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Tese

Métodos de prevenção de danos à superfície dental e manutenção da saúde bucal durante o tratamento ortodôntico.

Raíssa Coi de Araújo

Pelotas, 2020

RAÍSSA COI DE ARAÚJO

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Tese apresentada ao Programa de Pós-Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutora em Odontologia, Área de concentração Dentística.

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Notas Preliminares

A presente tese foi redigida segundo o Manual de Normas para Dissertações, Teses e Trabalhos Científicos da Universidade Federal de Pelotas de 2019, adotando o Nível de Descrição em Capítulos, descrita no referido manual (https://wp.ufpel.edu.br/sisbi/files/2019/06/Manual.pdf).

Resumo

ARAÚJO, Raíssa Coi de. **Métodos de prevenção de danos à superfície dental e manutenção da saúde bucal durante o tratamento ortodôntico.** Orientadora: Giana da Silveira Lima. 2020. 78f. Tese (Doutorado em Odontologia, Área de Concentração Dentística) - Programa de Pós-Graduação em Odontologia, Faculdade de Odontologia, Universidade Federal de Pelotas, Pelotas, 2020.

O objetivo deste trabalho foi verificar sistematicamente a literatura acerca do uso de probióticos frente aos principais microorganismos causadores da cárie (*Streptococcus mutans* e *Lactobacillus*), bem como propor e testar o uso de lâminas de bisturi (SB) como novo sistema de remoção de cimento ortodôntico residual, a fim de evitar danos irreversíveis na superfície dos dentes. Materiais e métodos: Estudo 1 - Quatro bancos de dados foram pesquisados até dezembro de 2019. Foram incluídos ensaios clínicos, envolvendo pacientes em tratamento ortodôntico fixo que receberam qualquer intervenção probiótica em comparação ao placebo. O risco de viés foi avaliado com a ferramenta RoB 2 Cochrane e o sistema GRADE. Meta-análises foram realizadas considerando as contagens bacterianas salivares antes e após o tratamento, usando odds ratio. Estudo 2 - Cinqüenta discos de esmalte foram cortados e polidos. A rugosidade superficial foi avaliada. Braquetes metálicos (Edgewise Standard; Morelli, Sorocaba, SP, Brasil) foram colados à superfície do esmalte usando Transbond XT (3M Unitek, Monrovia, CA, EUA) e armazenados em água destilada a 37°C por 24 horas. Os bráquetes foram removidos com um alicate específico. O remanescente adesivo foi removido usando quatro sistemas (TCB: broca de carboneto de tungstênio; DHpro: polidor de óxido de alumínio; MO: broca de zircônia para remoção de resina e SB: lâmina de bisturi) e a rugosidade superficial utilizando Microscopia de Força Atômica foi avaliada novamente. Após o polimento final, com sistema de polimento específico, a rugosidade superficial, a medição do brilho superficial do esmalte (usando o Glossmeter) e o Índice de Dano ao Esmalte foram avaliados. Resultados: Estudo 1 - No total, foram identificados 35 estudos, dos quais 8 foram incluídos. Baixo risco de viés foi determinado para a maioria dos estudos. A evidência que suporta a terapia probiótica foi classificada como muito baixa a moderada. Os resultados não mostraram diferença estatística na comparação entre probióticos e placebo para *Streptococcus mutans* salivar ou contagem de *Lactobacillus*. Estudo 2 - Para Rugosidade supercial, não houve diferença entre os sistemas de remoção nas diferentes etapas. Diferenças foram obtidas dentro dos grupos dos sistemas de remoção, nos diferentes tempos. Todos os grupos apresentam aumento da rugosidade da superfície após a remoção da resina, mas Dhpro e SB foram capazes de reduzi-la após o polimento. Em relação ao brilho, todos os grupos apresentaram valores inferiores ao esmalte polido. Entre os grupos testados, Dhpro e SB apresentaram os maiores valores de brilho. No EDI, o DHpro apresentou o maior número de amostras com score 0, ou seja, superfície com aspecto semelhante à superfície inicial. Conclusões: Estudo 1 - Não foram observadas diferenças no uso de probióticos em pacientes ortodônticos para diminuir o Streptococcus mutans e o Lactobacillus. Estudo 2 - De acordo com dados obtidos e imagens do AFM, podemos concluir que o DHPro obteve resultados adequados e o sistema proposto pelo estudo (SB) foi semelhante estatisticamente a este.

Keywords: Ortodontia, probióticos, cárie dentária, Streptococcus mutans, Lactobacillus, sistemas de polimento, Microscpia de Força Atômica

Abstract

ARAÚJO, Raíssa Coi de. **Methods for preventing damage to dental surface and maintaining oral health during orthodontic treatment.** Advisor: Giana da Silveira Lima. 2020. 78f. Thesis (PhD in Dentistry) - Graduate Program in Dentistry. Federal University of Pelotas, Pelotas. 2020.

The objective of this work was to systematically check the literature on the use of probiotics against the main microorganisms that cause caries (Streptococcus mutans and Lactobacillus), as well as to propose and test the use of scalpel blades (SB) as a new system for removing orthodontic cement. to avoid irreversible damage to the teeth surface. Materials and methods: Study 1 - Four databases were searched until December 2019. Clinical trials were included, involving patients undergoing fixed orthodontic treatment who received any probiotic intervention compared to placebo. The risk of bias was assessed with the RoB 2 Cochrane tool and the GRADE system. Meta-analyzes were performed considering the salivary bacterial counts before and after treatment, using odds ratios. Study 2 - Fifty enamel discs were cut and polished. Surface roughness was assessed. Metal brackets (Edgewise Standard; Morelli, Sorocaba, SP, Brazil) were glued to the enamel surface using Transbond XT (3M Unitek, Monrovia, CA, USA) and stored in distilled water at 37 ° C for 24 hours. The brackets were removed with specific pliers. The adhesive remnant was removed using four systems (TCB: tungsten carbide drill; DHpro: aluminum oxide polisher; MO: zirconia drill for removing resin and SB: scalpel blade) and surface roughness using Atomic Force Microscopy has been evaluated again. After final polishing, with a specific polishing system, the surface roughness, the measurement of the enamel surface gloss (using the Glossmeter) and the Enamel Damage Index were evaluated. Results: Study 1 - In total, 35 studies were identified, of which 8 were included. Low risk of bias was determined for most studies. The evidence supporting probiotic therapy was classified as very low to moderate. The results showed no statistical difference in the comparison between probiotics and placebo for salivary Streptococcus mutans or Lactobacillus count. Study 2 - For supercial roughness, there was no difference between the removal systems in the different stages. Differences were obtained within the groups of the removal systems, at different times. All groups showed increased surface roughness after removing the resin, but Dhpro and SB were able to reduce it after polishing. Regarding brightness, all groups showed lower values than polished enamel. Among the groups tested, Dhpro and SB had the highest brightness values. In EDI, DHpro presented the largest number of samples with a score of 0, that is, a surface with a similar aspect to the initial surface. Conclusions: Study 1 - There were no differences in the use of probiotics in orthodontic patients to decrease Streptococcus mutans and Lactobacillus. Study 2 - According to data obtained and images from AFM, we can conclude that DHPro obtained adequate results and the system proposed by the study (SB) was statistically similar to this one.

Keywords: Orthodontics, probiotics, dental caries, Streptococcus mutans, Lactobacillus, polishing systems, atomic force microscopy.

Sumário

1 Introdução

A ortodontia é uma área da odontologia que predispõe os pacientes a muitas alterações no decorrer do tratamento, já que para sua realização, inúmeros acessórios necessitam ser instalados. Entre as alterações citadas encontramos modificações na estrutura dental (JANISZEWSKA-OLSZOWSKA et al., 2014a) e na microbiota oral (LUCCHESE et al., 2018).

A partir da introdução dos sistemas adesivos na ortodontia, muito se tem estudado sobre o dano que tal procedimento poderia causar ao esmalte. Seja no momento da cimentação, através da profilaxia, do condicionamento ácido e aplicação do primer adesivo, ou na finalização do tratamento, através do descolamento do bráquete e/ou desgaste do cimento residual. Sabe-se que o dano causado à estrutura é inevitável e irreversível (PUS et al., 1980; FJELD; ØGAARD, 2006; JANISZEWSKA-OLSZOWSKA et al., 2014a).

No início do tratamento, para cimentação das peças, o esmalte recebe profilaxia, condicionamento ácido e aplicação de primer adesivo, na maioria das vezes. A profilaxia, etapa responsável pela limpeza da superfície, com remoção da material alba e da placa acumulada, geralmente é realizada com escova Robinson ou taça de borracha, associada à pasta de pedra pomes e água. Segundo Pus e Way (1980), a perda de esmalte durante esta etapa, utilizando taça de borracha, é de aproximadamente 5 µm. Assim como a profilaxia, o condicionamento ácido do esmalte causa perdas importantes de estrutura. Os mesmo autores relatam perdas entre 6,5 e 7,5µm de esmalte e outros estudos apresentam desmineralizações chegando a 50 µm, com formação de tags adesivos de até 20µm (FJELD; ØGAARD, 2006).

Na fase de finalização do tratamento, as etapas de descolagem de bráquetes e remoção do cimento residual também causam perdas de estrutura saudável. Várias são as técnicas, porém todos os métodos disponíveis até o momento causam danos importantes à estrutura dental como desgaste da superfície e arranhões.(JANISZEWSKA-OLSZOWSKA et al., 2014b)

Além das alterações superficiais causadas pelas pontas de remoção, sabe-se que muitas vezes esta “limpeza final” não é realizada completamente e pequenas porções de cimento ou adesivo permanecem sobre os dentes (GWINNETT;

GORELICK, 1977), levando a um aumento na rugosidade superficial, possibilidade de retenção de placa (BOLLEN; LAMBRECHTS; QUIRYNEN, 1997) e de pigmentação destes restos residuais (ELIADES et al., 2001).

A porção externa do esmalte apresenta-se mais mineralizada, é mais resistente às alterações de pH salivares e portanto menos suscetível à desmineralização. No momento em que se remove essa camada superficial, o esmalte torna-se mais fragilizado, mais rugoso, mais suscetível à desmineralização (JANISZEWSKA-OLSZOWSKA et al., 2014a), retenção e acúmulo de placa (BOLLEN; LAMBRECHTS; QUIRYNEN, 1997; PONT et al., 2010), pigmentação (ELIADES et al., 2001) e diminuição do brilho superficial (SILVA et al., 2018). Este fato torna-se extremamente importante quando se trata de Ortodontia. A desmineralização com condicionamento ácido previamente à instalação dos acessórios, a descolagem dos bráquetes e remoção do cimento remanescente levam a essa agressão e, portanto devem ser realizadas de maneira controlada.

A presença de múltiplos acessórios durante o tratamento ortodôntico faz com que a microbiota oral altere-se. Segundo Lucchese et al. (2018), em uma revisão sistemática, a aparelhagem ortodôntica provoca alterações significativas na microbiota, com aumento na contagem de *Streptococcus mutans* e *Lactobacillus*, microorganismos importantes na atividade e progressão da cárie. Os autores ainda relatam que a alteração na microbiota já ocorre após o primeiro mês de tratamento.

O uso de probióticos como prevenção e/ou tratamento de doenças causadas por microorganismos patogênicos vem crescendo amplamente, em especial na área médica. São definidos como microorganismos vivos que, administrados na dose correta, proporciona benefícios à saúde do hospedeiro, segundo a Organização Mundial de Saúde (Food and Agriculture Organization of United Nations; World Health Organization, 2001). Na Odontologia, os probióticos surgiram, então, como um possível adjuvante no controle dos microorganismos patogênicos causadores da cárie. (MEURMAN; STAMATOVA, 2007; MEURMAN, 2005; TEUGHELS et al., 2008) Revisões sistemáticas confirmam essa possibiidade, a redução na contagem de Streptococcus mutans e Lactobacillus em pacientes que fizeram uso deste tipo de produto. (LALEMAN et al., 2014; LALEMAN; TEUGHELS, 2015; NADELMAN et al., 2018; SEMINARIO-AMEZ et al., 2017)

Evitar ou tentar amenizar todas estas alterações torna-se uma medida de prevenção frente a cárie e doença periodontal. Por este motivo, o presente estudo

propõe um novo sistema de remoção do cimento residual, bem como uma estratégia que reduza os microorganismos causadores da cárie.

Com o objetivo de reduzir o dano superficial do esmalte, nosso estudo propõe o uso de lâmina de bisturi para remoção do cimento residual, bem como testar novos sistemas de remoção de resina e polimento de esmalte pós-ortodontia. A hipótese a ser testada é de que a lâmina não causa dano na superfície do esmalte e restabelece as condições do tecido próximo às condições iniciais. Ainda tem por objetivo revisar sistematicamente se o uso de probióticos pode influenciar a contagem de *Streptococcus mutans* e *Lactobacillus* na saliva e placa bacteriana em pacientes ortodônticos.

