International Journal of Interdisciplinary Dentistry

Effect Of Lacteal Products Containing Probiotic In The Progression Of Tooth Decay Around Orthodontic Brackets

Journal:	*International Journal of Interdisciplinary Dentistry*
Manuscript ID	REVISTA-2022-0040.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	12-Dec-2022
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Keywords:	Orthodontic brackets, Dental caries, Dental biofilm

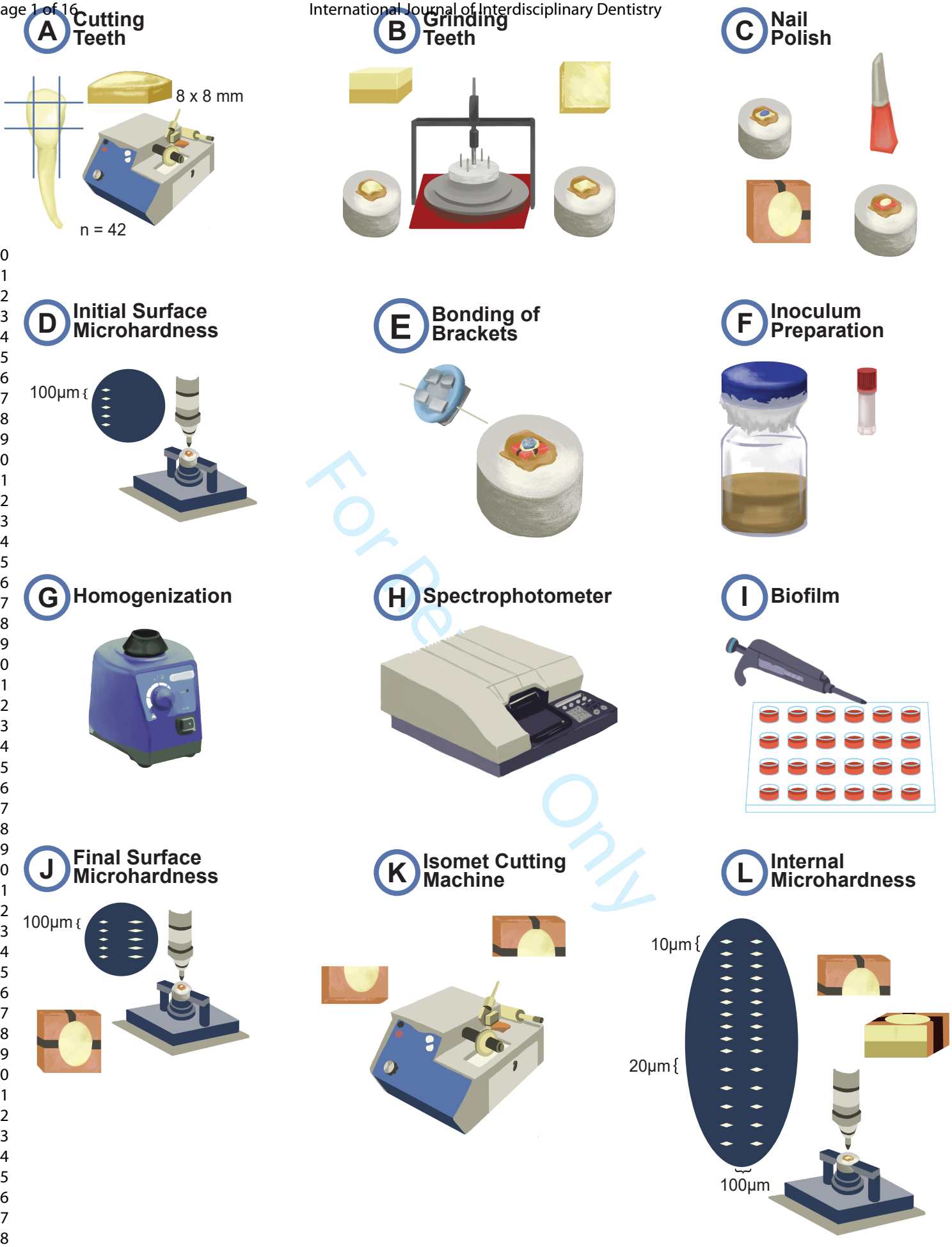

Table 1. Moisture. Protein Fat, and calcium of milk, probiotic milk, fermented milk and probiotic fermented milk.

