DO PROBIOTICS AFFECT ORAL HEALTH IN PATIENTS UNDER TREATMENT WITH FIXED ORTHODONTIC APPLIANCES? A SYSTEMATIC REVIEW

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ABSTRACT

DO PROBIOTICS AFFECT ORAL HEALTH IN PATIENTS UNDER TREATMENT WITH FIXED ORTHODONTIC APPLIANCES? A SYSTEMATIC REVIEW

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AIM: As the presence of fixed orthodontic appliances increases biofilm retention, a deterioration in periodontal clinical parameters can be observed and, under certain conditions, a cariogenic environment may develop, leading to enamel decalcification. The aim of the present review was to systematically investigate the available literature regarding the effects of probiotics on gingival inflammation and enamel decalcification development in patients under orthodontic treatment with fixed appliances.

MATERIALS AND METHOD: Searches without restrictions in eight databases and hand searching were carried out. Randomized controlled studies investigating the effect of probiotics on gingival health and enamel decalcification development in patients under orthodontic treatment with fixed applianceswere reviewed. Following study retrieval and selection, relevant data were extracted and the risk of bias was assessed according to the Cochrane Collaboration guidelines.

RESULTS: Out of the initially identified unique records, four studies fulfilled the selection criteria for inclusion in the systematic review. Three studies evaluating gingival inflammation after probiotic use for up to one month did not show any

statistically significant changes. The only study investigating enamel decalcification for a mean duration of 17 months of probiotic use did not demonstrate differences in the incidence of white spot lesions between the groups at debonding. No adverse effects were reported. Various problems were noted during risk of bias assessment.

CONCLUSIONS: Overall, short-term probiotic administration does not seem to exert an effect in the development of gingival inflammation and enamel decalcification in patients under treatment with fixed orthodontic appliances. More high-quality studiesinvolving different combinations of probiotic strains and of longer duration of intervention and follow-up are required.

DEDICATION

My deepest gratitude goes to God who has provided all that was needed to complete this thesis and the program. I would also like to dedicate my Master Thesis and express my greatest gratitude to my beloved parents and family, who have always stood by me, giving me the strength to reach here today. Thank you.

I would like to express my sincere gratitude to my advisors for their continuous support.

DECLARATION

I declare that all the content of the thesis is my own work. There is no conflict of interest with any other entity or organization.

Name: Riham HadjHamou

Signature:

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1. INTRODUCTION

The term probiotics comes from the Greek words“*προ*” (pro – promoting) and “*βίος*” (bios – life). According to the World Health Organization, probiotics are living microorganisms (including bacteria and yeasts) which, when administered in a sufficient manner, are found to be beneficial to the host **(**FAO/WHO, 2001**)**. Probiotics have been a subject of interest among the scientific communitysince the twentieth century when Elie Metchnikoff known as the "Father of Probiotics”introduced the concept in 1907 (Parvez et al., 2006; Meurman and Stamatov, 2009; Kour et al., 2015). The increase in the popularity of probiotics in recent years is believed to be due to their natural origin and a number of probiotic-induced benefits, mainly in the prevention and treatment of gastrointestinal diseases, as well as their perceived safety (Ried et al., 2003; Pujia et al., 2017).

Multiple reports have advocated the benefits of probiotics in association with oral diseases. Recent clinical studies have reported a possible impact on oral health by reducing the prevalence, incidence and severity of dental caries and periodontal disease (Meurman and Stamatova, 2007; Cildir et al., 2009; Pujia et al., 2017). As orthodontic treatment with fixed appliances has been linkedwith significant biofilm accumulation, thus putting patients at a higher risk of developing enamel demineralization and periodontal diseases, probiotics could be of benefit. However, studies solely targeting patients undergoing treatment with fixed orthodontic appliances are limited and have not, so far, been reviewed in an evidence-based manner.

The aim of the present thesis was to systematically investigate the available literature regarding the effects of probiotics on the oral health of patients under treatment with fixed orthodontic appliances.

2. REVIEW OF THE LITERATURE

2.1. Oral probiotics

The concept of probiotics was first introduced during the 20th century by Nobel Prize winner Elie Metchnikoff, in 1907. Metchnikoff had found that the population in a rural village in Bulgaria showed exceptional longevity. He speculated that the cause was related to the consumption of a fermented yoghurt drink containing *Lactobacillus bulgaricus* (Anukam and Reid, 2007; Kour et al., 2015). Following Metchnikoff's research, the French pediatrician Henry Tissier was the first to utilize probiotics as a therapeutic agent in infants suffering from diarrhea. Later, Lilly and Stillwell (1965) described probiotics as "micro-organisms stimulating the growth of other micro-organisms" (Lilly and Sitllwell, 1965). Probiotics are now considered as being a natural therapy for treating diseases of an infectious nature (Anukam and Reid, 2007; Kour et al., 2015).

According to the Food and Agriculture Organization of the World Health Organization probiotics are "living microorganisms which, when administered in a sufficient amount and manner, confer a health benefit on the host" (FAO/WHO, 2001). In order for probiotics to be fully effective they must involve an appropriate strain and be administrated in appropriate doses (Guarner and Schaafsma, 1998). Other essential properties are the abilities to adhere to, and colonize the oral cavity tissues, thus allowing a longer presence of probiotics in the oral cavity, and prolonging the demonstration of their effect (Haukiojaa, 2010; Oelschlaeger, 2010; Kour et al., 2015).

The most common genera used asoral probiotic bacterial strains belong to *Bifidobacterium* and *Lactobacillus* species, which are found naturally in the oral ecosystem (Saxelin et al., 2010; Haukioja, 2010; Kour et al., 2015; Rotimi and

Duerden, 1981; Kour et al., 2015). These species are also present in the breast milk indicating the early exposure of the oral cavity to these strains (Gueimonde et al., 2007; Abrahamsson et al., 2009; Kour et al., 2015). The oral cavity contains many *Lactobacillus* species that benefit oral microbiota, including *Lactobacillus acidophilus, Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus salivarius* and *Lactobacillus paracasei* (Ahrne et al., 1998; Colloca et al., 2000; Simark-Mattsson et al., 2007; Maukonenet al., 2008). Various *Bifidobacteria* specieshave also been isolated from the oral cavity, like *Bifidobacterium bifidum, Bifidobacterium dentium* and *Bifidobacterium longum* (Crociani et al., 1996; Beighton et al., 2008 Maukonen et al., 2008; Haukioja et al., 2010).

2.2. Mechanism of action

Probiotics act by interacting, directly or indirectly, with pathogens to create a more balanced microbial environment and induce positive changes in oral health (Parvez et al., 2006; Cildir et al., 2009; Haukioja et al., 2010; Kour et al., 2015).

Oral probiotic strains may act in a direct manner by competing with pathogens overblocking the adhesion sites and over nutrients. Also, some strains might secrete different antimicrobialsubstances such as hydrogen peroxide, organic acids, and bacteriocins that can inhibit or destroy pathogens (Meurman, 2005; Bonifait et al., 2009). Moreover, some oral probiotic strains act indirectly by means of immune modulation and modification of the surrounding environment (altering pH and/or oxidation reduction). In these ways the ability of pathogens to colonize the oral cavity is compromised. Finally, some strains might stimulate the secretion of non-specific

immunoglobin A production, thus promoting the colonization of less pathogenic bacterial species (Saavedraet al., 2004; Meurman et al., 2005; Niel et al., 2005; Gueimonde et al., 2006; Bonifait et al., 2009; Rastogi et al., 2011; Kour et al., 2015).