2 Capítulo 1

Does the use of probiotics during orthodontic treatment influence the count of salivary Streptococcus mutans and Lactobacillus?[1]

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Highlights: Review systematically the use of probiotics for orthodontic patients;

Identify if probiotics effectiveness against the main carie microorganisms;

Identify possible auxiliary treatments to combat caries in orthodontic patients.

Abstract: Introduction: To review systematically the literature to evaluate the influence of using probiotics against the main microorganisms that causes caries in a patient undergoing fixed orthodontic treatment. **Material and methods:** Four databases were searched up to December 2019. The eligible studies comprised clinical trials, involving patients undergoing fixed orthodontic treatment that received any probiotic intervention compared to placebo. The risk of bias was assessed with the RoB 2 Cochrane tool and the GRADE system. Meta-analyses were performed considering the salivary bacterial counts both before and after treatment, using odds ratio. **Results:** Overall, 35 studies were identified, of which 8 were included. Low risk of bias was determined for the majority of the studies. The evidence supporting probiotic therapy was graded as very low to moderate. The results showed no statistical difference both for the comparison between probiotics and placebo for salivary Streptococcus mutans or Lactobacillus count. **Conclusion:** No differences were observed in probiotics use for orthodontic patients to descrease *Streptococcus mutans* and *Lactobacillus*.

Keywords: Probiotics, dental caries, orthodontics, *Streptococcus mutans*, *Lactobacillus*.

1 Introduction

Orthodontics treatment predisposes patients to a complex alteration in the oral cavity and consequently significant changes occurs in the oral microbiota already in the first month after the installation of fixed appliances and other accessories which have irregular surfaces[1]. Also, the orthodontic appliances cause difficulty in maintaining hygiene making the patient more susceptible to plaque accumulation and development of caries and periodontal disease.[2]

There are many products to overcome these problems, such as the use of dentifrices with increased fluoride concentration, use of mouthwashes with antimicrobials, modified oral hygiene techniques, and the use of electric brushes[3]. Even so, the difficulty to control plaque remains. Probably the young age of patients favors negligence in hygiene, in addition to the lack of manual dexterity in children[2].

Caries is a multifactorial disease, of an infectious nature, formed by a complex structure composed of several microorganisms[4]. Among the main ones, we highlight *Streptococcus mutans* (SM) and *Lactobacillus* (LB), related to caries activity and lesion progression, respectively[5]. Probiotics are food supplements based on live microorganisms that, when administered in the correct dose, bring benefits to patients[6]. Among the main beneficial effects we can highlight the treatment of inflammatory bowel diseases, food allergies, diarrhea associated with rotavirus, ulcerative colitis[7]. Probiotics can also assist in the treatment and prevention of cancers, diabetes, obesity in addition to improved immune function[7,8] Although the mechanism of action is not clear, these microorganisms can offer direct interaction with pathogenic microorganisms or promote an immunomodulatory interaction[9]. Strain of probiotics have an ability to autoaggregate and coaggregate[10,11]. This ability promotes to the probiotics the capacity to adhere to host cells and form barriers

against pathogenic microorganisms colonization, and adherence to other bacterias, which can inhibit their pathogenic action[11].

In dentistry, the use of probiotics has also been researched and some positive results can be observed with regard to the benefits of its use in oral health[12]. Probiotics has demonstrated to provide a reduction in cariogenic pathogens, such as SM, and provides a reduction of gingival bleeding at probing, probing depth and gingival index[13].

Halitosis is a condition faced by many patients, characterized by malodor from the oral cavity. It is related to the production of volatile sulfur compounds by microorganisms such as *Fusobacterium nucleatum*. *Weissella cibaria* is a probiotic that, by coaggregating with *F. Nucleatum*, was able to inhibit the profiling of pathogen, thus reducing the production of sulfur compounds[14]. In the treatment of candidiasis in elderly patients, the use of a mix of probiotics associated with cheese was effective, resulting in a reduction in the prevalence of *Candida* in these patients, without producing side effects. It also reduced the risk of hyposalivation and a dry mouth sensation, demonstrating its beneficial effect on oral health[15].

The use of *Lactobacillus rhamnosus* GG added to milk resulted in a protective effect for children, mainly in the age group between 3-4 years. This probiotic cannot ferment sucrose and lactose, for this reason, it does not negatively affect the progression of caries and can be used as a protector. It showed an inhibitory effect for SM and LB, with a beneficial effect on caries.[16] *Bifidobacterium* DN-173 010 added to yogurt significantly reduced the levels of SM in the saliva of young adult patients[17].

In a randomized clinical trial, *Lactobacillus reuteri* was administered to patients with high counts of SM, for 10 days. There was a significant reduction in counts of

microorganisms due to the dissolution of this probiotic through lozenges.[18] *L. reuteri* added in chewing gums, straws or tablets also behave to reduce SM levels in saliva.[19,20]

Despite various studies have shown a positive effect of probiotics against caries microorganisms, some systematic reviews reveal that there is no difference in their use in non-orthodontic patients[21,22]. Aiming to obtain a targeted answer, the present study aims to review systematically the literature to evaluate the influence of using probiotics against the main microorganisms that cause caries in a patient undergoing fixed orthodontic treatment. The null hypothesis was that the use of probiotics for orthodontic patients would not reduce count of Streptococcus mutans and Lactobacillus.

2 Material and Methods

This systematic review was conducted following the recommendation of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA). The literature search was performed to answer the following focused question: “Does the use of probiotics influence the count of salivary SM and LB of patients undergoing fixed orthodontic treatment?” Therefore the PICO framework was centered in the following aspects:

- Patients: Individuals undergoing fixed orthodontic treatment;
- Intervention: Individuals that received any probiotic (local or systemic use);
- Comparison: Use of placebo substance or no probiotic (local or systemic use);
- Outcome: Count/density (salivary or dental plaque) of microorganisms (SM and/or LB);

The studies were selected when the title or abstract fulfilled the following inclusion criteria: Clinical trials (both parallel or crossover designs); Blind, double-blind or non-blind studies; Patients undergoing fixed orthodontic treatment; The intervention must comprise the use of a probiotic, by systemic or other administration routes; The control intervention had to have a placebo, but other groups with alternative treatments could be present; Studies that assessed the count or density of microorganisms (SM and/or LB) in saliva or dental plaque. Reviews (systematic or not), case reports, observational studies, *in vitro* or animal model studies and letters to the editor were excluded.

2.1 Search strategy

The literature search was carried out in October 2018 and last updated December 2019 in the following electronic databases: PubMed, Web of Science, Cochrane, and Scopus. No language or publication date restriction was applied. Seacrh strategy used to performed in PubMed is listed in Table 1. The above mentioned search strategy was adapted to the other databases. A hand search was performed on the reference list of every study selected. The gray literature was searched for additional eligible references, using the Google Scholar database. The identified studies were imported into a reference manager software (Endnote X7 software (Thompson Reuters; USA) to remove duplicates.

2.2 Studies selection

Studies resulting from the search strategy were screened independently by two researchers (RCA and CSS). Any discrepancy regarding the inclusion or exclusion of a study was discussed with a third researcher (GSL) when a consensus

could not be reached. Studies in which abstract was not available, but the title suggested any relation to the inclusion criteria of the present study were also screened for eligibility.

2.3 Data extraction

Two independently reviewers (RCA and CSS) performed the data extraction from the included studies using a spreadsheet in Excel format (Microsoft Corporation, Redmond, WA, USA). The following items were recorded: author, publication year, country, design of the study, wash-out period in cross-over trials, population type, size and recruitment of sample, age, number of males/females, test and control interventions including probiotic species, total dose (daily dose multiplied with consumption time in colony-forming units [CFU]), number of subjects in each treatment group, frequency and length of consumption; presence of adverse effects, assessment method and frequency, outcomes and conclusion. The authors were contacted by email in case of the need for additional data. When the study did not present the numerical values of interest in tables, but a figure was available, data were extracted from graphs using the WebPlotDigitizer website (https://automeris.io/WebPlotDigitizer).

2.4 Risk of bias assessment

The individual risk of bias assessment of the studies was performed using the RoB 2, the tool recommended by Cochrane to assess the risk of bias in randomized trials[23], which provides a framework for considering the risk of bias in the findings of randomized trials. Additionally, the overall quality of evidence for each of the main outcomes included in the meta-analyses was rated using the GRADE system[24].

Regarding the RoB 2 tool, when all these criteria were assessed as low risk of bias, the article was classified as having a low risk of bias. The risk of potential bias was high when one or more criteria had a high risk of bias. Two reviewers assessed the risk of bias and the overall quality of evidence independently (RCA and CSS). Any discrepancy was discussed with a third researcher (GSL) when a consensus could not be reached.

2.5 Statistical analysis

The meta-analyses were applied with RevMan 5.3 (RevMan 5.3, The Nordic Cochrane Centre, Copenhagen). The heterogeneity was assessed by the Q test and quantified with I^2 statistics. Count/density of microorganisms (SM and/or LB) in saliva was considered the main outcome, and the analyses were presented for each binary outcomes considering the number of patients in the probiotic versus the placebo group in the different thresholds of salivary bacterial counts both before and after treatment, using odds ratio. We consider an event the number of individuals who presented a certain range of microorganism count. In SM analysis, the following thresholds were considered: $<10^3$, $10^3<10^5$, 10^5-10^6 and $>10^6$. For LB analysis, different thresholds of bacterial counts were considered: $\leq10^3$, 10^4, 10^5 and $\geq10^6$. To all meta-analysis performed, a baseline and different follow-up periods were considered, such as 2 to 3 weeks.

3 Results

3.1 Study selection

Overall, 35 studies were identified by the electronic database search. Two additional studies were identified through hand search in the reference list of the selected studies. After duplicates removal, 20 studies were screened, of those 15 were assessed for eligibility. Six studies were excluded because they had an inappropriate design and one because the individuals were not under fixed orthodontic treatment (Fig. 1). Therefore, 8 studies fulfilled the inclusion criteria and were included in the present study.

3.2 Study characteristics

The final sample size of selected studies comprised a total of 330 patients between 10 and 30 years old with a predominance of female participants (63.5%), although one study did not report the proportion. Table 3 shows the main characteristics and results of the included studies. All included studies evaluated the probiotics consumption effect on salivary and/or dental plaque levels of SM and/or LB in patients undergoing fixed orthodontic treatment. Included studies were published between 2009 and 2019, and used parallel-group or cross-over design [25,26], considering 4 to 6 weeks of washout period between them. The selected studies were substantially heterogeneous since they used different probiotics and vehicles, different methods and frequency of evaluation of microorganism count and also, different ways of reporting the results (ordinal count).

The probiotics and protocol regimen used in the studies are described in Table 3. The majority of studies used LB[27–32]; other probiotic used were *bifidobacteria*[25,26]. Most of the included studies administered systemic probiotics: four used milk products (yogurt, curd, kefir)[25–27,32], one used milk[30] and two used lozenges vehicle [28,29]. One study administered a local probiotic in a mouthwash vehicle and also had

another mouthwash group containing chlorhexidine[31]. In all studies, a placebo substance or no intervention was administered compared to the administration of probiotics. Furthermore, two studies administered a toothpaste with probiotic content to a third group[27,32]. Overall, 4 studies stated to have monitored adverse events and none reported the occurrence of adverse events while conducting the clinical trials.

Seven of selected studies, considered as exclusion criteria, individuals under treatment with systemic or local antibiotics up to 2 weeks before the study start. In three studies[25,26,30], tooth brushing was not allowed for at least 1 h after administration of the probiotic, and the others did not report the time between brushing and the use of the probiotic product.

All included studies had as outcome salivary or dental plaque measures of SM and/or LB counts. Bacterial numbers for SM and LB were provided as ordinal counts (number of patients with $\leq 10^3$ CFU/ml before and after therapy). Different methods were used to assess the count/density of salivary microorganisms: Chair-side test (CRT bacteria, Ivoclar Vivadent AG, Schaan, Luechtenstein)[25,27,29], Real-time polymerase chain reaction (RT-PCR)[28,32] and laboratory microbiological evaluation[26,30,31]. All studies had an evaluation moment on baseline (before the intervention) and post-intervention, which ranged from 2 to 6 weeks.

One study assessed the influence of probiotic use on white spot lesion (WSL) formation[29] and no significant difference between the groups was found. Six studies[25,27,28,30–32] reported a significant reduction in SM when a probiotic was used and two reported a decrease in LB count[27,29]. In contrast, two studies reported no significant differences in SM count[26,29] and two reported no difference in LB count[25,26]. One study reported an increase in *lactobacillus* numbers when probiotics were used[30]. Two studies found a significant difference at the end of the study

between the SM count in the probiotic versus control group[27,32], this difference was not noticed at baseline. In contrast, two studies could not detect a statistically significant difference, neither at baseline nor at the end of the probiotic usage for SM count[26,28] and three for LB counts[26,27,29].

3.2 Risk of bias

The methodological qualities of the studies included were assessed to estimate the potential risk of bias (Fig. 2). The methodological quality of each study was summarized as low, high or some concerns. A substantial risk of bias was determined for two studies[31,32]. The results were not sufficiently reported in one study since it only reports whether there was a reduction or not in the levels of microorganisms[32]. In the other study, the study design is not well reported, as it does not report whether there was a loss of patients and whether patients and researchers were blinded or not[31]. Low risk of bias was determined for the other studies[25–30].