	Milk	Probiotic Milk	Fermented Milk	Probiotic Fermented Milk
Moisture	88.1[a] ±0.04	87.9[a] ±0.04	88.1[a] ±0.04	82.7[a] ±0.21
Protein	3.7[a] ±0.08	3.8[a] ±0.07	4.3[a] ±0.11	4.2[a] ±0.03
Fat	3.4[a] ±0.16	3.3[a] ±0.05	2.91[a] ±0.03	2.82[b] ±0.26
Calcium	124.4 [a] ± 0.25	123.9[a] ± 0.15	125.1 [a] ± 0.33	125.3[a] ±0.42

* Values are expressed ± standard deviation. Moisture, Protein and Fat are expressed in % w/w. Calcium is expressed in mg/100g. Analysis performed in triplicate. [ab] Different letters at the same line indicate statistical difference according the Tukey test ($p<0.05$).

Table 2. pH, *Lactococcus lactis* and *Lactobacillus casei* count of milk, probiotic milk, fermented milk and probiotic fermented milk

	Milk	Probiotic Milk	Fermented Milk	Probiotic Fermented Milk
pH	6.52[a] ±0.11	6.51[a] ±0.04	4.63[b] ±0.02	4.45[b] ±0.01
L. lactis	-----	7.2[a] ±0.27	7.5[a] ±0.11	7.7[a] ±0.03
L. casei	6.21[a] ±0.13	6.24[a] ±0.09	------	8.53[b] ±0.96

* Values are expressed ± standard deviation. pH is admensional. *L.lactis* and *L. casei* are expressed in log CFU/g. Analysis performed in triplicate. [ab] Different letters at the same line indicate statistical difference according the Tukey test ($p<0.05$).

Table 3. Surface microhardness of enamel around orthodontic brackets before and after treatments

Treatment	Surface microhardness		p-value*
	Before	After	
Group 1 (negative control)	310,60 ± 22,55	208,87 ± 58,77[a]	0,013
Group 2 (positive control)	302,10 ± 16,07	107,21 ± 22,45[b]	< 0,001
Group 3 (milk	328,70 ± 24,56	67,71 ± 24,39[c]	< 0,001
Group 4 (milk + probiotic)	300,83 ± 10,87	35,53 ± 9,04[d]	< 0,001
Group 5 (fermented milk)	325,47 ± 26,06	50,84 ± 23,41[cd]	< 0,001
Group 6 (fermented milk + probiotic)	311,87 ± 19,81	94,94 ± 50,38[bc]	< 0,001
p-value	0,066†	< 0,001‡	

Values are expressed as mean ± standard deviation. * Student t test for paired samples; † one-way ANOVA; ‡ Kruskal-Wallis test: abcd means followed by distinct letters vertically (column) are statistically different by the Mann-Whitney test.

Table 4. Percentage of loss of superficial microhardness and internal microhardness, according to the treatments.

Treatment	Parameters	
	%PMS	MI - ΔZ
Group1(negative control)	35,04 ± 39,68[a]	1669,72 ± 1225,09
Group 2(positive control)	65,33 ± 11,30[b]	728,51 ± 892,15
Group 3 (fermented cow's milk)	79,79 ± 7,95[c]	2027,19 ± 1190,70
Group 4(fermented cow's milk+ probiotics)	87,63 ± 5,14[d]	1875,58 ± 1214,27
Group 5 (yogurt)	87,44 ± 14,06[cd]	1703,68 ± 1522,18
Group 6(yogurt+probiotics)	66,80 ± 34,79[bcd]	1651,89 ± 1521,81
p-value	< 0,001*	0,423†

% PMS, percentage of loss of surface microhardness; MI, internal microhardness. Values are expressed as median ± interquartile range, except for MI - ΔZ which was expressed as mean ± standard deviation. * Kruskal-Wallis test: abcd averages followed by distinct vertical letters (column) are statistically different by the Mann-Whitney test; † ANOVA one-way.

Clinical relevance

Much has been said about the preventive effects of probiotics in dental caries lesions.This study showed that probiotics, administered through fermented cow's milk and yogurt, do not prevent the initiation or progression of white spots around orthodontic brackets. Given these results, other ways to prevent the emergence of dental caries should be adopted.