2.3. Safety in using probiotics

In order to ensure safety in their usage, probiotic strains must sustain genetic stability in the oral cavity and should not demonstrate properties such as pathogenicity, ability to stimulate the growth of other bacteria and ability to transfer genes leading to antibiotic resistance (Grajek et al., 2005).

It is logical to assume that the consumption of specific types of a probiotic strain will lead to an increase in the concentrations of these species in the host organism. However, a long-term follow-up study in Finland confirmed that with the administration of *Lactobacillus rhamnosus* no gradual increase in *Lactobacillus*concentration was noted (Salminenet al., 2002).On the other hand, some studies have reported adverse effects when using probiotics on individuals with pre-existing chronic conditions or systemic diseases such as diabetes, cardiovascular diseases, gastrointestinal disorders, malignancies, or in organ transplant patients (Husni et al, 1997; Cannon et al, 2005).

A recent systematic review that evaluated 74 randomized control studies on the safety of probiotics in children below 18 years of age, failed to establish any association between the use of probiotics and increased adverse effects or health risks. Regarding the development of antibiotic resistance, the authors concluded that further studies are warranted (Van den Nieuwboer et al., 2015).

2.4. Probiotics for oral health in the general dental population

The presence of oral biofilms is involved in the etiology of the most common oral diseases, like caries and periodontal diseases, and has been extensively documented in clinical and epidemiological studies (Rosenoer and Sheiham, 1995; Marsh, 2006; Filoche et al., 2010; Marsh and Devine, 2011; Wade, 2013; Jansson et al., 2014; Li et al., 2014; Benic, 2016). Recently, probiotics have been used adjunctively to prevent biofilm-related diseases of the oral cavity (Meurman, 2005; Twetman and Stecksen-Blicks, 2008; Stamatova and Meurman, 2009; Teughels et al., 2011; Cagetti et al., 2013; Laleman et al., 2015; Gruner et al., 2016).

2.4.1. Enamel demineralization

Dental caries is one of the most common diseases worldwide (Cagetti et al., 2013). With an increased consumption of sugary foods and drinks, a shift to an acidogenic environment occurs in the oral environment, ultimately leading to enamel demineralization and cavitation (Martinez et al., 2015; Coqueiro el al., 2018). Despite the extent of the problem, few studies have assessed the effect of probiotics on caries prevalence and incidence as the primary outcome. Thus, the current evidence is considered to be insufficient (Gruner et al., 2016).

In children, the administration of probiotics in dairy products, as well by other means, like tablets, has been shown to exert a beneficial effect in caries prevalence and incidence in the medium term (Nase et al., 2001; Stecksén-Blicks et al., 2009 Hedayati-Hajikand et al., 2015). However, in the long term, the results were contradictory (Hasslöf et al., 2013; Stensson et al., 2014).

Regarding adult populations, Petersson et al. (2011) conducted a trial aiming at

assessing root caries index reversal. They divided 160 healthy participants into 4 different groups; Group 1:placebo milk consumption group, Group 2:consuming milk containing 5 ppm of fluoride and probiotic bacteria *Lactobacillus rhamnosus LB21*with a concentration of 10^7 CFU/mL, Group 3:consuming milk with the probiotic bacterial strain only, and Group 4: consuming milk with fluoride only. Root caries index improved in all intervention groups, with Group 2 showing the most significant improvement.

2.4.2. Periodontal tissue health

Periodontal diseases can present in two forms, either gingivitis or periodontitis,both initiated by the formation of dental plaque (Armitage, 1995; Yanine et al., 2013). Gingivitis is the presence of gingival inflammation without loss of connective tissue attachment. On the contrary, periodontitis is characterized by the presence of gingival inflammation with loss of connective tissue attachment and the resorption of coronal portions of thetooth supporting alveolar bone (Armitage, 1995; Yanine, 2013). Two recent systematic reviews aimed to determine the effects of probiotics on various periodontal health parameters (Gruner et al., 2016; Jayaram et al., 2016).

Studies assessing gingival inflammation using the Gingival Index (Löe and Silness, 1963), demonstrated contradictory outcomes with some reported no statistically significant benefit from probiotic administration (Krasse et al., 2006; Shimauchi et al., 2008; Toiviainen et al., 2015, Shah et al., 2013; Szkaradkiewicz etal., 2014; Hallström et al., 2103; Laleman et al., 2015), while other trials showed a statistically significant difference between groups receiving probiotics and placebo (Riccia et al., 2007; Vivekanandaet al., 2010; Ince et al., 2015; Tecke et al., 2015; Laleman et al., 2015).

When reporting on bleeding on probing measurements, the literature was again divided by conflicting findings; some found no statistically significant differences between the experimental and control groups (Shimauchi et al., 2008; Hallström et al., 2104; Lee et al., 2014; Laleman et al., 2015), while other researchers noted a significant reduction in the bleeding on probing values after probiotic administration (Riccia et al., 2007; Twetman et al., 2009; Teughels et al., 2013; Szkaradkiewicz et al., 2014; Vicario et al., 2013; Ince et al., 2015).

Likewise, the assessment of the literature regarding morphological parameters of the periodontal tissues, like probing pocket depth (PPD), lead to the retrieval of studies reporting significant differences between groups receiving probiotics and placebo (Shimauchi et al., 2008; Iniesta et al., 2012; Laleman et al., 2015). On the contrary, other trials reported a statistically significant beneficial effect of probiotics (Vivekananda et al., 2010; Shah et al., 2013; Teughels et al., 2013; Szkaradkiewicz etl al., 2014; Vicario et al., 2014; Ince et al., 2015; Tecke et al., 2015). In a similar manner, the data on clinical attachment levels gain were conflicting. No statistically significant differences between the groups were noted in one study only (Vivekananda et al., 2010), while significant changes were observed in the other trials (Teughels et al., 2013; Ince et al., 2015; Tecke et al., 2015).

2.5. Oral health during orthodontic treatment

Patients undergoing orthodontic treatment have been linked with significant biofilm accumulation, placing them at a higher risk of developing white spot lesions and caries, as well as demonstrating deterioration in periodontal clinical parameters (Graber et al., 2004; Justus, 2015). Oral hygiene is a critical factor that can affect the quality and

timing of treatment and relies on patient motivation (Al-Jewair et al., 2011; Cozzani et al., 2016). Several studies have reported a rapid decline in oral hygiene after the initial bonding stemming from the greater difficulties in maintaining proper oral hygiene (Miller and Hobson, 1961; Katz, 1978; Cozzani et al., 2016; Al-Jewair et al., 2017). Achieving efficient oral hygiene requires clear professional instructions and patient motivation (Marini et al., 2014; Flemings, 2015).

Dental biofilm comprises of different bacterial strains that attach themselves to the soft and hard tissues for nutrition and protection (Socransky and Haffajee, 2005; Lombardo et al., 2013). Initial colonizers attach to the pellicle using different aggregation patterns (Socransky and Haffajee, 2005; Kolenbrander et al., 1993).With the continuous accumulation of biofilm, a gradual shift of bacteria from aerobic to anaerobic is observed. This shift, including *Spirochetes, Eubacterium nodatum, Peptostreptococcous, Fusobacterium, Prevotella intermedia, Campylobacter, Prophyromonas gingivalis, Tannerella forsythia* and *Treponema denticola* species, is associated with the development of periodontal inflammation (Diamanti-Kipioti et al., 1987; Huser et al., 1990; Atack et al., 1996; Naranjo, et al., 2006; Pan et al., 2007; Lucchese et al., 2018). Following the completion of orthodontic treatment and a rigorous oral hygiene regimen, the levels of these species have been measured to be lower than the pretreatment levels (Thronberg et al., 2009; Davis et al. 2014).