The present systematic review examined the quality of the evidence for each meta-analysis outcome and the strength of the recommendation was rated as low to moderate (Table 3). Most studies included few patients, which decreases the accuracy of the results, as whenever there are sample sizes that are less than 400, review authors should consider rating down for imprecision. Also, the heterogeneity observed in some of the meta-analyses was considered substantial.

3.3 Meta-analysis

Due to the high heterogeneity among the studies and the impossibility of standardizing the available data, only 3 studies could be included in the quantitative

analysis of the present systematic review. Additionally, the studies were grouped according to follow-up period assessment to do meta-analysis.

The comparison between probiotic versus placebo considering the distribution of patients, in which subgroup meta-analyses were performed because different thresholds of SM counts were evaluated is shown in Figures 3 and 4. In these analyses, no statistically significant difference could be found between the treatments at both baseline (OR: 1.09; 95% CI: 0.72/1.65) and follow-up period (OR: 0.95; 95% CI: 0.43/2.09).

Similarly, the comparison between probiotic versus placebo regarding the LB counts are shown in Figures 5 and 6 and no significant benefit was detected between the treatments at both baseline (OR: 1.13; 95% CI: 0.73/1.74) and follow-up period (OR: 1.01; 95% CI: 0.66/1.54).

4 Discussion

The null hypothesis was accepted. no difference were observed after this systemic review. This is the first systematic review to evaluate the influence of probiotics' uses on salivary/dental plaque microorganisms count in patients undergoing fixed orthodontic treatment. In this study, no significant benefit could be detected for the use of probiotics compared to placebo for salivary SM or LB count. Low risk of bias was determined for the majority of the studies, however the quality of

the evidence for each meta-analysis outcome was rated as low to moderate according to the GRADE system.

The use of many accessories in orthodontic treatment increases a number of microorganisms in oral cavity[1,33] and associated with the increase in the number of irregular surfaces and difficulty in hygiene, the patient is predisposed to the development of oral diseases[2]. Use of probiotics becomes an important alternative in an attempt to reduce the effects of changes, especially in caries development.

The cariogenicity of SM could be modified by the probiotic bacteria presence, as they coexist in the oral biofilm[34]. Also probiotic bacteria may compete with oral microorganisms and establish a healthy oral colonization[35]. The effects of probiotics have gained visibility over the years in therapeutic use on the processes of demineralization/remineralization of dental enamel especially in patients undergoing orthodontic treatment, where prevalence rates of white spot lesions are between 68.4%[2].

Of the studies included in this systematic review, six showed a significant reduction in the SM count, three in saliva[25,27,30] and three in plaque[28,31,32], and two in salivary LB count[27,29] after the use of probiotics. In contrast, two studies showed no reduction in SM, one in saliva and plaque and one only in saliva[26,29], and two in LB, one on saliva and plaque and one in saliva[25,26].

Cildir et al. (2009)[25] and Pinto et al. (2014)[26] used yogurts with *Bifidobacterium animalis* subsp. *lactis* with the same ingestion recommendation's. Cildir and other showed a reduction for salivary SM and not for LB. Pinto and others did not obtain a significant reduction for any of the two microorganisms in saliva and plaque, despite a tendency for LB reduction in plaque. Technique of microorganisms count was not the same for both and probably this justify the difference. According to Gizani et al.[29],

chair-side tests are practical methods for use, without laboratory involvement, but they estimate approximately the amount of microorganisms.

Chaturvedi et al.[28] used *Lactobacillus brevis* in tablets and obtained a significant reduction SM counts in plaque, agreeing with findings of Campus et al. (2013)[37], who used the similar methodology in children and found a reduction in plaque acidogenicity, SM counts in the plaque and bleeding on probing.

Lactobacillus reuteri in tablets, used in study of Gizani et al.[29], did not result in a reduction in salivary SM after long-term follow-up but significantly reduced the LB counts. Çaglar et al.[18] found a significant reduction in SM, but have a short-term follow-up (10 days). Type of patient (orthodontic) may have influenced the non-reduction of microorganisms. Although reporting in their studies that the effect of probiotics could decrease over time, other studies reports that the effects of probiotics in long-term, reduced caries and the risks of developing caries[16], but still need to have their mechanism of action elucidated[38].

Mechanism of action of probiotics is not yet well known, but it is likely that their performance in saliva occurs through competition with acidogenic bacteria. With the reduction of acid-producing bacteria, their levels will decrease in saliva and consequently the pH of the medium will increase, reducing the chance of developing caries[12].

Alp and Baka[27] found a significant reduction in SM and LB in saliva with the use of kefir. Kefir is a mix of lactic acid bacteria culture (*Lactococcus lactis* subsp, *Leuconostoc* sp, *Lactobacillus* sp, and *S. thermophilus*). When used the same product, in young adult patients, Cogulu et al.[39] found that kefir can inhibit the growth of SM and LB levels. They also suggests tested in children because they have

immature microflora and could benefit more easily from these probiotics, inhibiting the growth of cariogenic microorganisms.

Ritthagol et al.[30], using a food enriched with *Lactobacillus paracasei*, found a reduction in SM count in saliva. In a mixed culture with other *Lactobacillus*, *L. paracasei* inhibited SM growth, with total inhibition after 60 h, at pH 7 and glucose at 0%[40]. For *Lactobacillus*, Ritthagol et al.[30] saw an increase of LB counts, as well as Chuang and Huang[41] who observed a trend in the increase of these levels. A significant increase in the total LB counts and a reduction in the levels of SM suggests a competitive inhibition, one of the types of LB inhibitory effect, not being considered a negative effect.[30]

Shah et al. (2019)[31] demonstrated an effect of probiotic mouthwash in SM count in plaque, as well as chlorexidine mouthwash, demonstrating direct effect on microorganisms.[9]

Use of capsules with a mix of probiotics, which will directly degrade in the intestine, promoted an increase in the count of microorganisms in the oral cavity, demonstrating that direct contact is not the only means of action of probiotics[44].

The intestine is an organ of great importance in the immune response. It has a complex microbiota, composed mostly of diverse bacteria that, being in balance, result in the health of the host.[42] Reaching the intestine, the probiotics have the ability to promote homeostasis in unbalanced organisms, resulting in benefits to the host.[43] They perform antimicrobial activity, through competitive exclusion, the production of bacteriocins, lactic acid, hydrogen peroxide. Perform immunomodulatory activities, which are not yet well understood. Probably probiotics interact with intestinal epithelial cells, which are stimulated to produce IgA, T cell migration, in addition to the phagocytic activity of macrophages, thus indicating their systemic action.

Therefore, the use of capsules containing probiotics may be an alternative in an attempt to control the microorganisms that cause caries.

5 Conclusions

Although the meta-analysis failed to identify significant differences in the probiotic intake in the control of the microorganisms that cause caries, it is clear that its use has many benefits. Studies with more standardized methodologies should be carried out in order to better identify such results. Further research should be carried out to evaluate its benefits and more detailed action on *Streptococcus mutans* and *Lactobacillus*.

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Tables

Table 1. Search strategy used in PubMed (MEDLINE).

	Search terms
#1	Orthodontics[MeSH Term] OR Orthodontics[Title/Abstract] OR Orthodontic Appliances, Fixed[MeSH Term] OR Orthodontic Appliances, Fixed[Title/Abstract] OR Appliance, Fixed Orthodontic[Title/Abstract] OR Appliances, Fixed Orthodontic[Title/Abstract] OR Fixed Orthodontic Appliance[Title/Abstract] OR Fixed Orthodontic Appliances[Title/Abstract] OR Orthodontic Appliance, Fixed[Title/Abstract] OR Fixed Appliances[Title/Abstract] OR Fixed Appliance[Title/Abstract] OR Appliance, Fixed[Title/Abstract] OR Appliances, Fixed[Title/Abstract] OR Orthodontic Brackets[MeSH Term] OR Orthodontic Brackets[Title/Abstract] OR Bracket, Orthodontic[Title/Abstract] OR Brackets, Orthodontic[Title/Abstract] OR Orthodontic Bracket[Title/Abstract] OR Orthodontic Braces[Title/Abstract] OR Brace, Orthodontic[Title/Abstract] OR Braces, Orthodontic[Title/Abstract] OR Orthodontic Brace[Title/Abstract] OR Dental Braces[Title/Abstract] OR Brace, Dental[Title/Abstract] OR Braces, Dental[Title/Abstract] OR Dental 20 Brace[Title/Abstract])
#2	Probiotics[Mesh Term] OR Probiotic[Title/Abstract]
#3	Streptococcus mutans[Mesh Term] OR Streptococcus mutans[Title/Abstract] OR Lactobacillus[Mesh Term] OR Lactobacillus[Title/Abstract]
#4	Search #1 AND #2 AND #3

Table 2. Demographic data, methodological characteristics, and main results of the selected studies.

Author (Year), Country	Design	Age	Groups (n) and Recommendations	Outcome assessment; (Follow-up period)	Main findings
Alp (2018),[27] Turkey	Parallel RCT	12-17 years	Control group: individuals received no probiotic treatment (n=15). Kefir group: individuals consumed 100 ml of Kefir (mix of *Lactococcus lactis* subsp, *Leuconostoc* sp, *LB* sp, and *S thermophilus*) 2 times a day (n=15). The individuals in the toothpaste group brushed their teeth with toothpaste with probiotic content twice a day (morning and evening) (n=15).	SM and LB levels in saliva; baseline, 3 and 6 weeks after. Stimulated saliva samples were taken. The CRT bacteria was used to determine SM and LB levels in saliva.	Statistically significant decrease was observed in the salivary SM and LB levels in the kefir and toothpaste groups compared with the control group. The regular use of probiotics during fixed orthodontic treatment reduces the SM and LB levels in the saliva.
Chaturvedi (2016),[28] India	Parallel RCT	Not informed	Placebo group (n=15): 2 placebo lozenges in the morning and at night. Probiotic group (n=15): 2 probiotic lozenges in the morning and at night. Patients were instructed to brush their teeth with their regular toothpaste before taking the lozenges. They were also instructed to restrict intake of any food or beverage 30 min to 1 h, before and after having the lozenges and avoid chewing gums, mouthwashes, and antibiotics during the study.	SM levels in the plaque; Baseline and after 4 weeks. The samples were placed into individual microcentrifuge tubes for a real-time polymerase chain reaction.	After the use of the probiotic lozenges, 14 of 15 individuals showed a reduction in the SM levels and two individuals, there was no detectable SM after 30 days. In the placebo group, 3 of 15 patients showed a decrease in SM levels. This indicates that daily short-term ingestion of a LB brevis derived probiotic through a lozenge tablet could reduce the levels of SM in plaque around orthodontic brackets.
Cildir (2009),[25] Denmark	Crossover RCT	12-16 years	Probiotic group (n=24): 200g (2 x 10^8 *Bifidobacterium animalis* subsp. *lactis* DN-173010) fruit yogurt per day consumed at dinnertime. Control group (n=24): Yogurt without probiotic bacteria consumed at dinnertime. No tooth brushing was allowed for at least 1 hour after yogurt consumption.	SM and LB levels in saliva; baseline and 2 weeks. The saliva was collected directly into a graded test tube. The counts of salivary SM and LB were estimated with a chair-side test according to the manufacturer's instructions.	A statistically significant reduction of salivary SM was recorded after 2 weeks' consumption of the test yogurt, while no alterations were found in the control group. No significant alterations of the salivary LB counts were observed. Daily consumption of fruit yogurt with probiotics could reduce the salivary levels of SM in orthodontic patients with fixed appliances.
Gizani (2015)[29] Greece	Parallel RCT	Mean 15.9 ± 3.9 years	Probiotic group (n=42): One probiotic lozenge (two strains of LB reuteri) 1x per day. Placebo group (n=43): Identical lozenge without active bacteria; The patients were instructed to let the tablet slowly melt in the mouth after tooth cleaning and before bedtime.	SM and LB levels in saliva and white spot lesion formation; Baseline and immediately after brackets debonding. Saliva samples were collected for 5 minutes and the counts were estimated with CRT® chair-side tests according to the manufacturer.	A significant decrease in LB levels in both groups, but no difference was showed between the groups. MS levels remained unchanged over the study period. Daily intake of probiotic lozenges did not seem to affect the development of WSL during orthodontic treatment with fixed appliances.

