

Quantitative assessment of *S. mutans* and *C. albicans* in patients with Haas and Hyrax expanders

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Objective: To assess and compare the number of *Streptococcus mutans* and *Candida albicans* colonies in patients with Haas and Hyrax appliances before and after insertion.

Methods: The sample consisted of 84 patients requiring orthodontic treatment. For all patients a midpalatal suture expansion was indicated. Patients were randomly divided into Group HA, who used the Haas appliance (n = 42) and Group HY, who used the Hyrax appliance (n = 42). Initially and thirty days after appliance insertion all patients were submitted to saliva collections. The saliva was diluted followed by seeding in Mitis Salivarius and CHROMagar media, for growth of *S. mutans* and *C. albicans* respectively.

Results: Results showed statistically significant difference between groups HA and HY for *Streptococcus mutans* and *Candida albicans* ($p < 0.05$). Haas appliance promoted greater *S. mutans* and *C. albicans* proliferation when compared to Hyrax appliance.

Conclusion: The Haas appliance favored greater proliferation of *S. mutans* and *C. albicans* when compared with the Hyrax appliance. Insertion of the appliances resulted in greater buildup of microorganisms.

Keywords: Orthodontics. Orthodontic appliances. *Streptococcus mutans*. *Candida albicans*. Palatal expansion technique.

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INTRODUCTION

The majority of devices used for orthodontic treatment encumber patient's adequate dental hygiene, thus altering the quantity and composition of the oral microbiota. Several studies have reported the direct association between the presence of orthodontic bands,^{19,25,39} brackets^{13,19,32,43} and other appliances^{5,29} with greater biofilm accumulation, and consequently, a higher incidence of enamel demineralization and even carious lesions.^{31,34,36}

The process of biofilm formation begins immediately after insertion of orthodontic appliances, with fixation of the primary colonizing bacteria, in general streptococcus, and progresses, culminating in the appearance of strict anaerobic gram negative bacilli.⁶ The streptococci are associated with pathologic processes that range from dental caries in patients with poor oral hygiene and a diet rich in fermentable sugars, to bacteremias, when orthodontic bands are removed. The strict anaerobic microbiota is particularly associated with periodontal disease.^{6,32,29}

One of the fixed appliances frequently used in children and adolescents in orthodontic clinics is the palatal expander, used to correct skeletal posterior crossbites or just for maxillary arch expansion in cases of maxillary atresia. In the literature, there is an infinite number of appliances used for this purpose,^{4,9,10,12,23,26,37} however, those most frequently and widely used are the Haas appliance, recommended by Hass²² in 1961 and the Hyrax appliance.^{8,18}

The Haas appliance has an acrylic resin component which remains in intimate contact with the palate for a minimum period of approximately four months. Clinically, over appliance removal, a thick biofilm buildup is noted under the acrylic component.

Aiming at the achievement of favorable results, such as those achieved with the Haas type appliance, but without biofilm accumulation, Biederman⁸ developed the Hyrax appliance, which has the same indications as the Haas appliance, however, supported only by teeth, without the acrylic component. Due to the absence of the acrylic support, hypothetically the Hyrax appliance would promote less biofilm accumulation, and consequently, less *S. mutans* and *C. albicans* proliferation. In the attempt of confirming this hypothesis, the aim of the present study was to make a quanti-

tative assessment of the number of *C. albicans* and *S. mutans* colonies before and after these appliances are inserted.

MATERIALS AND METHODS

This study was conducted in compliance with the demands of the Research Ethics Committee of the Federal University of Rio de Janeiro – UFRJ. The volunteers were able to participate in the study after explanations with respect to the research and the express agreement of their guardians, which was obtained by signature of the terms of free and informed consent.

The sample consisted of a total of eighty-four patients yet to receive orthodontic treatment at the clinic of the Master's Course in Dentistry at the Federal University of Rio de Janeiro. The patients were in the age-range between 13 years and 04 months and 15 years and 9 months, 42 being girls and 42 boys. The patients were selected according to the need for orthodontic treatment associated with maxillary expansion in cases of unilateral or bilateral crossbite and cases of atresic maxilla without the occurrence of crossbite.

Appliances used

The expander appliances were fabricated by the same operator, and differed in design (related to the anatomy of the patient's palatine region), and the quantity of activations (related to the treatment plan) (Figs 1 and 2).