A recent study has reported that the highest levels of biofilm accumulation were found at the gingival margin of the maxillary lateral incisors and canines in males, and that young adults showed greater accumulations than older adult patients (Mei et al., 2017). The majority or this accumulation was found to be present beneath the arch wires (Mei et al., 2017). Moreover, appliance design can favor the accumulation of plaque and cause difficulty in accessing the area while brushing (Al-Jewair et al., 2011, Cozzani

et al., 2016). Some studies have shown an increase in biofilm retention on banded molars compared to bonded molars (Boyd and Baumrind, 1992; Mei et al., 2017). Moreover, other parameters like the application of different biomaterials and the alterations in the tooth surface when bonding fixed orthodontic appliances might lead to exacerbation of biofilm accumulation (Øilo and Bakken, 2015; Lucchese et al., 2018). Finally, when comparing labial and lingual fixed appliances, it was shown that lingual appliances are prone to more biofilm retention (Stammet al., 2005; Lucchese et al., 2018).

2.5.1. Changes in clinical periodontal parametersduring orthodontic treatment

Changes in clinical periodontal parameters during active orthodontic treatment with fixed appliances have been reported in several studies.Most studies describe that following the initial increase in biofilm accumulation, inflammation and bleeding is observed, but with some improvement over time (Page et al., 1976; Slots, 1977; Artun, 1987; Vacek et al., 1994; Lang et al., 1995; Davis et al., 2014). These changes can be observed as early as two weeks into treatment and the peak levels are noted at 3 months. Within 6 months a decrease is observed, but not to the pretreatment values (Ristic et al., 2007; Lo et al., 2008; Davis et al., 2014).

A consensus has yet to be agreed in the literature on whether probing depths increase or maintain the same level during the treatment phase (Alstad and Zachrisson, 1979; Sinclair et al., 1987; Naranjo et al., 2006; Liu and Dong, 2011; Davis et al., 2014). The deep probing depths frequently observed in orthodontic patients can simply be attributed to weakened connective tissues allowing a deeper insertion of the dental probe, or due to the formation of pseudopockets because of gingival enlargement (Van

Gastel et al., 2011; Davis et al., 2014). The posterior teeth and interproximal contacts may develop deep pseudopockets in children in the first couple of months due to the hyperplastic gingivitis. This could be attributed to chemical or mechanical irritation, poor oral hygiene, or food impaction. Kloehn and Pfeifer (1974) recorded almost four times higher rates of hyperplastic gingivitis in the inter-proximal area than in the middle of the crown, andfive times higher rates in the premolars and molars than those observed in the anterior teeth (Kloehn and Pfeifer, 1974; Zachrisson, 1976; Davis et al., 2014). Upon the completion of orthodontic treatment and a rigorous oral hygiene regimen, gingival inflammation and enlargement subside within the first month after the removal of the appliances (Sallum et al., 2004; Davis et al. 2014).

Regarding attachment loss, there is some disagreement on the possible effects of orthodontic treatment also. Several studies have compared the attachment loss between orthodontic patients and the non-orthodontic population and have demonstrated insignificant differences (Alstad and Zachrisson, 1979; Paolantonio et al., 1996; Davis et al., 2014). On the other hand, Zachrisson and Alnaes (1973) measured pocket depth from the CEJ to the base of the pocket and reported significant loss of attachment in some orthodontic patients (Zachrisson and Alnaes, 1973).

2.5.2. Enamel demineralization during orthodontic treatment

Orthodontic treatment is considered to increase the risk of enamel demineralization, with consequences possibly harmful to patients and potentially compromising treatment outcomes (Graber et al., 2004; Justus, 2015). Poor diet and high sugar consumption, lead to an acidogenic shift in the oral bacterial composition, involving bacteria like *Streptococcus mutans* and *Lactobacilli*.This sudden rise in the levels of

these specific bacteria is associated with a drop of pH to the critical level of demineralization. Consequently, the remineralization balance is disturbed and shifts towards enamel demineralization and caries formation. The higher risk of caries development in immature, newly erupted, teeth must also be considered (Mtaya et al., 2009; Martignon et al., 2010; Shrestha et al., 2013; Hriday et al., 2012).

Initial stages of enamel demineralization, like white spot lesions can appear as early as four weeks into orthodontic treatment, and like any other carious lesion can extend and reach the dentin layer of the tooth (Featherston, 2004; Willmot, 2008). White spot lesions present like white opacities on smooth surfaces with a well-defined shape, located in the middle of the tooth (Sangamesh and Amitabh, 2011). The development of such lesions is more frequently found on lateral incisorsthan canines, first premolars, second premolars, and molars (Øgaard, 1989; Chapman et al., 2010). In a more recent study, the authors reported that white spot lesions were found predominantly on the upper and lower premolars, first maxillary molars, upper and lower lateral incisors, upper canines, on the middle third of the tooth or cervical area, in areas surrounding or between the bracket and wire (Lovrov et al., 2007; Øgaard, 2008).

The prevalence among the orthodontically treated population varies in the literature. Reports have presented prevalence rates ranging from 2- 97%; the large variation seeming to be largely dependent on the method of examination used to detect the lesions (Zachrisson and Zachrisson, 1971; Gorelick et al., 1982; Mizrahi, 1982; Artun & Brobakken, 1986; Geiger et al., 1988; Øgaard, 1989; Mitchell, 1992).

Research investigating the association with gender found that the prevalence of white spot lesions is higher in young male than young female patients, maybe because females maintain better oral hygiene (Zachrisson and Zachrisson, 1971; Boersma,

2005; Chapmen et al., 2010; Al Maaltah et al., 2011; Karadas et al., 2011; Kuusela et al., 1996; Sakki et al., 1998; Ostberg et al., 1999). On the other hand, Gorelick et al. (1982) observed that young female patients exhibited higher white spot lesion incidence than young males.

Furthermore, the duration of orthodontic treatment has been suggested to exert an impact on the formation of white spot lesions; the longer the length of treatment, the greater their incidence. Some studies have reported that from 12 to 36 months, the prevalence increases by almost 3.5 times (Karadas et al., 2011; Khalaf, 2014).Tufekci et al.(2011) and Lucchese and Gherlone (2013) also reported prevalence of 38% and 40%, respectively, in the first six months of orthodontic treatment, that increased to 46% and 43% respectively, at 18 months of treatment (Tufekci et al., 2011; Lucchese and Gherlone, 2013; Julien et al., 2013). On the contrary, a few researchers have found that treatment duration was not a significant factor in white spot lesion development (Gorelick et al., 1982; Southard, 1986; Akin et al 2013; Sundararaj et al., 2015).

2.5.3. Oral health maintenance during orthodontic treatment with fixed appliances

It is important to highlight the role of the orthodontist in closely monitoring patients in maintaining proper oral hygiene, thereby reducing the side effects associated with fixed orthodontic treatment, such as decalcification and the development of gingival inflammation (Sinclair et al., 1987; Ristic et al., 2007).