Jose (2013)[32] India	Parallel RCT	14-29 years	Probiotic group (n=20): 200 mg of probiotic curd, instructed to eat it with their lunch for 30 days, and asked to brush twice daily with their regular fluoride toothpaste; Toothpaste group (n=20): Brush twice daily with probiotic toothpaste only for 30 days and to discontinue using their normal toothpaste; Control group (n=20): No probiotic treatment.	SM levels in the plaque (genomic expression); baseline and after 30 days. Plaque specimens were collected from the labial surfaces immediately surrounding the orthodontic brackets of the maxillary lateral incisors using a 4-pass technique. The presence of SM was evaluated using a real-time polymerase chain reaction.	The consumption of probiotic curd and the use of probiotic toothpaste cause a significant decrease in the SM levels in the plaque around brackets in orthodontic patients.
Pinto (2014)[26] Brazil	Crossover RCT	10–30 years	Probiotic group (n=26): 200g of yogurt (*Bifidobacterium animalis* subsp. *lactis* DN-173010) per day at a single sitting during dinner. Control group (n=26): 200g of yogurt without probiotic bacteria per day at a single sitting during dinner. Tooth brushing was prohibited for at least 1 h after yogurt consumption.	SM and LB levels in the plaque and saliva. Baseline and 2 weeks. Dental plaque samples were collected from around the brackets on the buccal surfaces of premolars and canines. Volunteers chewed paraffin film for 5 min to stimulate salivation, and the resultant saliva was collected in graded flasks.	There was no difference between the yogurt containing probiotic and the control yogurt for any of the studied variables. A reduction in counts of total cultivable microorganisms was observed in dental plaque samples after the ingestion of either yogurts, but not in saliva.
Ritthagol (2014)[30] Thailand	Parallel RCT	Mean 19.2 ± 3.6 years	Probiotic group (n=15): 10g of reconstituted milk powder with *LB paracasei* SD1 in 50ml of water, once a daily for 4 weeks. Control group (n=15): 10g of reconstituted milk powder without *L. paracasei* SD1 in 50ml of water, once a daily for 4 weeks. All subjects were asked to drink the received milk at breakfast time. No tooth brushing was allowed for at least 1 hour after milk consumption.	SM and LB levels in saliva. Baseline and once a week after the end of the administration period for 4 weeks. All microbial evaluations were made in duplicate at the same time by the same examiner. Using an oral rinse method with 10 mL of PBS.	A statistically significant reduction of salivary SM was detected following the 4-week consumption of probiotic in contrast to that of the control group. A statistically significant increase of salivary LB was also found following the 4-week consumption of probiotic compared with that of the control group.
Shah (2019)[31] India	Parallel RCT	Not informed	Group A (n=10): 0.2% chlorhexidine mouthwash twice daily after brushing; Group B (n=10): Probiotic mouthwash (2×10^8 CFU/g sporlac sachets dissolved in distilled water) twice daily after brushing; Group C (n=10): No intervention was administered.	SM counts in saliva and the effect of probiotics on the oral health status and gingival status; Baseline and once every week for 4 weeks. The saliva samples were spread over MSB culture media, and the CFU were measured. The SM colonies were identified by morphology under the microscope with ×10 magnification.	For SM count, the values of both probiotic and chlorhexidine groups showed a significant decrease as compared to the control group. The comparison of probiotics to chlorhexidine has proven that probiotics are as effective as chlorhexidine as an adjunctive chemical plaque control agent.

RCT: Randomized controlled trial; CFU: Colony-Forming Unit; PBS: Phosphate Buffer Solution; MSB: Mitis-Salivarius-Bacitracin; CRT: Caries Risk Test; SM: Streptococcus mutans; LB: Lactobacillus.

Table 3. Summary of the quality assessment of all outcomes included in the meta-analyses.

Certainty assessment							№ of patients		Effect			
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other consideration	Probiotics	Control	Relative (95% CI)	Absolute (95% CI)	Certainty	Importanc
Distribution of SM (CFU/ml) in subjects (baseline)												
3	randomised trials	not serious	not serious	not serious	serious [b]	none	70/272 (25.7%)	66/272 (24.3%)	**OR 1.09** (0.72 to 1.65)	**16 more per 1.000** (from 55 fewer to 103 more)	⊕⊕⊕◯ MODERATE	IMPORTAN
Distribution of SM (CFU/ml) in subjects (2-3 weeks)												
3	randomised trials	not serious	serious [a]	not serious	serious [b]	none	68/272 (25.0%)	65/260 (25.0%)	**OR 0.95** (0.43 to 2.09)	**9 fewer per 1.000** (from 125 fewer to 161 more)	⊕⊕◯◯ LOW	IMPORTAN
Distribution of LB (CFU/ml) in subjects (baseline)												
3	randomised trials	not serious	not serious	not serious	serious [b]	none	66/272 (24.3%)	61/272 (22.4%)	**OR 1.13** (0.73 to 1.74)	**22 more per 1.000** (from 50 fewer to 110 more)	⊕⊕⊕◯ MODERATE	IMPORTAN
Distribution of LB (CFU/ml) in subjects (2-3 weeks)												
3	randomised trials	not serious	not serious	not serious	serious [b]	none	67/272 (24.6%)	64/260 (24.6%)	**OR 1.01** (0.66 to 1.54)	**2 more per 1.000** (from 69 fewer to 88 more)	⊕⊕⊕◯ MODERATE	IMPORTAN

CI: Confidence interval; SMD: Standardised mean difference; OR: Odds ratio. While determining what constitutes a large I2 value is subjective, the following rule-of-thumb can be used: < 40% may be low; 30-60% may be moderate; 50-90% may be substantial; 75-100% may be considerable. b. Whenever there are sample sizes that are less than 400, review authors and guideline developers should certainly consider rating down for imprecision.

Figures

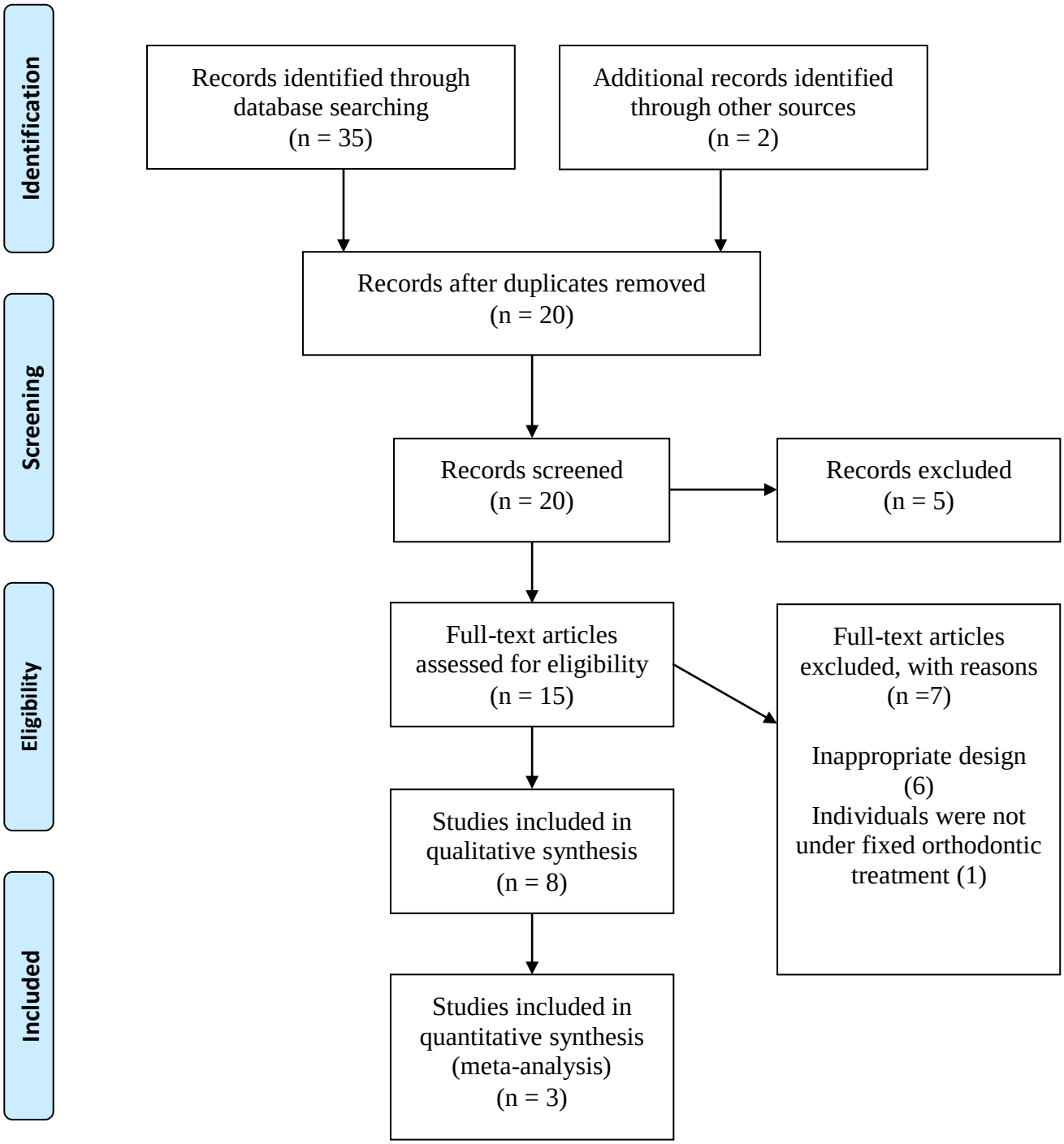

Fig. 1 - PRISMA flowchart of the study selection process.

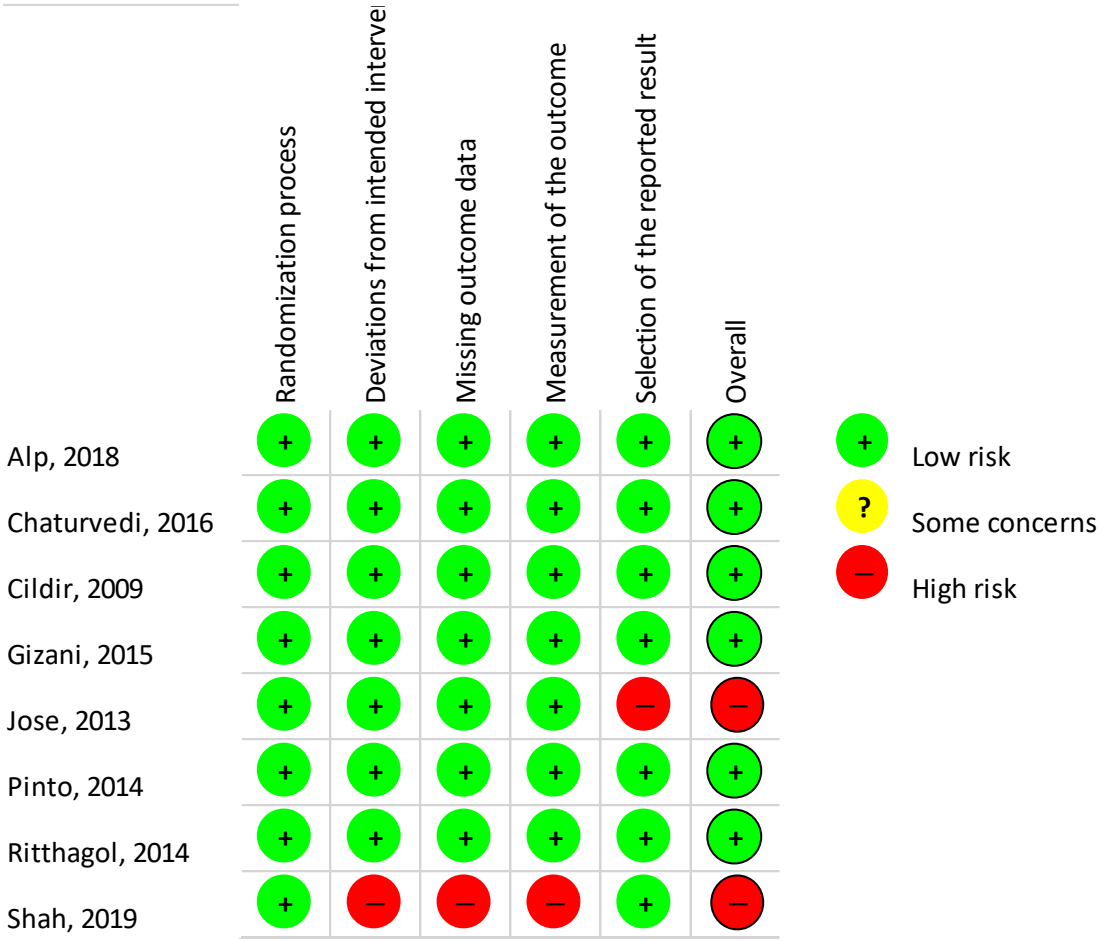

Fig. 2 - Risk of bias analysis: review authors' judgments about each risk of bias item for each included study using the COCHRANE criteria (RoB 2 tool).