Inserting the appliances

Before inserting the appliances, patients were instructed with regard to correct bacterial plaque control.

After the instructions had been given, the expanders were cemented to the patients' maxillary first premolars and first molars with glass ionomer cement (Vidrion C, Juiz de Fora, Brazil). The person who accompanied the patient was then instructed about how to activate the appliance, which varied according to each patient's individual requirements.

Groups evaluated

Two groups were formed for evaluation:

- » Group Ha: Which used the Haas appliance (n=42).
- » Group Hy: Which used the Hyrax appliance (n=42).

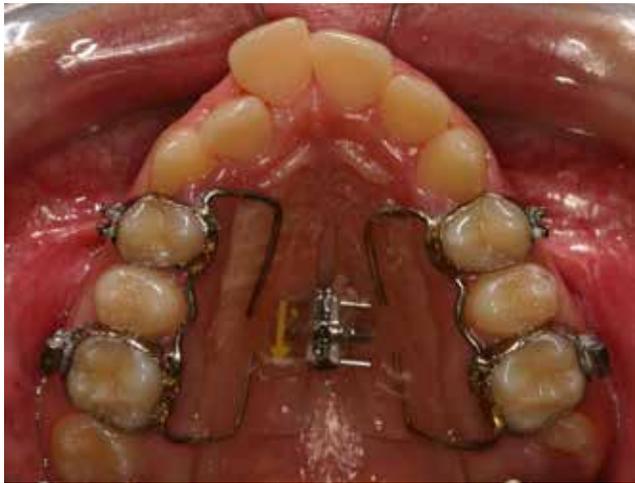


Figure 1 - Patient from group Ha, using Haas appliance.



Figure 2 - Patient from group Hy, using Hyrax appliance.

Saliva collection

Prior to appliance insertion and 30 days post-insertion saliva was collected. Unstimulated saliva collection was carried-out using sterilized plastic receptacles with lids. Patients were instructed to expel saliva into the receptacle until a volume of approximately 3 ml was collected. The flask containing saliva was transported in a thermal receptacle with ice, and was processed within 2 hours after collection. The same saliva sample was used for isolating both *C. albicans* and *S. mutans*.

Estimate of *S. mutans* in saliva

For the isolation of *S. mutans*, dilutions (10^{-1} and 10^{-2}) of the saliva samples were performed in peptonized water, homogenized in a magnetic agitator and 0.1 ml of each dilution was seeded in *Agar Mitis Salivarius* with bacitracin (30mg/ml of medium) and potassium tellurite (0.00005%), and incubated at 37 °C for 48 hours under a microaerophilic atmosphere, with the use of a Gaspack jar.

After incubation, estimated colony counts of *Streptococcus* of the *mutans* group were performed by multiplying the number of colonies in a standardized area of 1 cm² by the respective dilution factor.

Estimate of yeasts of the genus

Candida albicans in saliva

For the isolation of *C. albicans*, the saliva samples were diluted to 10^{-1} in Sabouraud broth, and homogenized in a magnetic agitator. From this dilution, 0.1 ml

of each sample was seeded in Agar Sabouraud Dextrose, and incubated at 300 °C for a 48 hours to 7 days period. The colonies developed in the culture medium were identified by their macroscopic and microscopic morphological characteristics.

An estimated count of the number of Colony Forming Units (CFU), was performed by multiplying the number of colonies by the dilution factor.

Statistical treatment

Statistical analysis were performed with the aid of SPSS 13.0 software (SPSS Inc., Chicago, Illinois, USA). The number of colony forming units (CFU) in each group was submitted to the analysis of variance (ANOVA) to determine whether there were statistical differences between the groups, and afterward to the Tukey test.

RESULTS

The results showed that proliferation of *S. mutans* (Fig 3) and *C. albicans* (Fig 4) was significantly higher in patients with Haas appliance when compared with those that underwent expansion with Hyrax appliance. There was statistically significant difference between Groups Ha and Hy for *S. mutans* ($p = 0.001$) and *C. albicans* ($p = 0.000$). Patients with Haas appliance displayed higher variations in colony counts for *S. mutans* and *C. albicans*.

After insertion of the appliances there was a significant increase in the number of *S. mutans* and *C. albicans* both in the group that used Haas appliance as in the one that used Hyrax appliance ($p < 0.005$).