Oral hygiene can be achieved mechanically by the physical removal of the biofilm using a toothbrush and interdental cleaning aids, or professionally, by a dentist or a dental hygienist performing scaling and polishing. Regarding mechanical methods of biofilm removal, the use ofelectric toothbrushes might aid in controlling gingival

inflammation, both in the general population, as well as, patients under fixed orthodontic appliance treatment (Yaacob et al., 2014; Al Makhmari et al., 2017. Regular dental flossing has been also been reported to lead to improvements in the Gingival Index (Zanatta et al., 2011).

However, many orthodontic patients fail to comply with the recommended oral hygiene instructions given by professional (Winterfeld et al., 2015). Thus, other means, including the use of substances with antimicrobial effects have been used as adjunctive strategies to maintain oral health during treatment with fixed orthodontic appliances. Chlorhexidine is considered to be the gold standard in chemical control and has a proven bactericidal effect (Øgaardet al., 1980; Gokce et al., 2017). It is used as an oral antiseptic mouth rinse with a concentration of 0.12% or 0.2%. Chlorhexidine gluconate has been found to reduce gingival inflammation and the levels of *Streptococci mutans*in the biofilm. However, using chlorhexidine for prolonged periods can lead to dental staining and alterations in the perception of taste (Gokce et al., 2017).

Nowadays, with the increased interest in, and popularity of, oral probiotics, several reports have discussed the effect of oral probiotics in enhancing oral health in the general dental population (Gruner et al., 2016; Jayaram et al., 2016). However, to date, studies solely targeting patients undergoing orthodontic treatment with fixed appliances are limited and have not been reviewed in an evidence-based manner.

3. AIM

3.1. Aim of the systematic review

To systematically investigate the available literature regarding the effects of probiotics on the oral health of patients undergoing treatment with fixed orthodontic appliances.

3.2. Objectives of the systematic review

To retrieve the existing data on the effects of probiotics on enamel decalcification and gingival inflammation development, in patients under treatment with fixed orthodontic appliances.

3.3. Null hypothesis

Oral probiotic administration does not prevent enamel decalcification and gingival inflammation development, in patients under treatment with fixed orthodontic appliances

4. MATERIALS AND METHODS

4.1. Protocol Development

The present review was based on a general protocol developed by following the guidelines outlined in the PRISMA statement (Shamseer et al., 2015; Moher et al., 2001) and the Cochrane Handbook for Systematic Reviews of Interventions (version 5.1.0) (Higgins and Green, 2011). The protocol was registered with PROSPERO - International prospective register of systematic reviews, which is produced by the Centre for Reviews and Dissemination (CRD) at the University of York, United Kingdom (UK), and is funded by the National Institute for Health Research (NIHR), UK (CRD42018118008).

4.2. Selection criteria applied for the review

The selection criteria for the domains of study design, participants' characteristics, intervention characteristics and principal outcome measures applied for the present review were as follows:

4.2.1. Types of study design

Studies included in the present thesis had to be Randomized Clinical Trials (RCTs) evaluating clinically gingival inflammation health and enamel demineralization development.Human studies that did not evaluate clinical outcomes, animal studies and non-comparative studies (case reports and case series), systematic reviews and meta-analyseswere excluded from the present review.

The type of study design was assessed by using the algorithm available from SIGN

(Scottish Intercollegiate Guidelines Network) available from http://www.sign.ac.uk (Appendix I).

4.2.2. Types of participants

The included studies had to involve healthy individuals of any age undergoing orthodontic treatment with fixed appliances.

Studies that included subjects with craniofacial anomalies or syndromes of the head and neck region, individuals with systematic disease or using antibiotics or antimicrobial agents were excluded from the present review.

4.2.3. Types of interventions

The included studies had to involve patients who had received any kind of probiotic and who were compared to groups receiving placebos or no administration at all.

4.2.4. Types of outcome measures

The studies included in the present review had primarily to provide clinical measurements on gingival inflammation and enamel demineralization development. Plaque measurements were not considered, as they are not representative of the level of gingival inflammation.

4.3. Search strategy for identification of studies

The principal investigator (RHH) developed detailed search strategies for each

database searched. These were based on the strategy developed for MEDLINE, but were revised appropriately for each database to take account of the differences in controlled vocabulary and syntax rules. The following electronic databases were searched (Appendix II): MEDLINE via PubMed, CENTRAL, Cochrane Systematic Reviews, Scopus, Web of Science™ Core Collection, Arab World Research Source, Clinical Trials registry and ProQuest Dissertations & Theses Global database.

No restriction was placed on the language, date or status of publications. In addition, efforts were made to obtain conference proceedings and abstracts where possible and the reference lists of all eligible studies for additional records were hand searched.

4.4. Selection of studies and data collection

The principal investigator and the thesis supervisor (EGK) assessed the retrieved records for inclusion independently. They were not blinded to the identity of the authors nor their affiliation. Any disagreement was resolved by discussion with the co-supervisor (AEA). The following details were extracted:

a. Bibliographic details of the study.

b. Details on study design, outcomes assessed and verification of study eligibility.

c. Participant characteristics (number, age, possible dropouts).

d. Intervention characteristics (experimental and placebo/control groups; type of probiotic used, duration of administration, mode of administration).

e. Details on outcomes assessed.

f. Data on adverse effects.

g. Additional information: a prior sample size calculation, methodology of

reliability assessment.

4.5. Risk of bias assessment

The principal investigator and the thesis supervisor assessed the risk of bias in the included studies independently and in duplicate during the data extraction process, using The Cochrane Collaboration's Risk of Bias assessment tool for RCTs (Higgins and Green, 2011). Any disagreements were resolved by discussion or consultation with the thesis co-supervisor. The Risk of Bias assessment tool includes the following domains.

a. Random sequence generation (selection bias).

b. Allocation concealment (selection bias).

c. Blinding of participants and personnel (performance bias).

d. Blinding of outcome assessors (detection bias).

e. Incomplete outcome data (attrition bias).

f. Selective outcome reporting (reporting bias).

g. Other sources of bias.

After the entering the information reported in each study in the data extraction form,every domain received a judgment of low, high or unclear risk of bias (indicating either lack of sufficient information to make a judgment or uncertainty over the risk of bias) (Higgins and Green, 2011).

Subsequently, studies were judged as being of low, unclear or high risk of bias.

a. Low risk of bias (plausible bias unlikely to seriously alter the results)

b. Unclear risk of bias (bias that raises some doubt about the results)

c. High risk of bias (bias that seriously weakens confidence in the results)

4.6. Summary measures and synthesis of results

Although a synthesis of the results was planned according to the research protocol, it was not, in the end, carried out due to the lack of an adequate amount of data as well as differences in the retrieved studies.

5. RESULTS

5.1. Results of the search

The flowchart of records through the reviewing process is shown in Figure 1. Initially, 259 records were identified. Of these, 21 were identified as duplicates, and 205 more were excluded on the basis of their title and abstract. Finally, four full-text reports were included in the systematic review (Gizani et al., 2016; Koharet al., 2015; Habib, 2016; Benic, 2016).

5.2. Study characteristics

The general characteristics of the studies included in the present systematic review, as well as their sample characteristics, are presented in Tables 1 and 2. All the studies were published between 2015 and 2016, and investigated the effect of oral probiotics on enamel demineralization and gingival inflammation in patients under orthodontic treatment with fixed appliances.