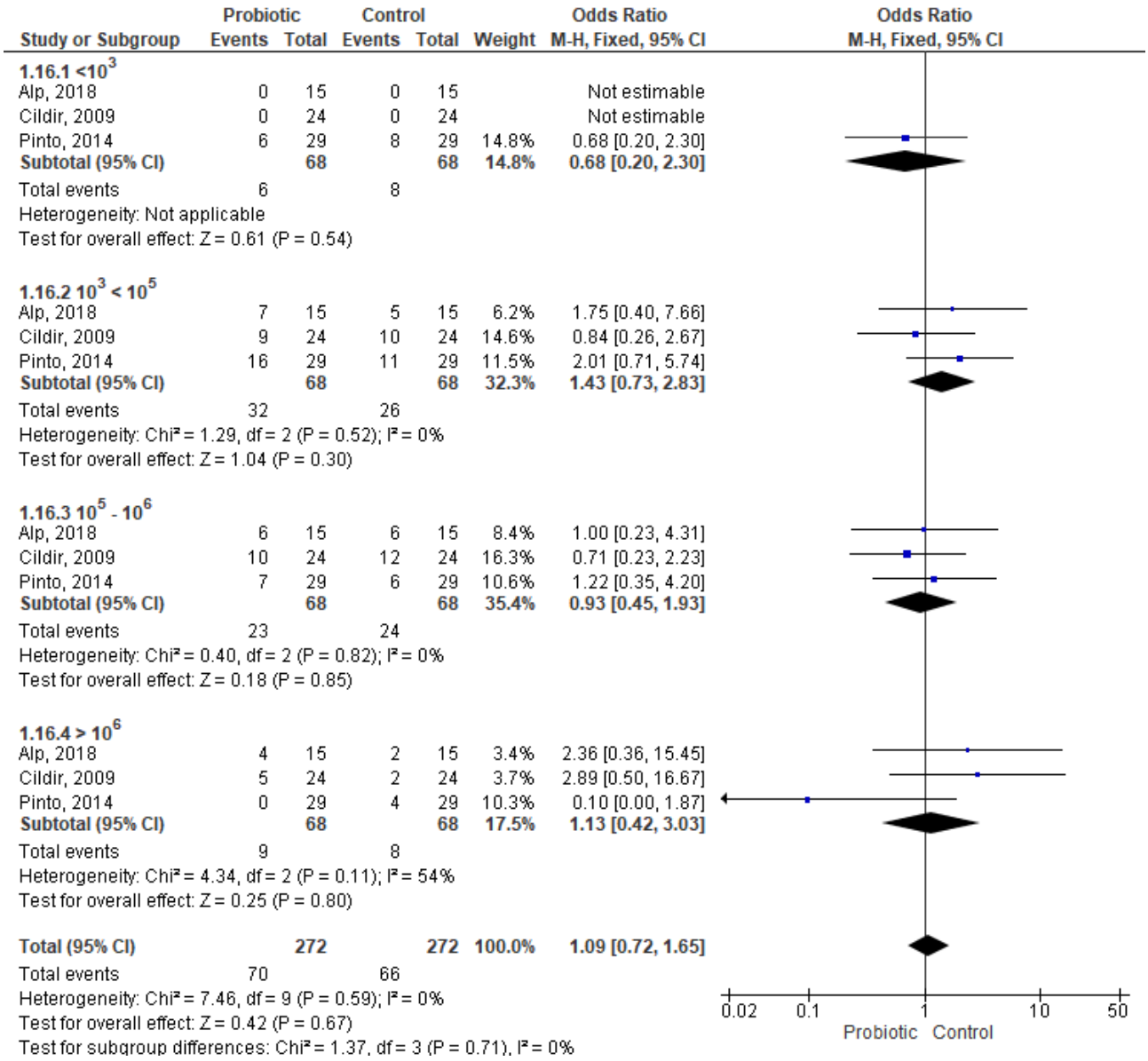

Fig. 3 - Forest plot for the comparison between probiotic and control groups, considering the distribution of patients in the different thresholds of SM salivary counts (CFU/ml) before treatment (baseline).

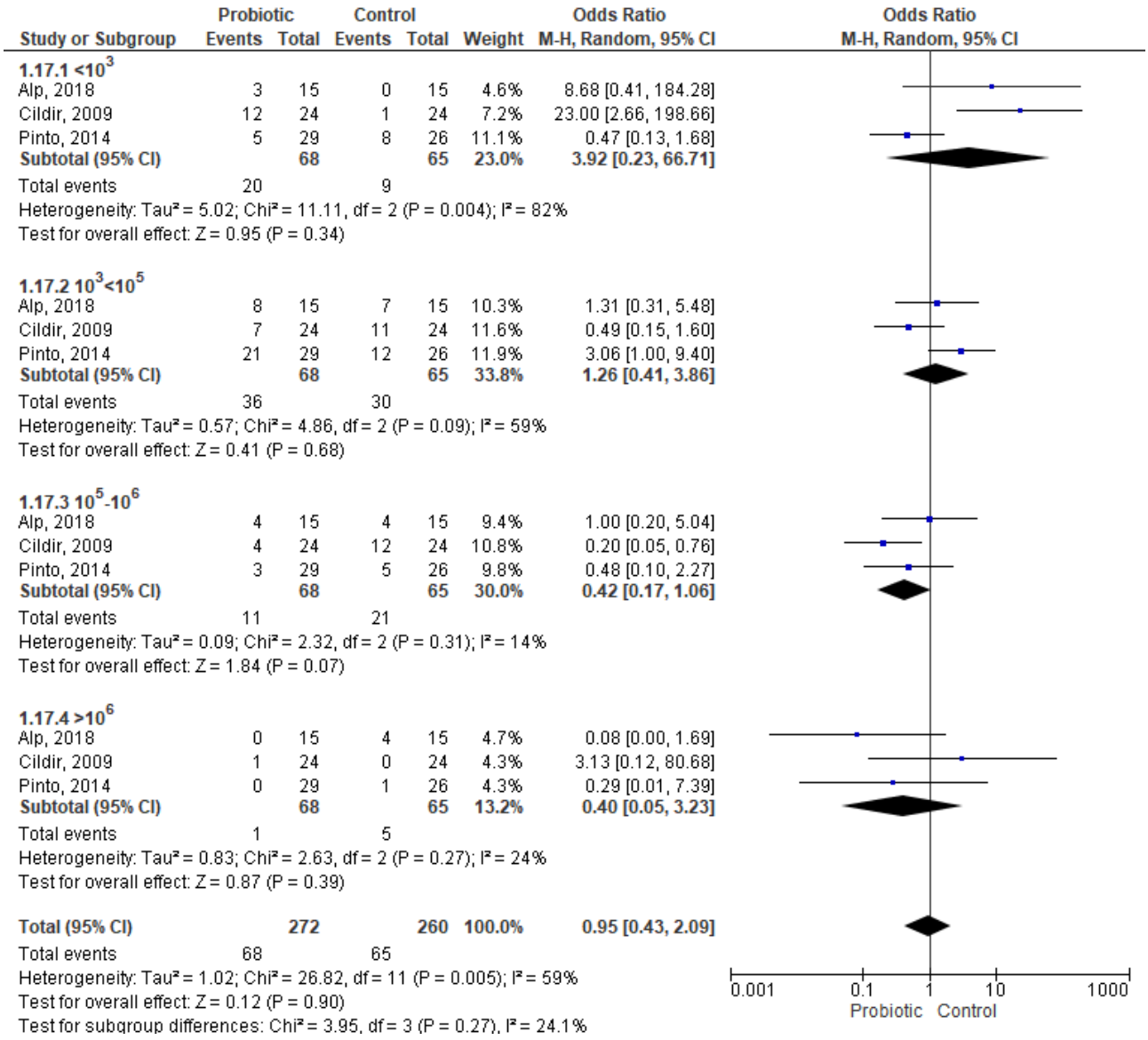

Fig. 4 - Forest plot for the comparison between probiotic and control groups, considering the distribution of patients in the different thresholds of SM salivary counts (CFU/ml) after treatment (between 2-3 weeks of follow-up period).

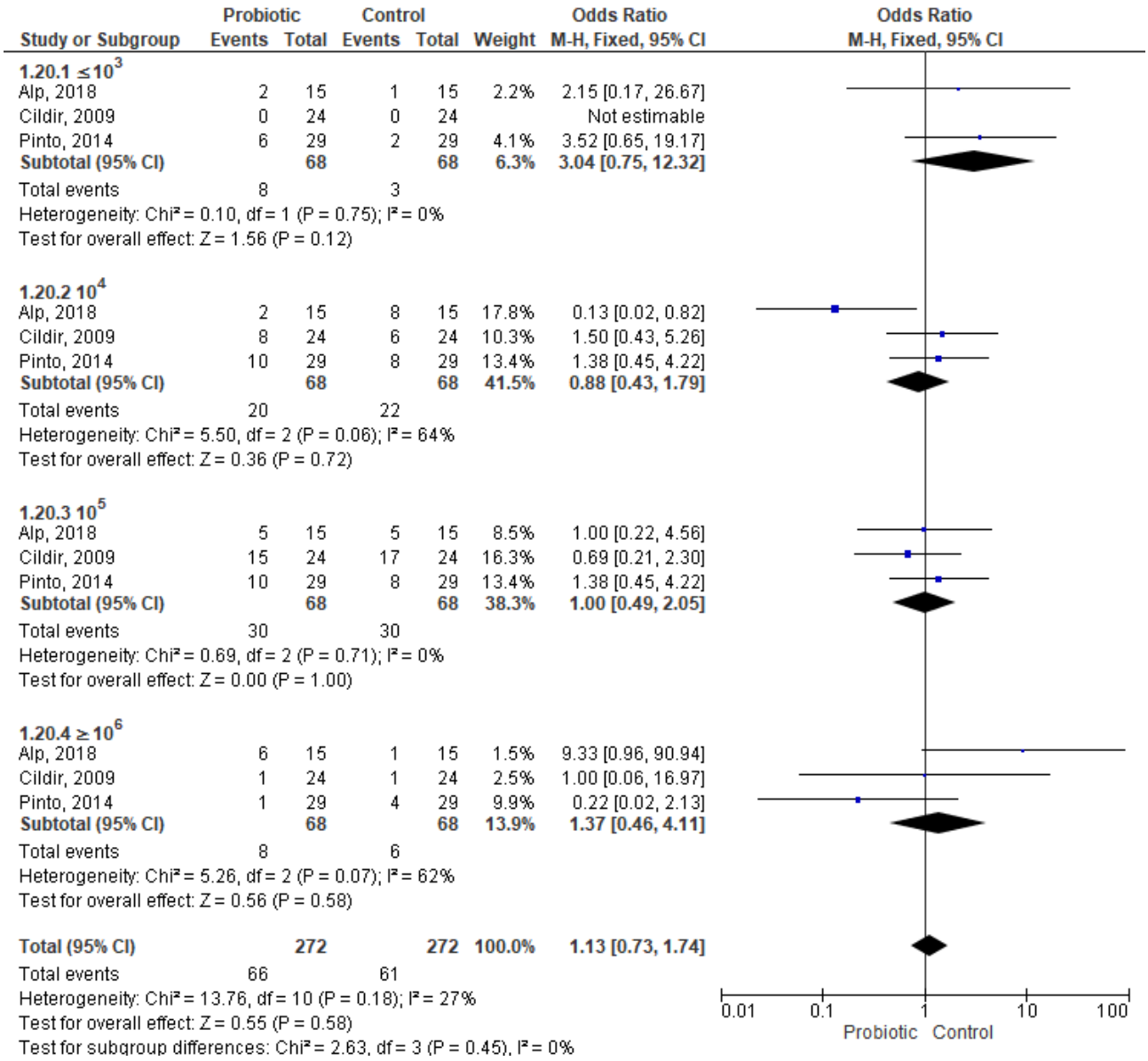

Fig. 5 - Forest plot for the comparison between probiotic and control groups, considering the distribution of patients in the different thresholds of LB salivary counts (CFU/ml) before treatment (baseline).