Effect Of Lacteal Products Containing Probiotic In The Progression Of Tooth Decay Around Orthodontic Brackets

ABSTRACT

Objective: To evaluate the progression of caries around orthodontic brackets after the enamel has been exposed to lacteal products containing probiotics. **Methods**: Orthodontic brackets were bonded to the enamel surfaces. The test specimens were randomly divided into six groups: **G1**–negative control; **G2**–positive control, exposed to culture environment only (without microorganisms); **G3**–exposed to the cariogenic environment and the fermented cow's milk without probiotic; **G4**–exposed to the cariogenic environment and fermented cow's milk with probiotic; **G5**–exposed to the cariogenic environment and yogurt without probiotic; and **G6**–exposed to the cariogenic environment and yogurt with probiotic. The groups were placed in brain heart infusion medium, supplemented with 2% sucrose and with $1x10^6$ cells/ml of *Streptococcus mutans* and *Streptococcus salivarius* (ATCC). The Shapiro-Wilk, Levene, Student t, Kruskal-Wallis, and Mann-Whitney tests were used. **Results**: all groups exposed to the ATCC strains showed lower final microhardness, compared to the negative control ($p<0.05$). The interventions with fermented milk and yogurt (fermented milk + probiotic) did not differentiate in relation to the positive control, nor in relation to the groups treated with milk and milk + probiotic ($p>0.05$). **Conclusions**: Lacteal products are not able to prevent the progression of caries around orthodontic brackets.

Keywords: Orthodontic brackets; Dental caries; Probiotics.

1. Introduction

The cavity process is initiated by bacterial fermentation of carbohydrates, leading to the formation of organic acids and a drop in the pH of the biofilm.[1] When microbial deposits remain adhered to the tooth for an extended period, there are further, sharp drops in pH, leading to a loss of integrity of dental enamel.[2]

The use of orthodontic devices makes it difficult to hygienize the teeth, thus increasing the susceptibility of dental enamel to caries. In orthodontic practice, white spot lesions are observed relatively frequently around orthodontic appliances, especially when oral hygiene is poor.[3] The prevention of demineralization during orthodontic treatment is one of the major challenges faced by clinicians, despite modern advances in caries prevention.[4]

Recently, a new class of products has been introduced as having the ability to control the initiation and progression of dental caries – probiotics.[5] A probiotic is defined by the World Health Organization as being living microorganisms that, when administered in adequate amounts, confer benefits to the health of the host.[6] The species most commonly used and researched belong to the genera *Lactobacillus* and *Bifidobacterium*[7]. These microorganisms are commonly found in the oral cavity, including in caries lesions.[8] They have been related to oral health benefits, such as the production of inhibitory substances in the growth of *Streptococcus sobrinus*, *S. mutans*, as well as a reduction in the risk of caries in 3- to 4-year-old children.[9]

With the professed benefits of probiotics on dental health in mind, the following question arises: are the probiotics present in fermented cow's milk and yogurt able to prevent the initiation and progression of white patches around orthodontic braces? In the search for an answer to this and related questions, this study was proposed to test the hypothesis that fermented cow's milk and yogurt with probiotics prevent the initiation and progression of white patches around orthodontic brackets.

2. Materials and Methods

Study Design

Forty-two blocks of 64 mm² bovine enamel were used. The teeth were selected based on initial surface microhardness value (340 ± 10%).

The total sample size (n = 42) was calculated based on the data obtained in a previous pilot study in which the formula for analysis of variance was applied in G*Power statistical software version 3.1.9.7 considering a significance level (α) = 0.05 and statistical power (1 − β) = 0.80, with an effect size 0.39 with 6 groups. The data for sample size calculation considered microhardness.

Orthodontic brackets were bonded to the enamel surfaces with orthodontic adhesive (Transbond XT, Monrovia, California, USA). The specimens were randomly divided into six groups (n=7). Except for the negative control group, all others were placed in brain heart infusion (BHI) medium, supplemented with 2% sucrose and with $1x10^6$ cells/mL of *Streptococcus mutans* and *S. salivarius* (ATCC) for 24 hours. Subsequently, they were washed in deionized water for 30 s, and then treated daily, for 5 min, for a total of four days. After the treatment, the external and internal microhardness was measured, and visual surface observations were made using scanning electron microscopy, and the protected and treated areas were compared (Figure 1).