A study by Gizani et al. (2016) assessed the formation of white spot lesions. The duration of the study was 7-24 months (mean±SD: 17±6.8 months) and involved 85 participants undergoing orthodontic treatment (mean age±SD: 15.9 ±3.9 years). The assessment of white spot lesions was made through photographs using the index proposed by Gorelickand co-workers (1982). The children comprising the test group were given lozenges containing two strains of the probiotic bacterium *Lactobacillus reuteri* (DSM 17938 and ATCC PTA 5289; 10^8 bacteria of each strain).

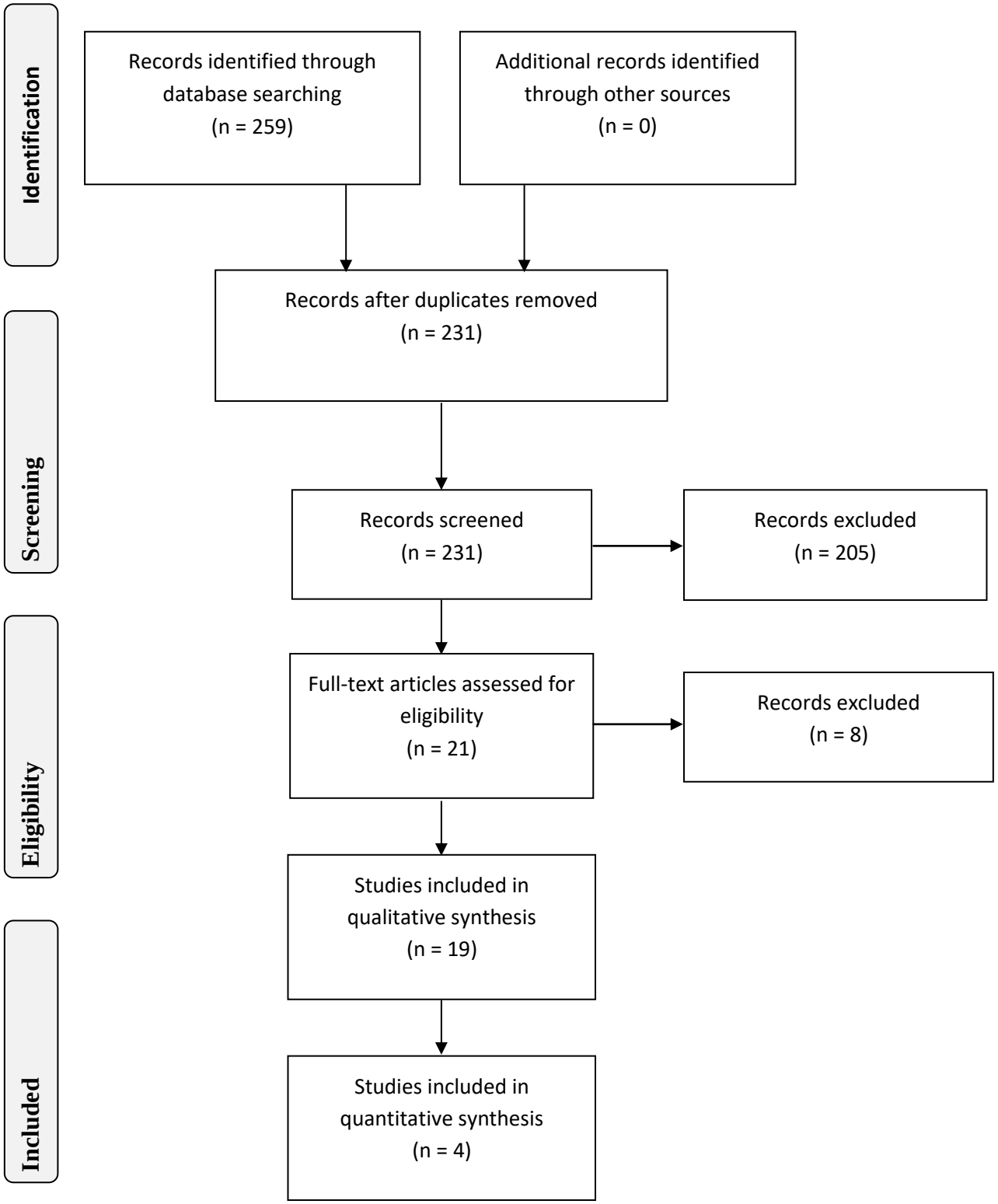

Figure1. Flowchart of records through the reviewing process.

The other three studies focused on gingival inflammation and used the Löe and Silness (1963) Gingival Index (Benic, 2016), the Lobene et al. (1986) modification of the Gingival Index (Habib, 2016) and the Papillary Bleeding Index developed by Saxer and Muhlemann (1975) (Kohar et al., 2015). The ages of the participants varied from children and young adults in two studies (Habib, 2016; Kohar et al., 2015), while Benic (2016) recruited participants of 10 to 30 years old. The duration of the interventions varied from two weeks (Kohar et al., 2015), up to one month of administration of the probiotics (Benic, 2016; Habib, 2016).

Regarding the types of probiotic used, only one trial used a single strain, *Streptococcus salivarius* M18 ($3x10^9$ CFU/lozenge), administered for one month (Benic, 2016). The other two studies used multi-strain probiotics. Habib (2016) reported on the use of a product in the form of lozengescontaining *Streptococcus salivarius* K12, *Lactobacillus paracasei*, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Lactobacillus reuteri* ($3x10^9$ CFU/lozenge) administered for 28 days. Finally, Kohar et al. (2015) selected two strains that were administered for 2 weeks; *Lactobacillus reuteri*in the form of lozenges ($2x10^8$ CFU/lozenge) and *Lactobacillus casei* strain Shirotain the form of fermented milk probiotic drink ($6.5x10^6$/bottle).

Three of the retrieved studies included an assessment of adverse effects (Gizani et al., 2016; Habib, 2016; Benic, 2016).

Table 1. Generalcharacteristics in the studies included.

Study	Intervention characteristics	Outcomes assessed	Others
Benic (2016) University of Otago New Zealand	**EG:** *Streptococcus salivarius* M18 **PG:** Identical lozenges without active bacteria Administration for 1 month	**Gingival inflammation:** Gingival index (Löe and Silness, 1963) **Adverse effects**	**Sample size calculation:** Yes, but not for GI **Reliability of measurements:** Not reported
Gizani et al. (2016) University of Athens Greece	**EG:** *Lactobacillus reuteri DSM 17938* and *Lactobacillus reuteri ATCC PTA 5289* **PG:**Identical lozenges without active bacteria Administration from the time of enrollment until debonding (mean ±SD: 17.0 ±6.8 months)	**Enamel demineralization:** Gorelick et al. (1982) White Spot Lesion Index assessed photographically **Adverse effects**	**Sample size calculation:** Yes **Reliability of measurements:** Yes
Habib (2016) University of Toronto Canada	**EG:** *Streptococcus salivarius* K12, *Lactobacillus paracasei, Lactobacillus plantarum,Lactobacillus acidophilus, Lactobacillus salivarius, Lactobacillus reuteri* **PG:** Lactitol, inulin, dicalcium phosphate, blueberry flavor (natural), dextrose, fructose, stearic acid, citric acid, vanilla flavor (natural), stevia rebaudioside Administration for 4 weeks	**Gingival inflammation:** Modified GI (Lobene et al., 1986) **Adverse effects**	**Sample size calculation:** Yes **Reliability of measurements:** Yes
Kohar et al. (2015) Trisakti University Indonesia	**EG$_1$:** *Lactobacillus reuteri* **EG$_2$:** *Lactobacillus casei* strain Shirota **CG** Administration for 2 weeks	**Gingival inflammation:** Papillary Bleeding Index (Saxer and Muhlemann, 1975)	**Sample size calculation:** Not reported **Reliability of measurements:** Not reported

CG: Control group, without placebo administration; EG: Experimental group; PG: Placebo group

Table 2. Participant characteristics of the studies included.

