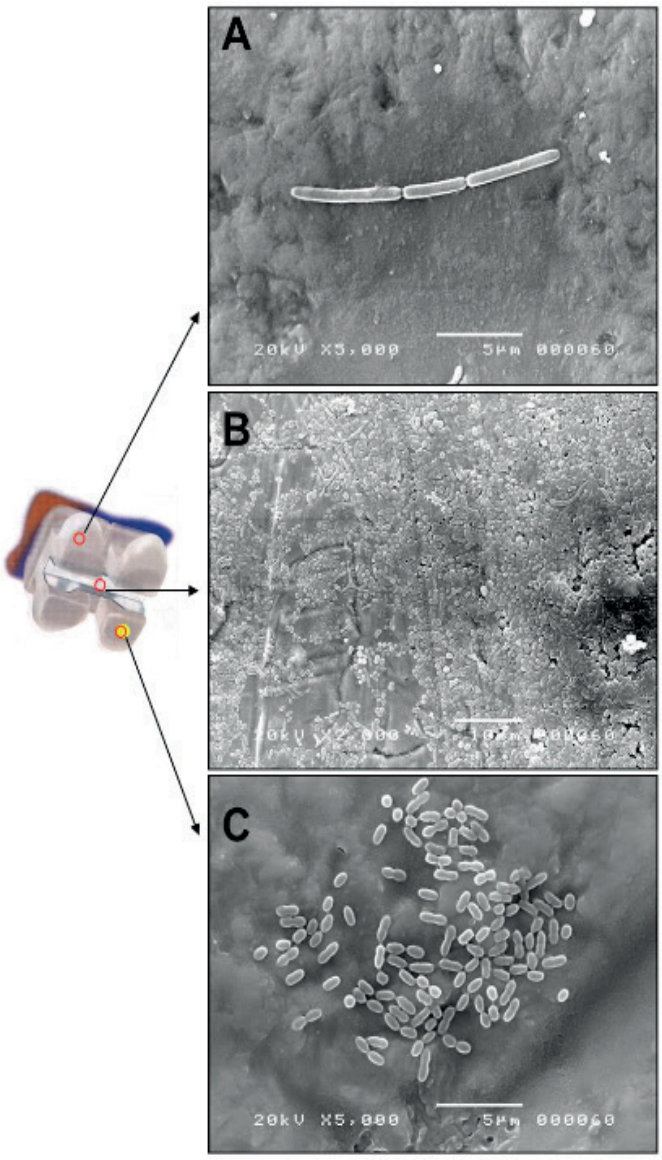

Figura 3 SEM images of the investigated areas on the ceramic bracket surface (patient 12, tooth 13), showing: A) isolated colonization by suggestive form of *streptobacillus*; B) composition of biofilm with predominant forms suggesting cocci and bacilli. C) sparse colonization of spherical forms (*cocci* and *diplococcus*) and rods. Magnification: A) 5,000x, B) 2,000x and C) 5,000x

4 I DISCUSSION

Orthodontic appliance is used in almost every part of the world. The studies show the microbial colonization, as well as the changes in the oral microflora before and after the orthodontic appliances installation (UETANABARO, 1980; MÜLLER, FLORES and JACOB, 1982). The purpose of this study was to obtain more illustrative data about the microbial population directly allocated on the surface of the metallic and ceramic brackets, as well as the colonization outlined by *Streptococcus mutans, Lactobacillus spp* and *Candida* albicans in relation to these two types of accessories.

When the three species studied were analyzed, it was observed that the

Streptococcus mutans were present on the dental surface of all the individuals, before the bonding procedure, in contrast to the numbers *Lactobacillus spp* and *Candida albicans*. Differences were noted in relation to *Lactobacillus spp* and *Candida albicans* whose presence of one species is marked by the absence of another. This finding was also observed by CAMPBELL, 2001, in which the increase in the CFU rate of *Lactobacillus spp* was accompanied by the decrease in *Candida spp*, but this was observed when the orthodontic appliance was installed and not on the surface dental practice. Such correlation is also present in others studies (Carlsson and Elgener 1975; KOGA, UNTERKIRCHER and JORGE, 1993).

The microbial colonization in the metallic brackets showed a marked predominance of Streptococcus mutans, followed by *Lactobacillus spp* and finally *Candida albicans.* This situation is in agreement with the results found by some studies (ANHOURY, NATHANSON and HUGHES et al., 2002), who studied the profile of bacteria, whose material was collected directly from the surface of metal and ceramic brackets, where these authors also found rates high levels of *Streptococcus mutans* accompanied by Lactobacillus spp. *Mycobacteria* such as fungi were not part of the study purposes of these authors.

In the scanning electron microscopy analysis, biofilm can be seen with forms suggestive of *cocci* and *bacilli*, but fungal forms are present in all the fields delineated for this observation. At the end of the analysis, small differences in the distribution of microflora and colonization of these microorganisms can be observed in the three stages studied.