Evaluation of initial surface microhardness and selection of enamel blocks

Prior to the biofilm formation experiment, the surface microhardness test was performed in order to select the enamel blocks. For this analysis, a microdurometer (Buehler, Micromet 5104, 679-MIT4-00335, Yokohama, Kanagawa, Japan) was used, with a Knoop-type diamond penetrator, under a load of 25g for 10s. Five indentations were made in the center of each specimen, spaced 100 µm apart[10], providing a value in kgf/mm^2 for each indentation.

The average of the five indentations was taken to represent the initial surface microhardness of the sample. All samples were stored in an environment moistened with Milli-Q water, until the beginning of the experimental phase.

Preparation of the inoculum

The inoculum used consisted of a pool containing $1x10^6$ cells/mL of *S. mutans* and *S. salivarius*, from previously selected ATCC strains. They were placed in BHI medium (Difco, Sparks, USA), supplemented with 2% sucrose.

The strains were suspended in saline solution and placed in a vortex shaker for 15s, after which the cell density was evaluated in a spectrophotometer

(Biospectro SP-220 UV-VIS spectrophotometer, Equipar Ltda., Curitiba, Brazil) at a wavelength of 625 nm. The cell density was adjusted by adding sufficient medium to obtain the equivalent transmittance of a standard solution of McFarland scale 1.0 – about $1x10^4$ CFU/ml.

Bracket bonding and splitting the sample into groups

Orthodontic brackets were bonded (Transbond XT, 3M Unitek, Monrovia, USA) to the enamel surfaces of the samples. The remaining area was covered with red nail polish (Risqué, São Paulo, Brazil). The samples were randomly divided into six groups (n=7), according to the following treatments:

G1 – negative control, sample immersed only in BHI plus 2% sucrose; **G2** – positive control, sample immersed in BHI plus 2% sucrose, with *S. mutans* and *S. salivarius* strains; **G3** – sample immersed in BHI plus 2% sucrose, with *S. mutans* and *S. salivarius* strains, followed by immersion, 1x per day for 5 min in fermented cow's milk without probiotics; **G4** – sample immersed in BHI plus 2% sucrose, with *S. mutans* and *S. salivarius* strains, followed by immersion, 1x per day for 5 min, in fermented cow's milk with probiotics; **G5** – sample immersed in BHI plus 2% sucrose, with *S. mutans* and *S. salivarius* strains, followed by immersion 1x per day for 5 min, in yogurt without probiotics; **G6** – sample immersed in BHI plus 2% sucrose, with *S. mutans* and *S. salivarius* strains, followed by immersion, 1x per day for 5 min, in yogurt with probiotics. Treatments were performed over the course of 3 days.

Cycle of biofilm formation on bovine enamel blocks

The enamel blocks were randomized, and fixed on polystyrene plates. This plate/block system was sterilized in ultraviolet light prior to microbiological testing.

The strains and each test specimen were added to 1,500 µL of the culture medium (BHI + sucrose 2%). This set remained in the medium for 24 hours. Subsequently, the specimens were removed from the medium, washed in deionized water for 30 s, and placed in contact with the experimental solution for 5 min daily, for a period of 3 days.

Probiotics (*Lactobacillus casei*) were incorporated into the fermented cow's milk and yogurt during processing. For both products, counts of the probiotics were carried out over time to verify their viability. After a total period of 4 days, the treated enamel was analyzed (Tables 1 and 2).

Analysis of final surface microhardness and calculation of hardness loss

After the biofilm formation test was completed, the blocks were removed from the medium, cleaned with gauze moistened with Milli-Q water, removed from the brackets and subjected to the final surface microhardness analysis. The same parameters from the initial surface microhardness test were used, wherein five new indentations were made, 150 µm from the initial indentations, also spaced 100 µm apart.[10] The average value of these five indentations was obtained, which was taken to represent the final hardness of the sample. Calculation of the percentage of hardness loss (% PHL) was carried out, following the equation: % PHL = (final hardness - initial hardness / initial hardness) × 100.