Study	Inclusion and exclusion criteria	Analyzed sample
Benic (2016) University of Otago New Zealand	**Inclusion Criteria:** Presence of at least 20 natural teeth; stainless steel brackets in both arches. **Exclusion Criteria:** Presence of systemic disease (e.g. diabetes); living in a non-fluoridated area; periodontal disease; antibiotic therapy; wearing lingual braces; using a toothpaste with supplementary antibacterial agents; using a non-fluoride toothpaste; dental fluorosis; smoking; using powered toothbrushes; lactose intolerance; allergy to dairy products; and participants being physically unable to brush.	**Age (range):** 10-30 years **EG:** 32 participants (20 F, 12 M) **PG:** 32 participants (21 F, 11 M) No dropouts occurred
Gizani et al. (2016) University of Athens Greece	**Inclusion Criteria:** Fixed appliances on at least eight maxillary front teeth (incisors, cuspids, and premolars); expected duration of treatment 7–24 months **Exclusion Criteria**: Individuals under treatment with systemic or local antibiotics up to two weeks before starting the study.	**Age (mean ±SD):** 15.9 ±3.9 years **EG:** 42 participants (23 F, 19 M) **PG**: 43 participants (33 F, 10 M) Nine out of 94 patients originally enrolled, were excluded due to technical errors with the follow-up photographs.
Habib (2016) University of Toronto Canada	**Inclusion Criteria:** Age 11 to18 years; mild to moderate gingivitis; understands English; informed consent; fixed appliances on both arches with attachments on at least 20 teeth, for at least five months; complete eruption of teeth #16, 21, 23, 36, 41, 43; Inactive caries before starting treatment; healthy; no use of antimicrobial mouth rinses, probiotics, antibiotics or anti-inflammatory drugs within one month before the trial; undergone standard orthodontic bonding procedure. **Exclusion Criteria:** Inability to make informed consent or communicate fluently in English; allergies or sensitivity to ingredients present in the probiotic complex; Immunocompromised; major underlying medical condition or ENT problem; pregnancy; smoking, alcohol consumers; Oral diseases or conditions; History of surgery within the past 45 days or planned within the next 90 days; use of medications such as; antibiotics, anti-inflammatory, ongoing or recent use of probiotics unrelated to the study one month before starting the study; nausea, fever, vomiting, bloody diarrhea or severe abdominal pain within the past one month; presence of molar bands.	**Age (mean ±SD):**15.69 ± 1.70 years **EG:** 15.75±1.67 **PG:** 15.64 ± 1.75 **EG:** 29 (13 F and 16 M) **PG:** 29 (20 F and 9 M) One participant from each group was lost from the final analysis

CG: Control group, without placebo administration; EG: Experimental group; F: Females; M: Males; PG: Placebo group

Table 2. Participant characteristics of the studies included in the systematic review. [continued]

Study	Inclusion and exclusion criteria	Analyzed sample
Kohar et al. (2015) Trisakti University Indonesia	**Inclusion Criteria:** Healthy individuals, no medication; age range 18-25 years; undergoing fixed orthodontic appliance treatment for at least one year; individuals whom volunteered after verbal and written information. **Exclusion Criteria**: Habitual consumers of xylitol chewing gums and mouthwash; smokers; Pregnancy; use of systemic antibiotic or topical fluoride treatment.	**Age (range):**18-25 years **EG_1:** 10 participants **EG_2:** 10 participants **CG:** 10 participants No dropouts occurred

CG: Control group, without placebo administration; EG: Experimental group; F: Females; M: Males; PG: Placebo group

5.3. Results of risk of bias assessment

The results regarding risk of bias assessment can be found in Table 3. One study was assessed to be of low risk (Benic, 2016) and three of unclear risk of bias (Gizani et al., 2016; Kohar et al., 2015; Habib, 2016).

Table3. Summary of the risk of bias assessment (Domains examined: 1: Random sequence generation 2: Allocation concealment, 3: Blinding of participants and personnel, 4: Blinding of outcome assessment, 5: Incomplete outcome data, 6: Selective outcome reporting, 7: Other potential threats to validity).

Domain	Benic, 2016	Gizani et al., 2016	Habib, 2016	Kohar et al., 2015
1	Low	Low	Low	Unclear
2	Low	Unclear	Unclear	Unclear
3	Low	Low	Low	Unclear
4	Low	Low	Low	Unclear
5	Low	Low	Low	Low
6	Low	Low	Low	Low
7	Unclear	Unclear	Unclear	Unclear
Summary	**Low**	**Unclear**	**Unclear**	**Unclear**

5.4 Results of individual studies and synthesis of results

The results of the studies included in the present review are presented below.

5.4.1. Enamel demineralization development

Only one study investigated the formation and development of white spot lesion in orthodontic patients after probiotic administration (Gizani et al., 2015). There was no statistically significantdifference between the groups. At debonding, no new lesions

were found in 22 out of 42 patients in the probiotic group, whereas in the placebo group in 26 outof 43 individuals. The mean number of new white spot lesions was 2.2 (SD 3.2) lesions in the experimental group and 1.7 (SD 2.5) in the placebo group.

5.4.2. Gingival inflammation development

The three studies evaluating gingival inflammation after probiotic use that varied from to two week upto four weeks did not show any statistically significant differences between the experimental and the control groups.In the Benic (2016) study, no significant differences between the two groups were noted regarding Gingival Index measurements (Löe and Silness, 1963) (p=0.867). The same was observed by Habib (2016) that assessed gingival inflammation with the Lobene et al. (1986) modification of the Gingival Index (p=0.797), as well as Kohar et al. (2015) that used the Papillary Bleeding Index by Saxer and Muhlemann (1975) (p=0.053).

5.4.3. Adverse effects

Noadverse effects were noted in any of the studies included in the present systematic review. Gizani et al. (2016) reported that 8participantscould not tolerate the taste of the lozenges. In the study of Habib (2016), one participant reported gastrointestinal pain and diarrhea after two weeks of taking the lozenges on a daily basis, but it was later shown that this individual belonged to the placebo group.

6. DISCUSSION

6.1. Summary of evidence

Patients undergoing orthodontic treatment are linked with a significant biofilm accumulation, thus exposing them to a higher risk of developing enamel demineralization and inflammation of the periodontal tissues (Graber et al., 2004; Justus, 2015; Miller and Hobson, 1961; Katz, 1978; Cozzani et al., 2016; Al-Jewair et al., 2011). Nowadays, with the increased use of probiotics, several reports have investigated their effects in enhancing oral health in the general dental population (Gruner et al., 2016; Jayaram et al., 2016). However, studies solely targeting patients undergoing orthodontic fixed appliances have not beenpreviously reviewed in an evidence-based manner. Based on the information retrieved in the present review, short-term probiotic administration does not seem to have an effect on gingival inflammation and enamel decalcification development in patients under treatment with fixed appliances. The three studies evaluating gingival inflammation after probiotic use for up to one month did not show any statistically significant differences between the two groups. The only study investigating enamel decalcification for a mean duration of 17 months of probiotic use, also failed to demonstrate statistically significant differences in the incidence of white spot lesions between the groups at debonding. Consequently, the null hypothesis was not rejected. Due to extensive clnical heterogeneity subgroups analyses could not be carried outAlso, no markedadverse effects were noted in any of the studies included in the present systematic review.