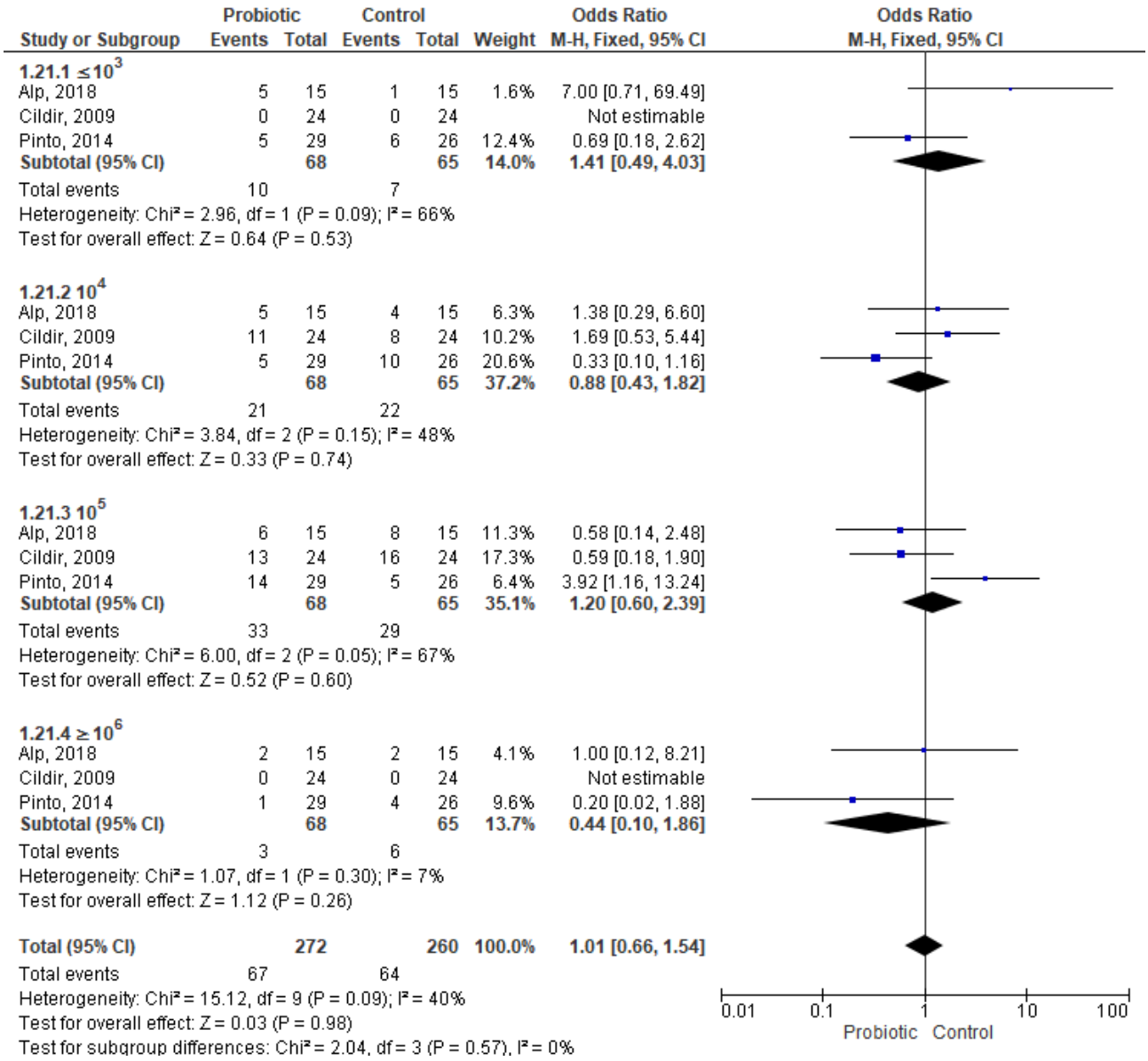

Fig. 6 - Forest plot for the comparison between probiotic and control groups, considering the distribution of patients in the different thresholds of LB salivary counts (CFU/ml) after treatment (between 2-3 weeks of follow-up period).

3 Capítulo 2

A new system for cement residual removal post orthodontic treatment[1]

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Highlights

Novel removal system of adhesive remnant facilitates the process;
Novel removal system may be avoid enamel surface damage;
Novel removal system may be avoid plaque accumulation.

Abstract

Objective: The objective of this study is to test the feasibility of using a scalpel blade to remove adhesive remnant after debonding of orthodontic brackets to avoid damage to the enamel surface. Methods: Fifty discs of enamel were obteined and polish. Roughness surface were evaluated with Atomic Force Microscopy. Then, metallic brackets (Edgewise Standard; Morelli, Sorocaba, SP, Brazil) were bonded to the polish enamel surface using Transbond XT (3M Unitek, Monrovia, CA, USA). Samples were stored in distilled water at 37°C for 24 hours. Brackets were removed with bracket removal plier. Adhesive remnant on the surface was removed using four systems (TCB: Tungsten carbide bur; DHpro: Aluminum oxide polisher; MO: Zirconia bur for resin removal and SB: Scalpel) and roughness surface were evaluated again. After polish, roughness surface, gloss measurement of the enamel surface using Glossmeter and Enamel Damage Index were evaluated. Results and conclusion: No difference were obtained between groups on different times. Differences were obtained into the groups of systems removal on different times. All groups presents increase of roughness surface after resin removal but only DHpro, SB and ARI0 was able to reduce the surface roughness after polishing, obtaining values statistically similar to baseline. Gloss measurement in all groups were different to enamel polish, but DHpro, SB and ARI0 showed the highest gloss values. On EDI, DHpro showed the largest number of samples with score 0. According to datas obtained and images of AFM we can conclude that DHPro system presented a more satisfactory result compared to the others and that the new system proposed by this study (SB) was similar to it for surface roughness and gloss. To the system proposed by us, new tests must be carried out in order to improve the technique of use, as well as associate an adequate polishing system to improve the results obtained.

Keywords: Orthodontics, polish system, atomic force microscopy

3.1 Introduction

Orthodontics is an area of dentistry that leads patients to many changes during the treatment because for the treatment to be performed, numerous accessories need to be installed. At the beginning of the treatment, enamel receives prophylaxis, acid conditioning and application of adhesive primer and these steps can cause important loss of structure[1,2].

On the final phase of the treatment the steps of detachments of brackets and removing residual cement cause loss of healthy structure. There are several techniques, but all methods could cause important damage to the dental structure as surface wear and scratches.[3,4,13,14,5–12]

Diamond burs are efficient but highly destructive, responsible for severe tissue damage resulting in an unacceptable surface.[4,15–18] Tungsten carbide burs are widely used to remove residual post-orthodontic cement. They result in a smoother surface than the obtained with diamond tips, with less significant damage. However they still cause wear and surface roughness. A polishing sequence is necessary to smooth the surface and the apparent roughness.[19]

Other systems are used for this purpose. Fiber-reinforced composite bur[20], Sof-Lex discs[21,22], Green rubber wheel[1,16], Ultrassonic scaler[23], Er: Yag laser[18] are examples of instruments used for resin removal. However, regardless of the method of removal, none can restore the integrity of the enamel or promote the restoration of this surface as it had before the treatment.[5,7,10,19,24,25] In addition to the changes caused by the removal tips, it is known that this "final cleaning" is often not carried out completely and small portions of cement or adhesive remain on the teeth[7], leading to an increase in surface roughness, may favour plaque retention[26] and pigmentation of these residual remains[27].

Besides surface roughness can also cause changes in the enamel surface gloss as evidenced by Silva and collaborators (2018)[28]. Thus, to investigate methods to remove residual cement with minimal superficial damage in the enamel, our study proposes the use of a new system of orthodontic cement removal, with scalpel blade, as well as evaluates systems for removing residual cement and polishing enamel after orthodontics treatment. The hypothesis to be tested is that the scalpel blade does not increase the surface roughness and does not alter the gloss surface

restoring the conditions of the tissue close to the initial conditions.

3.2 Materials and methods

3.2.1 Experimental design

Enamel roughness surface was evaluated and then, metallic brackets (Edgewise Standard; Morelli, Sorocaba, SP, Brazil) were bonded to the enamel surface using Transbond XT (3M Unitek, Monrovia, CA, USA). Samples were stored in distilled water at 37°C for 24 hours. Brackets were removed with bracket removal plier. Adhesive remnant on the surface was evaluated, classified and then removed using four systems, as described below and in more detail in table 1:

- TCB: Removal with Tungsten carbide bur (24 blades) in high speed and polishing with pumice paste and rubber cup;
- DHpro: Removal with Aluminum oxide polisher and polishing with ultrafine silicon carbide polisher, in low speed;
- MO: Removal with Zirconia bur for resin removal and polishing with disposable tip for finishing, in low speed and
- SB: Removal with Scalpel blade and polishing with pumice paste and rubber cup.

In this study were evaluated: roughness surface using Atomic Force Microscopy, Gloss measurement of the enamel surface using Glossmeter, Adhesive Remnant Index and Enamel Damage Index. Surface roughness was measured in three different phases: after the initial polishing (baseline), Immediately after the bracket removal and residual adhesive wear (t1) and after final polish (t2).

The specimens that had all the adhesive removed from the enamel surface with the bracket debonding were categorized as a new group called ARI 0. The experimental design was summarized in figure 1.

3.2.2 Preparation of the samples

Fifty bovine incisors were obtained, cleaned and stored in a 0.5% chloramine T disinfectant solution for seven days. After this disinfection period, root and pulp were removed from each tooth and stored in distilled water, stored in a freezer,

where they will remain frozen until the moment of use. The procedures described here will be based on the ISO TS 11405: 2003 standardization[29].

Bovine incisors were used in the form of discs, with 8mm diameter, made with the aid of a bench bur. Discs were flat and polished with sandpaper grit SiC #600, #1200 and #2500. After, they were fixed on the glass slide to facilitate the position and capture of the sample in the equipment.

3.2.3 Atomic Force Microscopy (AFM)

Surface rougheness measurement was performed in 50µm x 50µm area, using a AFM equipment (Agilent 5500 Equipment, Agilent Technologies, Santa Clara, CA, USA) (figure 2), in non-contact mode and with a Nanosensors PPP-NCL probes (N=48N/m). This mode of measurement allowed to observe a surface in nano-scale level. The advantage of this methodology is the ability to provide quantitative roughness data, as well as a three-dimensional and topographic image of this surface. Measurements were realized at three moments: at baseline, at T1 and at T2. Three points of the surface were analyzed (the first point chosen randomly in the cementation area of the bracket, the second was made 1 mm to the right of the first and the third 1 mm upwards in relation to the second). an average of these values was calculated and this value was used to represent the roughness of that sample. Average surface roughness (Sa) and Root mean square roughness (Sq) were analyzed. In addition, topographical images were also collected. A scanning probe microscopy data analysis software Gwyddion T (version 2.33, GNU, Free Software Foundation, Boston, MA, USA) was used to analyze AFM micrographs.[30,31] Statistical analisys was performed using Two Way Repeated Measures ANOVA to compare a roughness between three moments and Two Way ANOVA to compare the differences between Sq2-Sq1 (difference of roughness surface at T1 and baseline), Sq3-Sq1 (difference of roughness surface at T2 and baseline) and Sq3-Sq2 (difference of roughness surface at T2 and T1). Post hoc were performed using Tukey's test. ($p<0.05$)

3.2.4 Gloss measurement

Surface gloss (UB) was measured using a Gloss Meter (CS 300, CHN Spec, Jianggan District, Hangzhou City, China), measurement aperture 2mm x 3mm, geometry of light incidence of 60°. To eliminate an influence of external light,

equipment was covered with a dark cloth, with reduced ambient light. Three readings were taken on each sample and an average was calculated for each specimen. Polished enamel (polished with sandpaper grit SiC #600, #1200 and #2500) not submitted to treatment also had to be measured surface gloss and served as a comparison for the systems.[32] [31] Statistical analisys was performed using One Way ANOVA and Tukey's Test. ($p<0.05$)

3.2.5 Enamel Damage Index (EDI)

After t2, enamel surfaces were classified by Enamel Damage Index, suggested by Schuler and Van Waes (2003)[33] and used for other researchers[4,34], by following scores:

* Score 0: Smooth surface without scratches, and perikymata might be visible;

* Score 1: Acceptable surface, with fine scattere scratches;

* Score 2: Rough surface, with numerous coarse scratches or slight grooves visible;

* Score 3: Surface with coarse scratches, wide grooves, and enamel damage visible to the naed eye.

A descriptive analysis was performed to describe the results.

3.2.6 Adhesive Remnant Index (ARI)

After debond of brackets to the teeth surfaces, the adhesive remnant index was evaluated, as described for Artun and Bergland (1984)[35]. The remnants were classified following the scores:

* Score 0: no material remnant on the surface

* Score 1: less than half material on the surface;

* Score 2: more than half material on the surface;

* Score 3: all bonding agent on the surface, with the impression of the base of the bracket on the material remnant.

A descriptive analysis was performed to describe the results.

3.3 Results

Average surface roughness (Sa) and root mean square roughness (Sq) of different systems is shown in table 2. There was no statistical difference between the systems at each time (Sa0, Sa1 and Sa2 and Sq0, Sq1 and Sq2). All the groups had their roughness increased significantly after the detachment of the brackets and removal of residual cement (t1) with the referred system. Only DHpro, SB and ARI0 groups reached a surface after polishing (t2) similar statistically to the initial surface.

Alteration in roughness surface between times baseline, t1 and t2, for each system, are shown in table 3.

Figure 2 presents a 3D image of the surface roughness in the three stages, in all systems. In baseline it is possible to identify more homogeneous surfaces. At t1, heterogeneous surfaces are evident in all groups tested, with a greater number of peaks (orange and red) and valleys (green and blue) evident. At 2, a more heterogeneous surface than the baseline, but more homogeneous than t1, is evident.

Surface gloss of the samples is shown in table 4. Polished enamel had the highest gloss and is statistically significant in relation to the systems. Dhpro and SB had similar surface gloss. TCB and MO systems had the lowest gloss and are similar to each other.

Scores of Enamel Damage Index are presented in table 5. DHpro system was the only one who could obtain the most surfaces with score 0. SB system also obtained a score 0 in three samples, different from the other systems. ARI0 group presented 50% of the samples with score 0. Figure 3 presents a 2D image of a surface roughness in the final stage to characterize the findings.