The colonization pattern evidenced by *Streptococcus mutans*, in relation to ceramic brackets, showed the lowest mean of variation when comparing the three types of surfaces: dental, metallic and ceramic. These data seem to be in disagreement with the results shown by (FOURNIER, PAYANT, 1998), who, in studying the adhesion and affinity of *Streptococcus mutans* for metal, plastic and ceramic orthodontic brackets, verified, *in vitro*, the higher affinity of these microorganisms for the surface of ceramic brackets than by the surface of metal or plastic brackets. The behavior of *Streptococcus mutans* and *Lactobacillus spp* in this research seem to agree with the results found by ANHOURY, NATHANSON, HUGHES et al., 2002, who say that there are no significant differences between the colonization of metal and ceramic brackets, with a predominance of *Streptococcus mutans* and *Lactobacillus spp*, in this decreasing order.

The scanning electron microscopy images of the ceramic bracket surfaces showed the colonization pattern of microorganisms with densely inhabited areas whose structures suggest *cocci, bacilli* and filamentous fungal forms. The metallic slot area shows itself as a field of greater co-agglomerations between species (Figura 4).

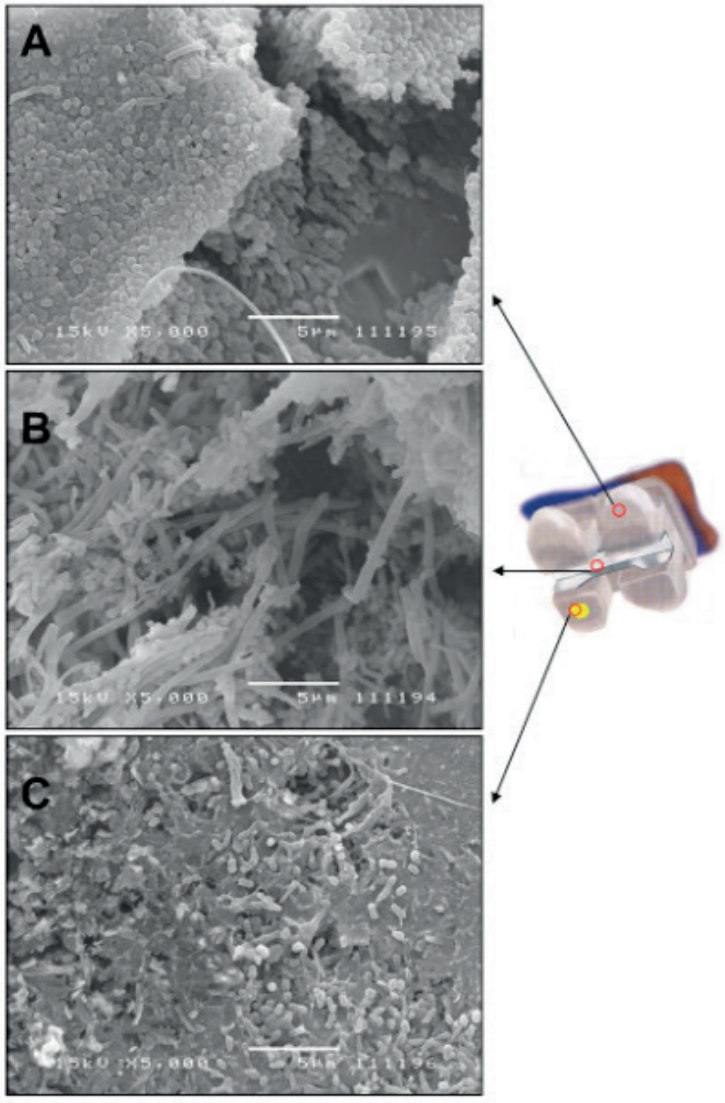

Figura 4 SEM images of predetermined fields on of the ceramic bracket surface (patient 16, tooth 24). A) microbial composition mainly with spherical forms that suggest *cocci* and rods; B) co aggregation of spherical microorganisms (*cocci*), in filamentous sticks forms and *fungi* (yeasts and hyphae) in the whole slot region; C) surface layer, partially removed, with exposure of structures suggesting rods and *cocci*. Magnification: A) 5,000x, B) 5,000x and C) 5,000x.

5 I CONCLUSIONS

There are no significant differences when comparing the colonization of *Streptococcus mutans, Lactobacillus spp* and *Candida albicans* by metallic and ceramic brackets.

On the surface of metallic and ceramic brackets, the distribution of colonies was marked by a decreasing scale of Streptococcus mutans, constituting the highest expression group, followed by Lactobacillus spp and Candida albicans.

The slot areas presented greater accumulation and were colonized by microorganisms whose forms showed the co aggregation of cocci, bacilli and fungi, being these, the zones of greater microbial composition in the ceramic brackets. The fins did not show sites of preference in colonization.

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ÍNDICE REMISSIVO

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