Numerous *in vitro* studies have shown the potential beneficial effects of various probiotic strains on oral pathogens (Chuang et al., 2011; Lee et al., 2011; Haukioja et al., 2008; Hedberg et al., 2008). Two recent systematic reviews aimed to determine the effects of probiotics on various periodontal health parameters (Gruner et al., 2016;

Jayaram et al., 2016). Insufficient data were found for recommending probiotics for the management of dental caries (Gruner et al., 2016). While a growing number of studies supportedthe useof probiotic therapy to prevent or treat gingivitis and periodontitis, there were also conflicting findings (Gruner et al., 2016; Jayaram et al., 2016). The non-significant results found in the studies included in the current systematic review might be attributed to various causes, including: the use of inappropriate and ineffective bacterial strains, ineffective concentrations of bacteria and administration methods,ineffectiveness of the selected probiotic strain to colonize the oral cavity or the inability of the strain to compete with the bacteria and biofilm accumulation present in the oral cavity, in addition to the short duration of administration of oral probiotics.

Up to the present time, no consensus has been reached about what bacteria strain is most appropriate and effective. Some reports have found that the *Lactobacillus* specieshave shown positive effects in the treatment of periodontal diseases, including *Lactobacillus reuteri* (DSM17938, ATCC PTA 5289 and TCC 55730,221-223), *Lactobacillusparacasei* (224), *Lactobacillussalivarius* (TI 2711183 and WB21), *Lactobacillus plantarum* and *Lactobacillus rhamnosus* (Lozo et al., 2004; Shimauch et al., 2008; Mayanagi et al., 2009; Simark-Mattsson et al., 2007; Ahmed et al., 2014; Contreras et al., 2017). *Lactobacillus brevis*has also been suggested to be potentially beneficial in view of its proven anti-inflammatory properties (Ricci et al., 2007Bhardwaj and Bhardwaj, 2012). *Bifidobacterium* is another species that has been found to exert a positive impact on periodontal disease (Hojo et al., 2007). Up to our knowledge, the *Streptococcus salivarius* K12 and M18 strainsused in two of the studies included in the present review, had never been previously assessed for their effects in treating gingivitis (Habib, 2016; Benic, 2016).

In principle, probiotics products including many strains could possess synergistic and

symbiotic properties because of the interactions of each strain with each other. However, some very limited data suggests that probiotic strains may also exhibit inhibitory properties against each other. For instance, the production of hydrogen peroxide and bacteriocin-like substances may induce the desired effect when inhibiting endogenous strains such as *Streptococcus mutans*, while, simultaneously they might also disable other probiotic strain in the same formulation, thus reducing its effectiveness (Kailasapathy et al., 2000).

The concentrations required for producing the optimal effects of oral probiotics have not been widely investigated. It is important to be certain about the exact dose required to initiate a dose-response reaction during the administration of probiotics. In the field of medicine, the industry standard for the counts of viable bacteria should range from $1x10^6$ to $1x10^9$ CFU (Imran et al., 2005). However, when using oral probiotics, itis logical to assume that a lower dose or concentration would be required, since it does not have to pass through the gastrointestinal system. The vast majority of probiotic studies evaluating various oral health parameters have used concentrations of $1x10^8$ CFU. Moreover, it is important to remember that each individual strain has a different potential for oral colonization. All doses shouldbe selected according to the specific strain used.

In addition, the administration method may also modulate the effect of a probiotic product. Various vehicles for oral probiotics have been employed, including chewing gums, lozenges, tablets, oil drops and drinks (Gruner et al., 2016; Jayaram et al., 2016). It has been suggested, for example, that the use of vehicles derived from milk that contain calcium, could potentially increase the anti-cariogenic effect. Milk derived products produce also ammonia that helps increase pH and delay biofilm formation, as well as preventing the adhesion of the bacteria to the tooth surface (Cochrane et al.,

2010; Li et al., 2014; Contreras et al., 2017).

Furthermore, for a probiotic strain to be effective, it must first be able toadhere to, and colonize, the surfaces of the oral cavity (Jivraj, 2015). These processes could be compromised in the cases of a mature biofilm that is difficult to penetrate, orin the existence of an oral pH that is not compatible with bacterial viability (Sookkhee et al., 2001). Moreover, the capacity of a probiotic strain to colonize might vary between members of the same species, as it has been demonstrated for *Lactobacilli* (Krasse et al., 2005; Çaglar el al., 2009; Yli-Knuuttila et al., 2006).

Finally, there is the possibility that the administered strains are unable to compete with the quantity of the bacteria and plaque accumulation present in the oral cavity, as is possibly the case with orthodontic patients. In such cases, higher concentrations of probiotics or administration for a longer duration may be required to demonstrate any potential to produce aclinical improvement.

Apart from factors associated with specific probiotic characteristics or the mode of administration, other parameters that could affect the observed results primarily include patients' compliance. Although compliance was found to vary from good to excellent, it should not be overlooked that assessment was performed on the basis of self-report, using tracking calendars (Gizani et al., 2016; Benic, 2016; Habib, 2016). Finally, the diet of participants during the interventions, the potential use of antibacterial or antiseptic products, changes in brushing/flossing technique and swallowing or chewing the lozenge rather than sucking it, thereby washing-out the probiotic from the mouth, could have affected the reported changes.

6.2. Strengths and limitations

The strengths of the present review include the use of well-established guidelines. As far as we can know, until now, there has been no other systematic review conducted on the possible effectiveness of probiotics on different clinical parameters in patients undergoing orthodontic treatment.

The search strategy utilized in this present review covered all electronic, manual, and gray literature material up to August 31st 2018, and was comprehensive, including every available study, irrespective of language, date, and status of publication. Every effort to decrease bias in the methodology employed was made. Screening, verification of eligibility, abstraction of information, assessment of risk of bias and of the quality of evidence were all performed in duplicate, and any disagreement was resolved by discussion or consultation until a final consensus was achieved.

There are also some limitations to the present review, arising mainly from the nature and the characteristics of the data retrieved during the review process. Furthermore, exploratory subgroup analyses and analyses for "small-study effects" and publication bias (Higgins and Green, 2011), could not be carried out, even though they were incorporated as possibilities according to the review protocol. Finally, the short duration of most interventions and the use of specific strains, concentrations, dose regimens or modes of administration, may have confounded the results of the included studies.

6.3. Recommendation of future studies

The employment of probiotics for oral health has been widely accepted and used by the general population by virtue of their natural source. However, further research is needed in order to optimize probiotic use and quantify the extent of clinical benefit. In order to take full advantage of using oral probiotics, a more completeunderstanding regarding their mechanism of action in the areaof adhesion and colonization and the capabilities of the all different strains is required. Although nowadays, more and more research is being focused on the use of probiotics, the literature is still unable to arrive at a consensus on the optimum duration requiredor theideal concentration or dose regimen and mode of administration for each probiotic strain. It is essential to understandtheefficacy of each strain, both when used alone, just as it is important to evaluate the potential synergistic effects of combining probiotic strains into a single entity.