For Adhesive Remnant Index, we only obteined scores 0 (no material remnant on the surface) and 3 (all bonding agent on the surface, with the impression of the base of the bracket on the material remnant). With the samples score 0 we formed a independent group, that were evaluated separately in all methodologies. With the samples score 3 we divided into 4 groups (removal systems).

3.5 Discussion

There is no doubt that the fixation of orthodontic devices causes irreversible

damage to dental enamel[19] and this is confirmed once again by our study. All groups, including the system proposed by our study (SB) resulted in an increase in roughness and surface gloss, rejecting the null hypothesis.

Regarding surface roughness, all groups showed an increase in roughness after T1 (after wear residual orthodontic cement) compared to the baseline. All of them presented a surface roughness reduction in t2 (after polish) compared to t1, demonstrating the importance of the finishing and polishing phase. DHpro, SB and ARI0 (group formed with the samples that had all cement removed together with the bracket in step t1) groups showed a reduction in surface roughness with values statistically similar to baseline. Janiszewska-Olszowska et al. (2016)[36] concluded that adhesive removal after orthodontics cause increase on roughness surface, independent to the system, varying the degree of the rough. In the study by Ferreira et al. (2014), the researchers identified that the final roughness was less than the initial. This probably may occur due to wear and polishing at the costs of enamel, as the result of this study shows - a loss of enamel volume[9].

In T1, where the samples not received polishing yet, there was a significant roughness increase and can be justified by the permanence of the primer layer on enamel surface or fracture and loss of enamel prisms.[15,21,24,35]. Polishing (t2) may have removed the coarse residues of primer that remained on the tooth or smoothed the enamel prisms that fractured on the surface. Surface irregularities may have occurred due to surface wear or incomplete removal of the adhesive material.[7,22,37] Another cause of a roughness increase is fracture of enamel on debonding[24] and maybe this justifies this increase in ARI0.

ARI0 group that had no interference from any type of instrument in t1 showed an increase of roughness. Facts presented by Pont et al. (2010)[24] probably justify this. Through X-ray spectroscopy by energy dispersion the authors detected a presence of calcium adhered to the cement that remained adhered to bracket base. Superficial enamel has greater hardness than the deeper enamel, in addition to the higher concentration of minerals. With loss surface, the rough enamel is more fragile and more susceptible to plaque acids and consequently to demineralization[38].

After polishing, all groups reduced the roughness, but only for DHpro, SB and ARI0 groups this reduction was significant. These groups showed roughness values at t2 statistically similar to the baseline, although we didn't achieve a roughness

equal to the previos one[15,19,36,39]. Final roughness is extremely important because this condition retains more plaque[26,39] and may increase the risk of demineralization, caries and gingivitis[24]. Roughness should not exceed 0.2µm as this value is considered a limit for bacterial adhesion[26] and all groups showed a roughness below ou next to this. In addition, the increased roughness results in a change in the color of the enamel[27,40]. System proposed in this study, scalpel blade, showed with DHpro an adequate performance.

When comparing the removal systems, within each stage (baseline, t1 and t2), we didn't find statistically significant differences. Into each system, differences were observed. All groups showed an increase in time 1 in relation to baseline, demonstrating that, regardless of the removal system, there will always be an increase in roughness, in agreement to Janiszewska-Olszowska et al.[19] and Mohebi et al.[39].

DHpro is a system that use aluminum oxide tips and ultrafine silicon carbide polisher. According to manufacturer, the system is ideal because wear and already polish since they are composed by silicone and aluminum oxide in ideal granulometry. It is likely that the characteristics of flexibility and fine granulometry of the polishing tip are plausible justifications for reducing surface roughness and improving the final surface after t2. Ulusoy (2009)[41] had positive results for aluminum oxide tips, with less damage to the enamel compared to Sof-Lex discs. Sigilião et al.[42] used the same DH polisher to remove the resin (without the final polisher) and found that the final roughness reduced compared to the initial, different from ours, where we had an increase in roughness. The author reports a loss of perikymata with fine scratches caused by polishers[42]. Possibly this smooth surface may have occurred at the expense of superficial enamel, not just cement.

Stainless steel scalpel blade (SB) was added in an experimental way, to check if it could be used as an orthodontic cement removal system without damaging the enamel. In addition to being used in surgical procedures, this instrument is used in finishing resin restorations.[43] As a residual adhesive removal instrument associated with polishing with a rubber cup and pumice, it demonstrated a behavior similar to a commercially available product (DHpro) or superior to two other systems (TB and MO). Scalpel blade system, despite the increase in t1, showed a reduction in the final roughness with the polishing with rubber cup and pumice. Removal the cement with

an scalpel blade can be done in two ways: cutting/scraping the cement or promoting a "lever" between the cement and the tooth and detached it. This could justify the increase in roughness at t1. It is possible that, when the cement is detached, it takes with it small portions of already weakened enamel prisms[24]. “Cutting/scraping” may have kept residual cement on the surface, another possibility to increased roughness in t1. Still, it is possible that due to the composition of the blade, there was no definitive damage to the surface and the polishing was able to reverse the increase in roughness at t1. For this reason, the technique still needs to be improved and studied, since it’s easy to perform, low cost and could be an alternative to superficial wear. Polishing should be improved. We must also emphasize that the technique uses a cutting instrument, so the professional must have a lot of accuracy, dexterity and care in the procedure.

A Tungstein carbide bur (TB) is multiblade bur, manufactured with inverted blades for preserving tooth enamel. This material is widely used for this purpose, and very related in the literature as the material of choice for removing residual cement[9,15,44]. In addition, we use a Morelli system (MO). According to the manufacturer, MO is made of yttria-stabilized zirconia, 18 blades and does not wear enamel and the polishing tip is made of polyamide combined with copolyamide enriched with fiber glass, has low abrasiveness, recovers its characteristics brightness and smoothness of the enamel reducing the possibility of retaining bacteria and accumulating plaque on the treated enamel.

According to Karan et al.[20], the use of tungsten carbide bur promoted an increase of approximately 70% in surface roughness when compared to baseline, agreeing with our study, in which the roughness more than doubled after the removal of cement. This same author also reports that the use of fiber-reinforced bur resulted in a smoother surface than tungsten carbide bur[20]. MO is manufactured with zirconia which is a high-strength ceramic and has the ability to wear enamel[45,46]. We believe that this is the reason for the high surface roughness in MO group.

In our study, the surface gloss of the polished enamel was 59GU, statistically different from all the groups evaluated. The DHpro, SB and ARI0 groups were similar, with the highest values for surface gloss. These same groups were statistically similar for surface roughness with the lowest values for this data. The groups with the lowest gloss values showed the highest roughness after polishing (t2). These findings

corroborate the findings of other studies that concluded that the surface roughness caused by the removal systems is strongly related to the gloss[28] and the other optical properties of the enamel[47]. In our study, the brackets were cemented with acid conditioning and application of adhesive primer. It is known that acid etching promotes profound demineralization in the enamel[2] and this superficial change promotes a significant increase in surface roughness[14], which can alter the translucency of the enamel[28].

The penetration of the primer and formation of resinous tags in this irreversible surface alteration, results in color variation[27] and consequently the gloss. According to Sifakakis et al.[47], the techniques for bonding brackets with acid conditioning promote important irregularity, with a greater change in surface gloss.[47]

This study used Enamel Damage Index to characterize the surfaces resulting from the use of the systems. The TCB group presented 40% of the surfaces with score 1 and 60% of the surfaces with score 2 agreeing with Alessandri Bonetti et al. (2011)[4], who used tungsten carbide burs with Sof-Lex discs and obtained most samples with a score different than 0. Another study also showed that most of the scores presented using tungsten carbide burs were 0 and 1, emphasizing that the use of dental loups should be part of the removal protocol[34]. They found that the use of dental loupes resulted in scores reduction, with less surface damage and less adhesive residual left on the structure[34]. Leaving adhesive residues on the enamel can result in roughness surface increase, favors plaque accumulation[20], and pigmentation of these remnants and causing an aesthetic problem[48]. Mohebi et al.[39] also used a loups on removal stage and found that, even using the accessory, there was an increase in surface roughness, confirmed by the use of AFM, as used by us. Surface irregularities, whether due to cement residues or enamel wear, can be influenced by the performing professional, that is, operator-dependent. This may be the reason for the difference in results between the authors. In our study, to reduce the chances of bias due to the action of more than one operator, the methodologies were always performed by the same person.[19]

The use of scalpel blade resulted, for the most part, in score 1, but the system also showed scores 0 and 2. It is a system that is being proposed at this time and is necessary new tests are necessary to identify the best way to use it and what is the best polishing system should be associated, considering the importance of the last

stage[48]. Because it is a material widely used in dental offices and low cost, the use of scalpel blades could be an alternative, especially when compared to the systems already established in the literature as the choice. DHpro system resulted in surfaces predominantly score 0. As in the SB system, it presented a significant increase in roughness before polishing, but significantly reduced after t2. This reduction, associated with the better average of superficial gloss among the systems and lower scores on Enamel Damage Index demonstrate and reinforce the importance of using a suitable polisher. Instruments of this system are made of aluminum oxide and ultrafine silicon carbide and have given a satisfactory result without increasing surface roughness as showed in other study[42]. In this study, there was an increase in roughness but the initial and final roughness were statistically similar.

3.6 Conclusion

Considering the limitations of this study it is possible to verify that the DHpro system presented a more satisfactory result compared to the others and that the new system proposed by this study (SB) was similar to it for surface roughness and gloss. Further studies should be carried out in order to identify the most appropriate technique for the use of SB in addition to the most suitable polishing system for this purpose.

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Tables

Table 1. Description and characteristics of the removal systems that compose the experimental groups.

System/Composition	Manufacturer	Indication / Protocol of use
Tungsten Carbide Bur – 24 Blades (TCB)	Orthometric – Indústria e Comércio de Produtos Médicos e Odontológico Ltda	Indicated to orthodontic cement removal. Used in high speed.
Zirconia Multilablade bur for resin removal and Disposable Tip for Finishing (MO)	Dental Morelli Ltda.	Indicated for removal of orthodontic adhesive residue after bracket removal and finishing the enamel. Rotation between 10,000 and 20,000 RPM, in low speed, pressure against the dental surface should be moderate so as to avoid excessive heating of the enamel.
Aluminum Oxide Polishers and Ultrafine Silicon Carbide Polisher (DHpro)	DHpro	Indicated for Resin Removal and polishing enamel. Maximum rotation: 12,000 RPM in low speed.
Scalpel Blade Stainless Steel (SB)	LAmedid Comercial e Serviços Ltda	Used mounted on scalpel handle.

* Terms in parentheses represents the acronyms that will be used throughout the text.

Table 2. Root Mean Square Roughness (Sq) and Average Surface Roughness (Sa), in nm, at baseline (Sq0/Sa0), after residual adhesive removal (Sq1/Sa1) and after polishing (Sq2/Sa2). (n=10)

System	Sq0	Sq1	Sq2	Sa0	Sa1	Sa2
TCB	115.3(±12.2)^{b}	304.5(±116.9)^{a}	238.2(±86.8)^{a}	84.2(±10.2)^{B}	226.4(±93.0)^{A}	179.1(±68.4)^{A}
DHpro	112.3(±28.3)^{b}	249.2(±54.5)^{a}	157.0(±48.1)^{b}	82.3(±24.5)^{B}	182.1(±40.0)^{A}	112.8(±29.7)^{B}
MO	113.1(±45.8)^{b}	275.9(±70.9)^{a}	218.3(±70.4)^{a}	79.88(±29.2)^{B}	201.1(±54.0)^{A}	160.5(±53.7)^{A}
SB	116.1(±38.5)^{b}	261.7(±34.9)^{a}	179.3(±72.3)^{ab}	82.3(±20.4)^{B}	182.4(±21.5)^{A}	129.2(±44.5)^{AB}
ARI0	119.2(±39.5)^{b}	215.1(±65.4)^{a}	196.9(±81.4)^{ab}	81.5(±32.8)^{B}	163.6(±49.0)^{A}	139.1(±23.7)^{AB}
Grouped averages	115.2^{c}	261.3^{a}	197.9^{b}	82.0^{C}	191.1^{A}	144.1^{B}

No differences were observed between systems at any time for Sq and Sa parameters. Different lowercase letters in lines represents differences between Sq0, Sq1 and Sq2. Different uppercase letters in lines represents differences between Sa0, Sa1 and Sa2. ($p<0.05$)

Table 3. Comparison of differences in Root Mean Square Roughness (Sq) and Average Surface Roughness (Sa) at baseline, t1 and t2, in nm. (n=10)

System	Sq1-Sq0	Sq2-Sq0	Sq2-Sq1	Sa1-Sa0	Sa2-Sa0	Sa2-Sa1
TCB	189.2^{a}	122.9^{a}	-66.3^{b}	142.2^{A}	94.8^{A}	-47.4^{B}
DHpro	136.9^{a}	44.7^{a}	-92.2^{b}	99.8^{A}	30.5^{A}	-69.4^{B}
MO	162.9^{a}	105.3^{a}	-57.6^{b}	121.2^{A}	80.7^{A}	-40.6^{B}
SB	145.6^{a}	63.2^{a}	-82.4^{b}	100.1^{A}	46.9^{A}	-53.2^{B}
Ari0	95.9	77.7	-18.2	82.0^{A}	57.5^{AB}	-24.5^{B}
Grouped averages	146.1^{a}	82.7^{b}	-63.4^{c}	109.1^{A}	62.1^{B}	-47.0^{C}

Sq0/Sa0 - baseline, Sq1/Sa1 - after residual adhesive removal Sq2/Sa2 - after polishing. No differences between groups, at any times, for differences in Sq and Sa. (Two way ANOVA, $p<0.05$) Different lowercase letters in lines represents differences between Sq1-Sq0, Sq2-Sq0 and Sq2-Sq1. Different uppercase letters in lines represents differences between Sa1-Sa0, Sa2-Sa0 and Sa2-Sa1. (Two way ANOVA and Tukey Test, $p<0.05$)

Table 4 – Average gloss values (GU) and standard deviations for each removal system.

