CHANGES IN THE ORAL MICROBIOME BY THE USE OF ALIGNERS: PROSPECTIVE IN-VIVO STUDY

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Conclusion

Based on the results obtained in this study, it can be concluded that there is a quantitative increase in microorganisms (hemolytic and non-hemolytic bacteria), namely, *Streptococcus mutans* and *Enterococcus faecalis,* following the use of orthodontic aligners.

Introduction

Orthodontic aligners have gained prominence in recent decades, presenting themselves as aesthetically more harmonious alternatives to fixed orthodontic appliances.(1) Although several studies have emphasized the easy relationship between these devices and oral hygiene, questions remain regarding their interaction with the microflora of the oral cavity.(2) In this context, the formation of biofilms in dental practice is relevant and requires control, since contamination of orthodontic materials can influence the oral balance.(3,4,5) **The aim of this study was to investigate whether the use of aligners in orthodontic therapy induces changes in the subgingival oral microbiome and in the space/aligner tooth surface that could potentiate the development of oral pathology.**

Materials and Methods

The sample consisted of patients who started orthodontic treatment with aligners at Clínica Universitária Egas Moniz and at a private clinic in the region of Lisbon between December 2024 and April 2025. After applying the inclusion and exclusion criteria, the sample consisted of 16 patients (12 women and 4 men) with an average age of 31,3 years.

t0

1 - Samples of maxillary lateral incisor collected by cone and swab on the **tooth**

2 - Samples of maxillary second premolars collected by cone and swab on the **tooth**

t1

1 - Samples of maxillary lateral incisor collected by cone on the **tooth** and swab on the **aligner**

2 - Samples of maxillary second premolars collected by cone on the **tooth** and swab on the **aligner**

Analysis of the oral microbiome

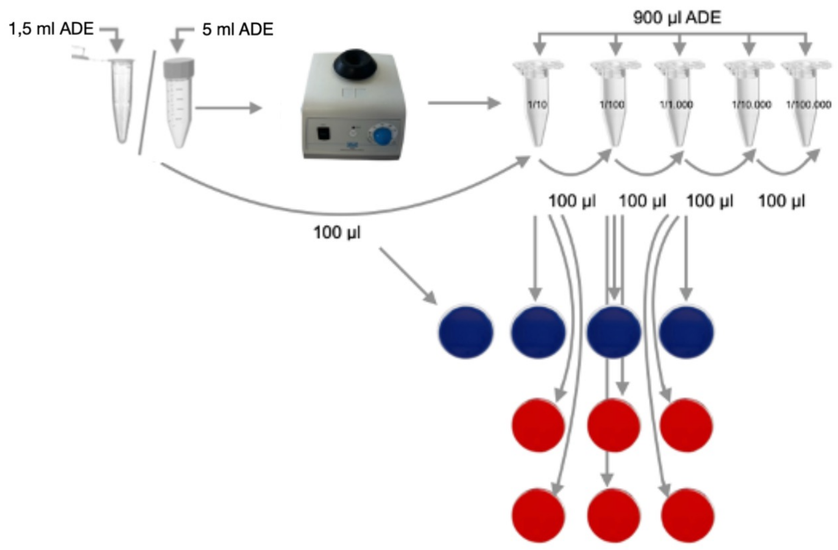


Figure 1 – Laboratory protocol for quantifying microorganisms (t0 and t1)

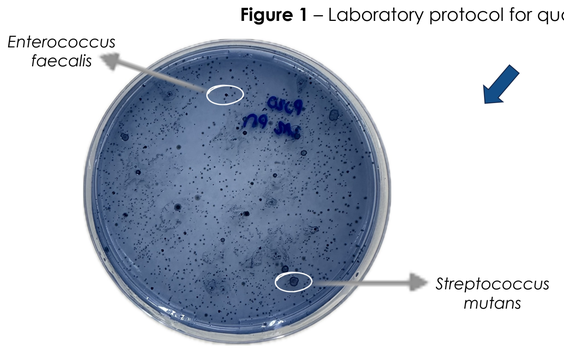


Figure 2 - S. mutans and E. faecalis colonies in Mitis Salivarius agar

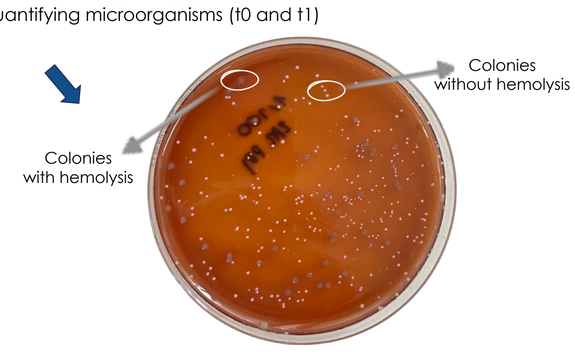


Figure 3 - Colonies with and without hemolysis in Columbia agar 5% blood

The data were subjected to descriptive and inferential statistical analysis techniques (parametric and nonparametric comparative tests). A significance level of 5% ($p < 0.05$) was established for the inferential analysis.