Transverse (internal) microhardness

To evaluate the transverse microhardness, the blocks were longitudinally sectioned. Measurements were made using a microdurometer with a Knoop indentator with a load of 25g per 10s. Ten indentations were made in the center of each test specimen, spaced 100 µm apart, and five indentations spaced 200 µm apart[10], obtaining a value in kgf/mm^2 for each indentation.

Statistical Analyzes

The normality of the data was evaluated using the Shapiro-Wilk test, and the homogeneity of variance by the Levene test. The Student t test for paired samples was used to compare the surface microhardness before and after the treatments. The differences between the groups were tested using one-way ANOVA or the Kruskal-Wallis test, and for the latter, when a significant difference was verified, the Mann-Whitney test was used for comparisons between peers. The level of significance was 5% (α=0.05). The data was tabulated and analyzed in IBM SPSS Statistics for Windows (IBM SPSS, 21.0, 2012, IBM Corp., Armonk, NY)

3. Results

Table 3 shows the enamel analyzes from around the brackets in relation to surface microhardness. No significant difference was observed between groups in the initial values of superficial microhardness, demonstrating that all groups presented the same initial conditions; however, all groups presented

mineral loss by the end of the experiment, compared to the baseline. Final surface microhardness analysis revealed that all groups exposed to the ATCC strains showed lower final microhardness, compared to the negative control (G1). The treatments with fermented cow's milk only (G3), fermented cow's milk plus probiotics (G4), and yogurt only (G5) resulted in lower final microhardness values, compared to the positive control (G2). The treatment with yogurt plus probiotics (G6) did not differentiate between the positive control or the other treated groups.

Table 4 shows a comparison between the groups, with respect to percentage loss of surface microhardness with hardness. Analysis of surface microhardness loss revealed that all groups exposed to the ATCC strains showed higher mineral loss, compared to the negative control; the treatments with fermented cow's milk and fermented cow's milk plus probiotics aggravated the loss of superficial microhardness, whilst the application of yogurt and yogurt plus probiotics could not be differentiated from the positive control or the groups treated with milk and milk plus probiotics. No significant differences were observed between the groups in terms of microhardness.

4. Discussion

Enamel demineralization often occurs in patients with fixed orthodontic appliances.[11] Several studies have attempted to evaluate materials and methods developed with the aim of reducing white spot problems in orthodontic patients.[12-14] Some studies report improvements[14], others ineffectiveness, and still more that the situation worsens[15]. In face of the dichotomy of results, systematic reviews have been performed in order to determine a useful conclusion; however, to date, these studies[16] have not been able to establish the best and most effective way to prevent the development of white patches during orthodontic treatment with fixed appliances, although some evidence of moderate and low quality has been suggested with the use of fluoride varnish and frequent professional cleaning of teeth.[16]

Faced with these findings, the need for innovative approaches, such as the use of products containing probiotics, has arisen. The use of probiotics has gained strength in recent years because of their natural origin and general health benefits.[17] In the literature, there are a few studies[11] that have

evaluated the action of probiotic-containing foods on the progression of enamel dental caries lesions in orthodontic patients. As a result, the idea of the present study was to evaluate the efficacy of the application of fermented cow's milk and yogurt, containing probiotics, in arresting the progression of caries around orthodontic brackets, using an *in vitro* model.

To carry out this study, bovine teeth were used due to their similarity with human enamel..[18] According to Ayoub et al[19] human or bovine enamel can be used in microbial in vitro caries models to study biofilm's maturation and anticaries agentes.

Many studies have shown that probiotics have a positive effect on dental caries[20-22], leading to a reduction in the concentration of *S. mutans* in saliva. The exact mechanism by which probiotics exert their influence is unknown. According to Petti et al.[23], probiotic-containing yogurts exhibit activity against microorganisms of the salivary microbiota, but they do not appear to possess the ability to colonize the oral cavity; however Fernandez et al.[24] suggested that probiotics alter the cariogenicity of *S. mutans*. It is now known that *S. mutans* is not the main causative agent of caries, but it is among the main agents, as demonstrated by the present study, wherein it was used in association with *S. salivarius* during the cariogenic challenge.