System	GU(±SD)
Enamel	59.1 (1.3)^{A}
DHpro	42.5 (7.9)^{B}
SB	41.5 (7.9)^{B}
ARI0	32.5 (13.0)^{BC}
TCB	25.0 (2.4)^{C}
MO	18.7 (3.6)^{C}

* Different uppercase letters in column represents differences between removal systems for Gloss values. (One way analysis of variance and Tukey Test, $p<0.05$)

Table 5. Distribution of Enamel Damage Index (EDI) scores, after treatment.

System	0	1	2	3
TCB	**0**	**4**	**6**	**0**
DHpro	**6**	**2**	**2**	**0**
MO	**0**	**8**	**2**	**0**
SB	**3**	**6**	**1**	**0**
ARI0	**5**	**4**	**1**	**0**

Figures

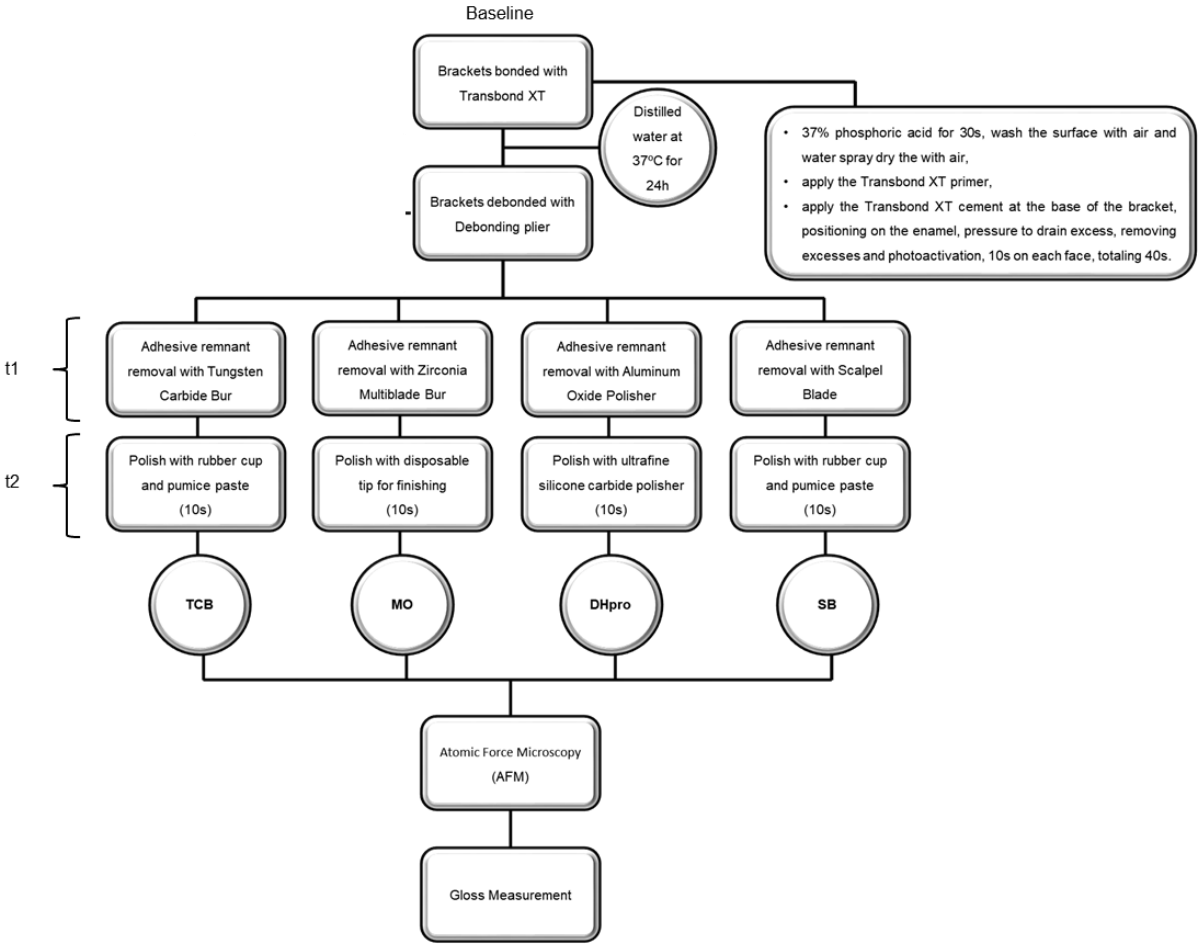

Fig. 1 – Experimental design.

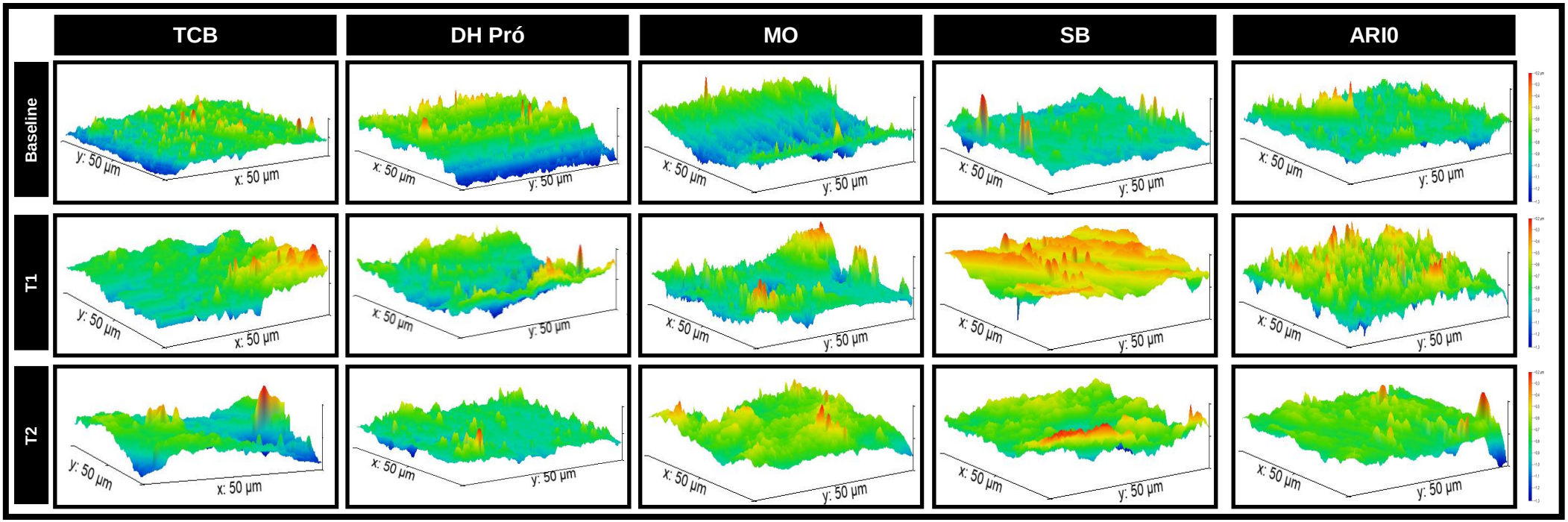

Fig. 2 – Comparative image of roughness surface of systems, on different times.

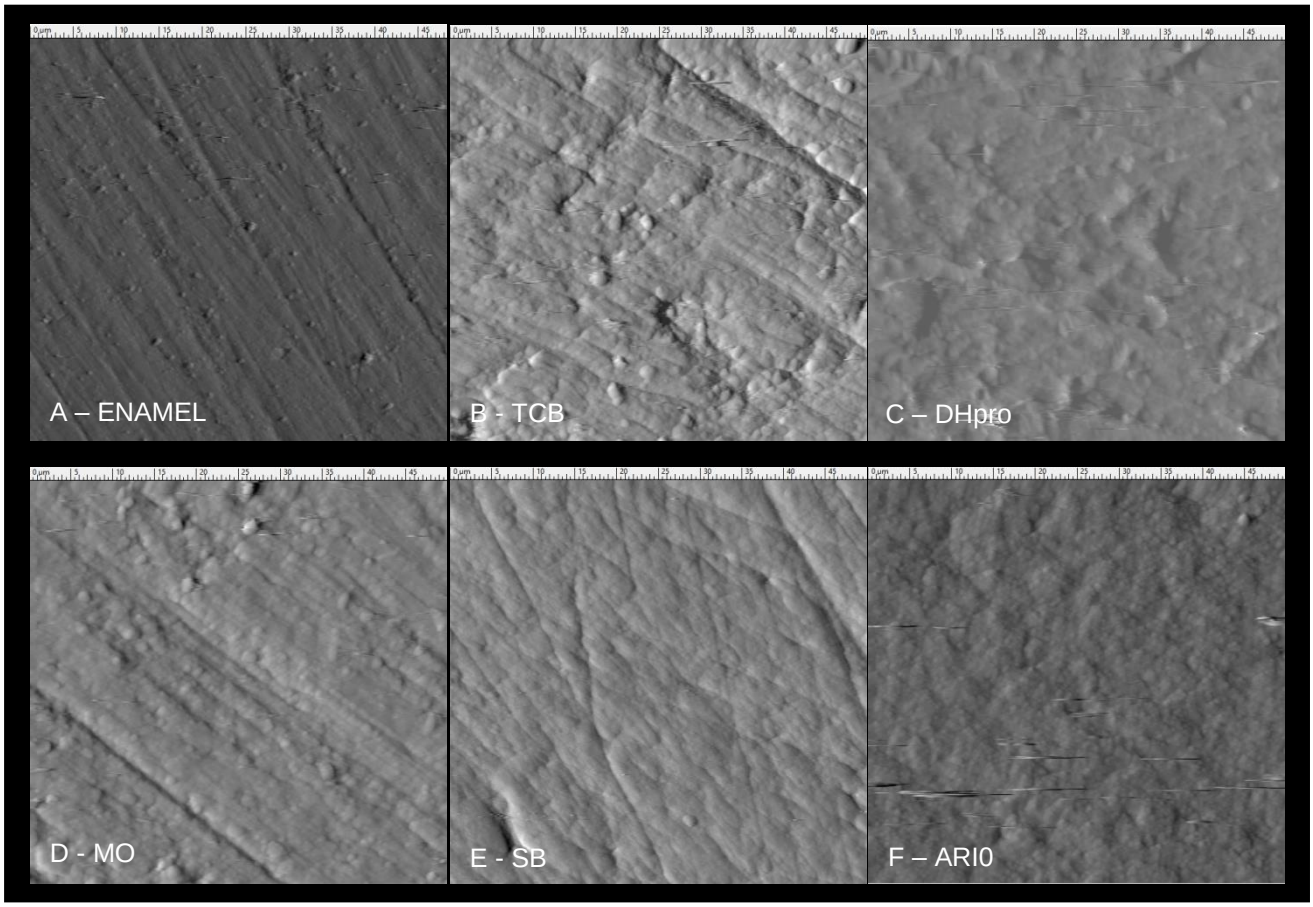

Fig. 3 – Characterization of enamel by Atomic Force Microscopy. A – represents enamel, previously to treatments. B, C, D, E and F – represents enamel after polish with the removal systems (TCB, DHpro, MO, SB and ARI0, respectively)

4. Considerações finais

A manutenção da saúde bucal e da integridade do tecido dental após a ortodontia é uma preocupação de muitos especialistas, e alternativas para estes fins vem sendo amplamente buscadas. Através da revisão sistemática concluímos que, apesar de não haver diferença significativa entre o tratamento com probiótico e com placebo, mais estudos devem ser realizados, com metodologias mais padronizadas, tempo de acompanhamento mais longo, já que individualmente a maioria dos estudos apresentou resultados significativos. Através da pesquisa *in vitro* concluímos que, independente da técnica utilizada, sempre há um aumento na rugosidade superficial. A nova técnica proposta por este estudo apresentou resultados satisfatórios e promissores, mas necessita ser aperfeiçoada e novos testes realizados.

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