As orthodontic patients require continuous and rigorous oral hygiene control, caries prevention and maintenance of gingival health, more high-quality studies, involving different combinations of probiotic strains and of longer durations of intervention and follow-up are warranted. Moreover, instead of testing the use of probiotics to combat established gingivitis, research could be conducted on the possibility of preventing gingivitis by using probiotics prior to the bonding oforthodontic brackets. Although much is knownabout probiotics in the gastrointestinal field, there is a great deal of knowledge tobe learned pertaining to probiotics for oral health.

7. CONCLUSION

Overall, short-term probiotic administration does not seem to have an effect on the gingival inflammation and enamel decalcification development in patients under treatment with fixed orthodontic appliances. High quality studies involving different combinations of probiotic strains and of longer duration of intervention and follow-up are required.

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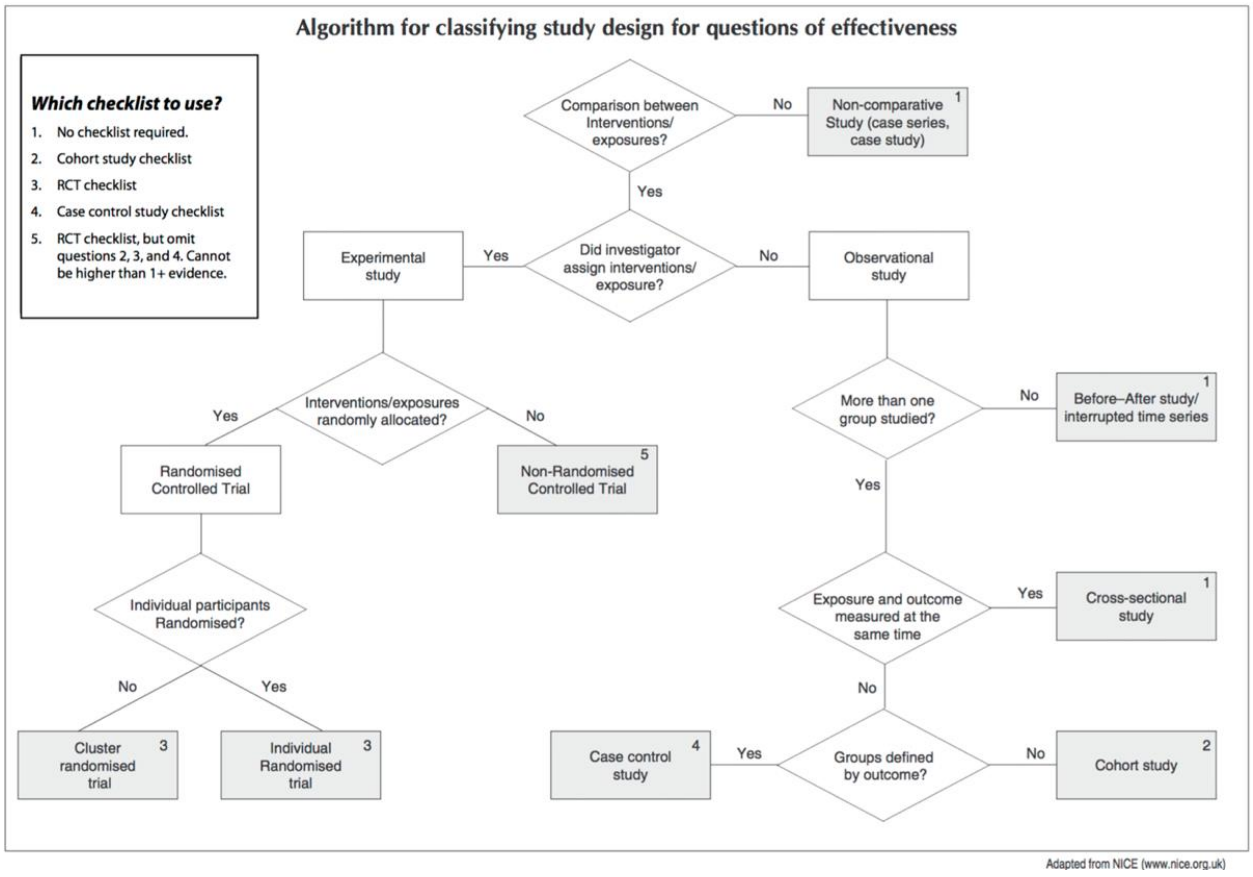
9. Appendices

Appendix I. Scottish Intercollegiate Guidelines Network (SIGN) algorithm for classifying study design for questions of effectiveness.



Appendix II. Strategy for database search (up to August 31st, 2018).

Database	Search strategy	Hits
PubMed http://www.ncbi.nlm.nih.gov/pubmed	((Probiot*) OR (Lactobacillus acidophilus) OR (ATCC 4356) OR (Bifidobacterium bifidum) OR (ATCC 29521) OR (Lactobacillus rhamnosus) OR SP1 OR (Streptococcus salivarius) OR (Lactobacillus plantarum) OR (Lactobacillus paracasei) OR (Lactobacillus reuteri) OR (Streptococcus uberis) OR (Streptococcus oralis) OR (Streptococcus rattus) OR (Bifidobacterium animalis)) AND ("fixed appliance" OR orthodon* OR "fixed orthodontic" OR bracket* OR multibracket)	**108**
Cochrane Central Register of Controlled Trials http://onlinelibrary.wiley.com/cochranelibrary/search	((Probiot*) OR (Lactobacillus acidophilus) OR (ATCC 4356) OR (Bifidobacterium bifidum) OR (ATCC 29521) OR (Lactobacillus rhamnosus) OR SP1 OR (Streptococcus salivarius) OR (Lactobacillus plantarum) OR (Lactobacillus paracasei) OR (Lactobacillus reuteri) OR (Streptococcus uberis) OR (Streptococcus oralis) OR (Streptococcus rattus) OR (Bifidobacterium animalis)) AND ("fixed appliance" OR orthodon* OR "fixed orthodontic" OR bracket* OR multibracket) in Title Abstract Keyword - (Word variations have been searched)	**20**
Cochrane Database of Systematic Reviews http://onlinelibrary.wiley.com/cochranelibrary/search	((Probiot*) OR (Lactobacillus acidophilus) OR (ATCC 4356) OR (Bifidobacterium bifidum) OR (ATCC 29521) OR (Lactobacillus rhamnosus) OR SP1 OR (Streptococcus salivarius) OR (Lactobacillus plantarum) OR (Lactobacillus paracasei) OR (Lactobacillus reuteri) OR (Streptococcus uberis) OR (Streptococcus oralis) OR (Streptococcus rattus) OR (Bifidobacterium animalis)) AND ("fixed appliance" OR orthodon* OR "fixed orthodontic" OR bracket* OR multibracket) in Title Abstract Keyword - (Word variations have been searched)	**0**
Scopus https://www.scopus.com/search/form.url?zone=TopNavBar&origin=searchbasic	TITLE-ABS-KEY ((Probiot*) OR (Lactobacillus acidophilus) OR (ATCC 4356) OR (Bifidobacterium bifidum) OR (ATCC 29521) OR (Lactobacillus rhamnosus) OR SP1 OR (Streptococcus salivarius) OR (Lactobacillus plantarum) OR (Lactobacillus paracasei) OR (Lactobacillus reuteri) OR (Streptococcus uberis) OR (Streptococcus oralis) OR (Streptococcus rattus) OR (Bifidobacterium animalis)) AND ("fixed appliance" OR orthodon* OR "fixed orthodontic" OR bracket* OR multibracket) AND (LIMIT-TO (SUBJAREA,"DENT"))	**170**
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