According to Comelli et al.[25], *Lactococcus lactis* and *S. thermophilus* are able to integrate with the supragingival biofilm, and *L. lactis* is also able to modulate the growth of *S. sobrinus*, leading to a decrease in the cariogenic potential of the dental biofilm. Based on these findings, we used *L. lactis* as a probiotic. The results showed that the addition of probiotics to the fermented cow's milk and yogurt did not reduce the cariogenic potential of *S. mutans* or *S. salivarius*, as demonstrated in previous studies. This result may be due to the fact that the *in vitro* model used did not reliably simulate the oral cavity, since the previous positive results were found from *in vivo* studies[11]. Another justification for the present findings is that those studies where favorable results were found used other types of probiotics, such as bifidobacteria.

In 2006, Basyigit et al.[26] analyzed the viability and degree of survival of *L. acidophilus* as a probiotic organism, and observed that the probiotic culture remained stable for up to six months. This justified the use of fermented milk in the present study, with lactea culture plus probiotic *L. acidophilus*.

When the enamel around the brackets was analyzed, all of the groups displayed the same initial conditions; however, all groups presented mineral loss by the end of the experiment. *In vitro* assays have reported an inhibitory effect of lactobacillus on different strains of *S. mutans*.[27] As in this work, Fernadez et al.[24] also reported that they could not detect any inhibitory effect by probiotics. It is possible that probiotics are more effective at achieving remineralization than preventing demineralization.

The limitations of the present study are inherent to all *in vitro* studies, as this method does not accurately simulate what happens in the oral cavity, due to its complexity, and therefore further studies *in vivo* should be developed to elucidate the real mechanism of probiotics in preventing dental caries.

5. Conclusion

This study showed that probiotics, administered through fermented cow's milk and yogurt, do not prevent the initiation or progression of white spots around orthodontic brackets.

References

1. Gugnani N, Gugnani S. Remineralisation and arresting caries in children with topical fluorides. Evid Based Dent. 2017 Jun 23;18 (2):41-2.
2. Zhou J, Jiang N, Wang Z, Li L, Zhang J, Ma R, et al. Influences of pH and Iron Concentration on the Salivary Microbiome in Individual Humans with and without Caries. Appl Environ Microbiol. 2017 Feb 15;83 (4).
3. Pithon MM, Dos Santos MJ, Andrade CS, Leao Filho JC, Braz AK, de Araujo RE, et al. Effectiveness of varnish with CPP-ACP in prevention of caries lesions around orthodontic brackets: an OCT evaluation. European journal of orthodontics. 2015 Apr;37 (2):177-82.
4. Sanpei S, Endo T, Shimooka S. Caries risk factors in children under treatment with sectional brackets. The Angle orthodontist. 2010 May;80 (3):509-14.
5. Jasberg H, Tervahartiala T, Sorsa T, Soderling E, Haukioja A. Probiotic intervention influences the salivary levels of Matrix Metalloproteinase (MMP)-9 and Tissue Inhibitor of metalloproteinases (TIMP)-1 in healthy adults. Archives of oral biology. 2018 Jan;85:58-63.