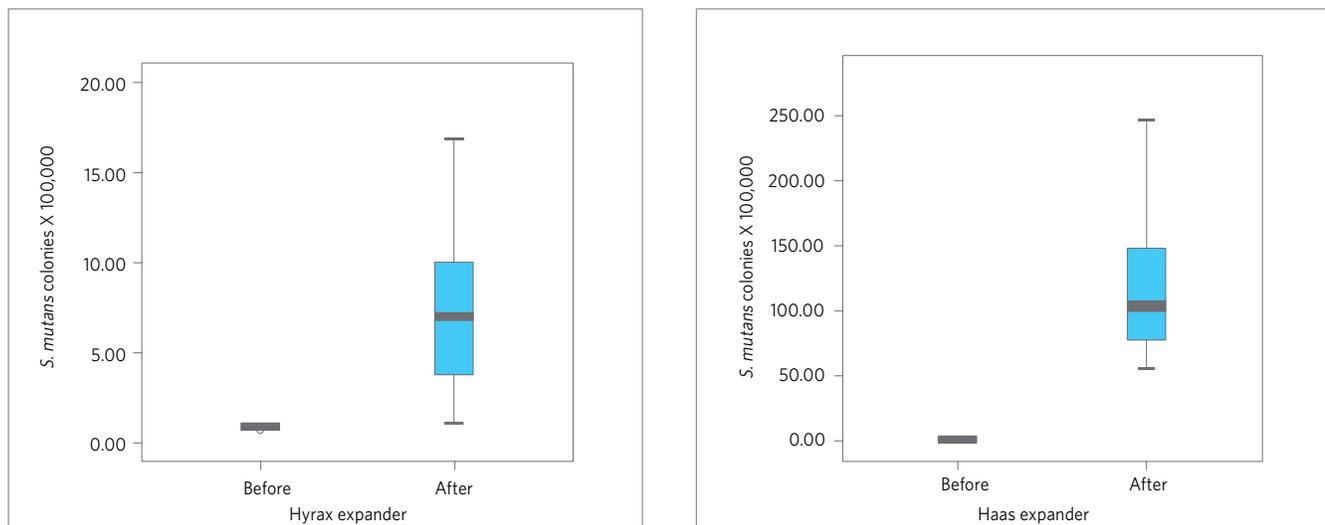


Figure 3 - *S. mutans* colonies count (CFU / ml) collected from patients with Hyrax and Haas expanders.

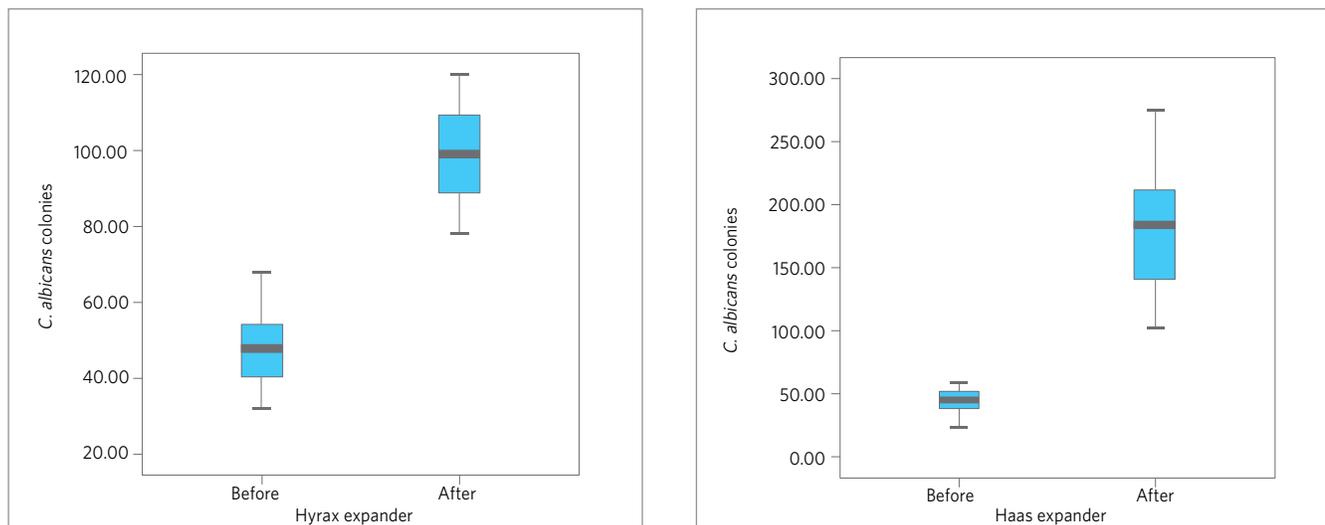


Figure 4 - *C. albicans* colonies count (CFU / ml) collected from patients with Hyrax and Haas expanders.