Results

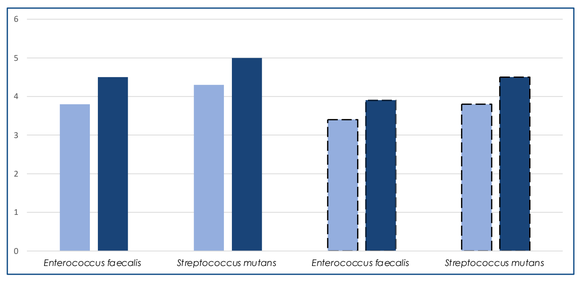


Figure 4 - Colonies of *E. faecalis* and *S. mutans* (*log* UFC/mL) on Mitis Salivarius agar, at t0 and t1. Maxillary lateral incisor samples on the buccal surfaces and in the crevicular grooves.

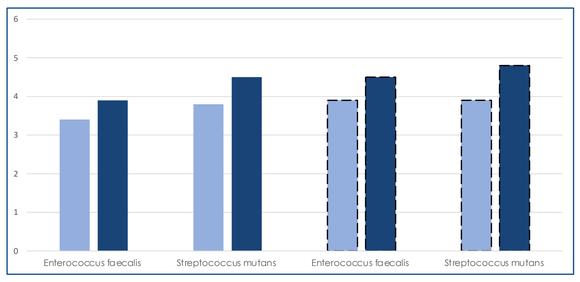


Figure 5 - Colonies of *E. faecalis* and *S. mutans* (*log* UFC/mL) on Mitis Salivarius agar, at t0 and t1. Maxillary second premolars samples on the buccal surfaces and in the crevicular grooves.

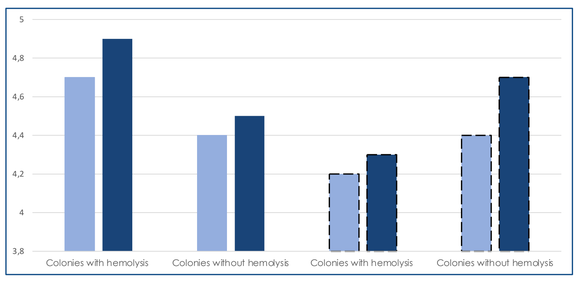


Figure 6 - Colonies with hemolysis and without hemolysis (*log* UFC/mL) in Columbia agar 5% blood in anaerobiosis, at t0 and t1. Maxillary lateral incisor samples on the buccal surfaces and in the crevicular grooves.

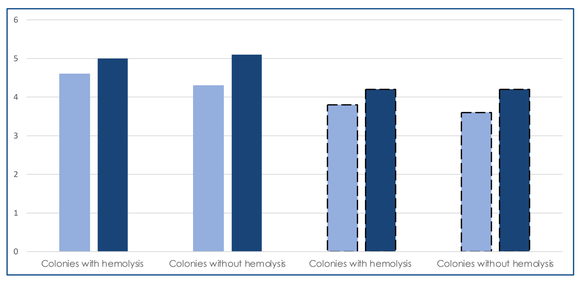


Figure 7 - Colonies with hemolysis and without hemolysis (*log* UFC/mL) in Columbia agar 5% blood in aerobiosis, at t0 and t1. Maxillary lateral incisor samples on the buccal surfaces and in the crevicular grooves.

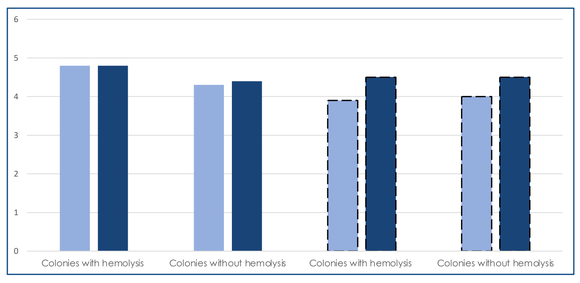


Figure 8 - Colonies with hemolysis and without hemolysis (*log* UFC/mL) in Columbia agar 5% blood in anaerobiosis, at t0 and t1. Maxillary second premolars samples on the buccal surfaces and in the crevicular grooves.

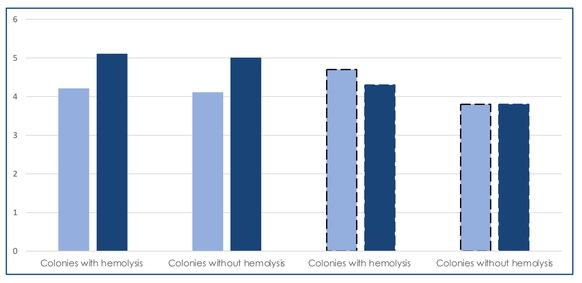
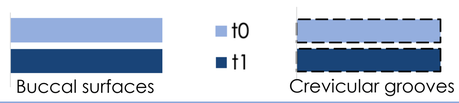


Figure 9 - Colonies with hemolysis and without hemolysis (*log* UFC/mL) in Columbia agar 5% blood in aerobiosis, at t0 and t1. Maxillary second premolars samples on the buccal surfaces and in the crevicular grooves.



Discussion

This study revealed statistically significant increasesin the relative abundance of viable microorganisms between t0 and t1, with increases in *Enterococcus faecalis, Streptococcus mutans* and in hemolitic and non-hemolytic bacteria. The results of the microbiome analysis are pending and are expected to offer a more comprehensive understanding of the alterations occurring within the oral environment. However, the data obtained so far already allow us to conclude that orthodontic aligners influence oral balance.

Aknowledgments

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References