6. Yang J, Qian K, Wang C, Wu Y. Roles of Probiotic Lactobacilli Inclusion in Helping Piglets Establish Healthy Intestinal Inter-environment for Pathogen Defense. Probiotics and antimicrobial proteins. 2017 Mar 30.
7. Magro DO, de Oliveira LM, Bernasconi I, Ruela Mde S, Credidio L, Barcelos IK, et al. Effect of yogurt containing polydextrose, Lactobacillus acidophilus NCFM and Bifidobacterium lactis HN019: a randomized, double-blind, controlled study in chronic constipation. Nutrition journal. 2014 Jul 24;13:75.
8. Chhour KL, Nadkarni MA, Byun R, Martin FE, Jacques NA, Hunter N. Molecular analysis of microbial diversity in advanced caries. Journal of clinical microbiology. 2005 Feb;43 (2):843-9.
9. Nase L, Hatakka K, Savilahti E, Saxelin M, Ponka A, Poussa T, et al. Effect of long-term consumption of a probiotic bacterium, Lactobacillus rhamnosus GG, in milk on dental caries and caries risk in children. Caries research. 2001 Nov-Dec;35 (6):412-20.
10. de Mazer Papa AM, Tabchoury CP, Del Bel Cury AA, Tenuta LM, Arthur RA, Cury JA. Effect of milk and soy-based infant formulas on in situ demineralization of human primary enamel. Pediatric dentistry. 2010 Jan-Feb;32 (1):35-40.
11. Cildir SK, Germec D, Sandalli N, Ozdemir FI, Arun T, Twetman S, et al. Reduction of salivary mutans streptococci in orthodontic patients during daily consumption of yoghurt containing probiotic bacteria. European journal of orthodontics. 2009 Aug;31 (4):407-11.
12. Beerens MW, Ten Cate JM, van der Veen MH. Microbial profile of dental plaque associated to white spot lesions in orthodontic patients immediately after the bracket removal. Archives of oral biology. 2017 Jun;78:88-93.
13. Knosel M, Bojes M, Jung K, Ziebolz D, Renger S. [Increased susceptibility for white spot lesions by surplus orthodontic etching exceeding bracket base area]. L' Orthodontie francaise. 2015 Sep;86 (3):233-44.
14. Restrepo M, Bussaneli DG, Jeremias F, Cordeiro RC, Magalhaes AC, Palomari Spolidorio DM, et al. Control of white spot lesion adjacent to orthodontic bracket with use of fluoride varnish or chlorhexidine gel. TheScientificWorldJournal. 2015;2015:218452.
15. Kalha AS. Lack of reliable evidence of the effectiveness of remineralising agents for the treatment of post orthodontic white spot lesions. Evidence-based dentistry. 2013 Sep;14 (3):76-7.
16. Benson PE, Parkin N, Dyer F, Millett DT, Furness S, Germain P. Fluorides for the prevention of early tooth decay (demineralised white lesions) during fixed brace treatment. The Cochrane database of systematic reviews. 2013 Dec 12(12):CD003809.
17. Maltz M, Beighton D. Multidisciplinary research agenda for novel antimicrobial agents for caries prevention and treatment. Advances in dental research. 2012 Sep;24 (2):133-6.
18. Teruel Jde D, Alcolea A, Hernandez A, Ruiz AJ. Comparison of chemical composition of enamel and dentine in human, bovine, porcine and ovine teeth. Archives of oral biology. 2015 May;60 (5):768-75.
19. Ayoub HM, Gregory RL, Tang Q, Lippert F. Comparison of human and bovine enamel in a microbial caries model at different biofilm maturations. Journal of dentistry. 2020 May;96:103328.

20. Cagetti MG, Mastroberardino S, Milia E, Cocco F, Lingstrom P, Campus G. The use of probiotic strains in caries prevention: a systematic review. Nutrients. 2013 Jul 5;5 (7):2530-50.
21. Teanpaisan R, Piwat S. Lactobacillus paracasei SD1, a novel probiotic, reduces mutans streptococci in human volunteers: a randomized placebo-controlled trial. Clinical oral investigations. 2014 Apr;18 (3):857-62.
22. Petersson LG, Magnusson K, Hakestam U, Baigi A, Twetman S. Reversal of primary root caries lesions after daily intake of milk supplemented with fluoride and probiotic lactobacilli in older adults. Acta odontologica Scandinavica. 2011 Nov;69 (6):321-7.
23. Petti S, Tarsitani G, D'Arca AS. A randomized clinical trial of the effect of yoghurt on the human salivary microflora. Archives of oral biology. 2001 Aug;46 (8):705-12.
24. Fernandez CE, Giacaman RA, Tenuta LM, Cury JA. Effect of the Probiotic Lactobacillus rhamnosus LB21 on the Cariogenicity of Streptococcus mutans UA159 in a Dual-Species Biofilm Model. Caries research. 2015;49 (6):583-90.
25. Comelli EM, Guggenheim B, Stingele F, Neeser JR. Selection of dairy bacterial strains as probiotics for oral health. European journal of oral sciences. 2002;110, :218-24.
26. Basyigit G, Kuleasan H, Karahan AG. Viability of human-derived probiotic lactobacilli in ice cream produced with sucrose and aspartame. Journal of industrial microbiology & biotechnology. 2006 Sep;33 (9):796-800.
27. Hasslof P, Hedberg M, Twetman S, Stecksen-Blicks C. Growth inhibition of oral mutans streptococci and candida by commercial probiotic lactobacilli--an in vitro study. BMC oral health. 2010 Jul 2;10:18.

Legends

Figure 1. Schematic representing the laboratory stages developed.