DISCUSSION

The oral environment offers ideal conditions for colonization of its anatomic parts by a very complex microbiota that coexists in equilibrium with the host.¹⁵ However, when changes in the oral environment occur, the microbiota also changes, resulting in imbalance, with the possibility of diseases development.³⁰

Orthodontic treatment, particularly by means of fixed appliances, predisposes to specific alterations in the oral environment,³ including pH reduction, increase in dental biofilm accumulation³³ and elevation in the salivary levels of microorganisms.²⁷

Considering that orthodontic appliances are composed of a variety of solid and elastic materials,⁴² after they are inserted into the oral cavity, specific salivary proteins will adsorb on their different surfaces,^{2,42} conditioning them and playing an important role in bacterial adhesion. In addition, the irregular surfaces of brackets, bands, wires and other accessories function as bacterial biofilm retention areas,^{2,27} encumber dental hygiene³⁵ and limit the occurrence of mechanical self-cleaning produced by saliva and musculature movement.³⁸

These factors favor a drop in the pH of dental biofilm in the presence of fermentable carbohydrates,

accelerating the accumulation and maturation of cariogenic biofilm, fundamentally composed of streptococcus of the *mutans* group, which are aciduric and acidogenic microorganisms,¹⁹ considered the primary etiologic agents of dental caries.^{7,28}

Although the oral cavity presents countless species of microorganisms¹⁵, this study assessed the presence of streptococcus of the *mutans* group, as they are considered the primary etiologic agents of dental caries¹⁵ and because they are the microorganisms that most commonly contaminate orthodontic appliances;^{1,41} and *Candida albicans*, which are frequent microorganisms in the oral cavity, and may or may not be associated with pathologies.

For microbiological evaluation the dilution technique was employed, in which the specimens were submitted to mechanical agitation for desorption of the microorganisms, and the resultant suspensions were diluted and seeded in a solid culture medium. Different solid culture media have been used for detecting streptococcus of the *mutans* group, such as Agar SB₂₀,¹¹ MS-MUTV⁴⁴ and TYCSB,⁴⁵ among others. In this study, Agar MSB¹⁷ was used, as occurs in various studies.^{20,21,24,46,47} For *candida* the Agar Sabouraud Dextrose medium was used, one of the most frequently used media when studying *C. albicans*.^{14,16,40}

In this study the proliferation of *S. mutans* and *C. albicans* was significantly higher in patients that underwent expansion with Haas appliance when compared to the Hyrax appliance.

The Haas expander is a dental-mucous-supported appliance which, in addition to orthodontic bands and an expander screw, has an acrylic structure that differentiates it from the Hyrax appliance.

The acrylic structure may influence on the proliferation of microorganisms, when it acts as a food deposit, depending on its size and smoothness. In this study it was observed that patients with Haas expander presented the greatest variations in colony counts for *S. mutans* and *C. albicans*. In addition to the acrylic structure, factors such as the difficulty in dental hygiene, palate depth, mastery of tooth brushing techniques and frequency of brushing performed by the patient may have influenced the proliferation of *S. mutans* e *C. albicans*.

To verify to what extent these appliances would favor bacterial accumulation, saliva was evaluated before and 30 days after insertion of the appliances. A significant increase in microorganisms count could be noted both in the individuals who were using Haas and Hyrax appliances ($p < 0.005$).

It should also be taken into consideration the lower standard deviation found in the first collection when compared to the second one. This fact could be justified by the appliances design, which in spite of having been fabricated by the same operator, had particular characteristics for each individual, also by the individual response of each host to the new intraoral device, and by personal dental hygiene.

CONCLUSIONS

By conducting this study, it could be concluded that:

- » the insertion of Haas and Hyrax expanders favors greater accumulation of microorganisms (*S. mutans* and *C. albicans*).
- » The Haas expander favored greater proliferation of *S. mutans* and *C. albicans* when compared to the Hyrax expander.

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