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Efficacy of Brushing with Probiotics for the Reduction of Gingivitis

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Efficacy of Brushing with Probiotics for the Reduction of Gingivitis

A Thesis

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Degree of Masters of Science

in

Dental Hygiene

in the

College of Graduate Studies

Eastern Washington University

by

Cheri L. Barton RDH, BS

Spring 2018

Major Professor: Lisa Bilich RDH, MSEd CHSE

EFFICACY OF BRUSHING WITH PROBIOTICS ii

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EFFICACY OF BRUSHING WITH PROBIOTICS iii

MASTER'S THESIS

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Date 4/26/2018

Human Subjects Approvals

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RG

TO: Cheri L. Barton, Department of Dental Hygiene

FROM: Ruth A. Galm, Human Protections Administrator

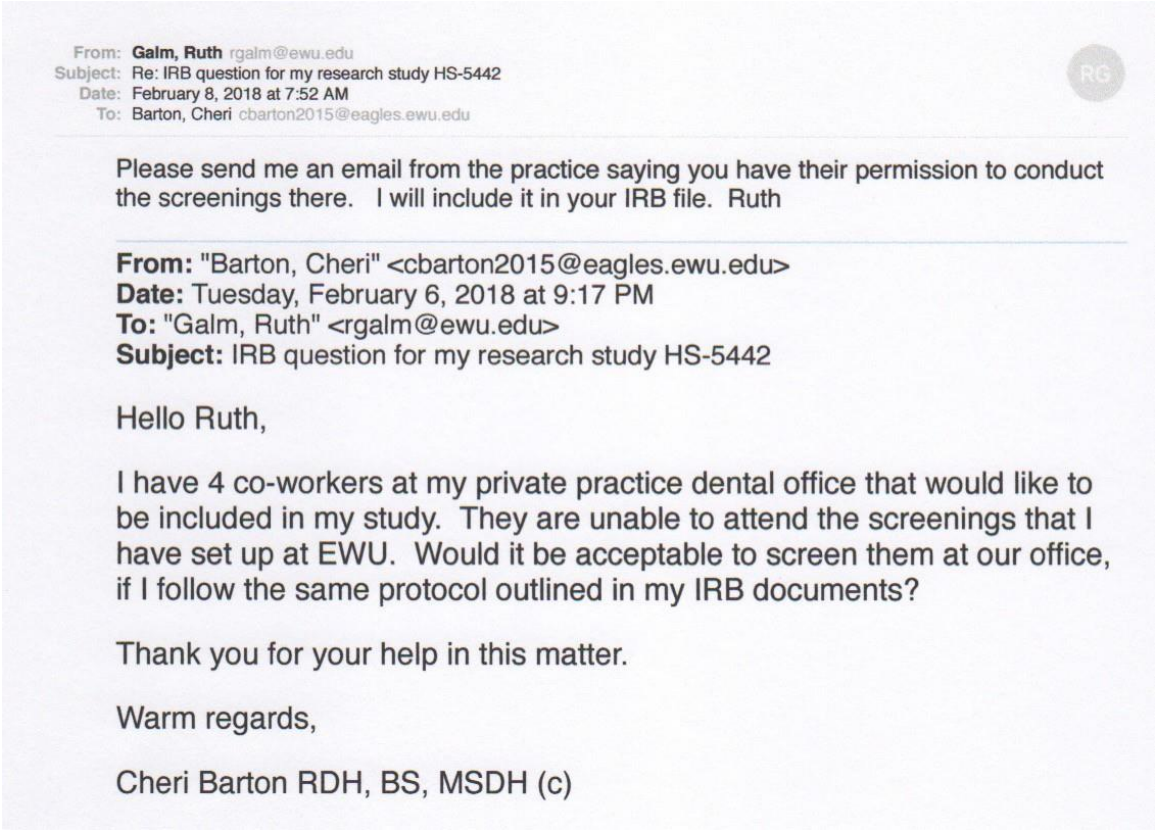
DATE: January 22, 2018

SUBJECT: Efficacy of Brushing with Probiotics for the Reduction of Gingivitis (HS-5442)

With the amendments provided on January 18 and 21, 2018, human subjects protocol HS-5442 entitled "Efficacy of Brushing with Probiotics for the Reduction of Gingivitis" has been approved by an expedited IRB review.

Student research qualifying for an expedited IRB review is valid for a period of one year. If subsequent to initial approval, the research protocol requires minor changes, the Office of Grant and Research Development should be notified of those changes. Any major departure from the original proposal must be reviewed through a Change of Protocol application submitted to the IRB before the protocol may be altered. Please refer to HS-5442 on future correspondence as appropriate as we file everything under this number.

Cc: HS-5442 file
Prof. Lisa Bilich, RPI
Prof. Ann O'Kelley Wetmore, Dept. Chair
Graduate Office



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Date: February 8, 2018 at 7:52 AM
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Please send me an email from the practice saying you have their permission to conduct the screenings there. I will include it in your IRB file. Ruth

---

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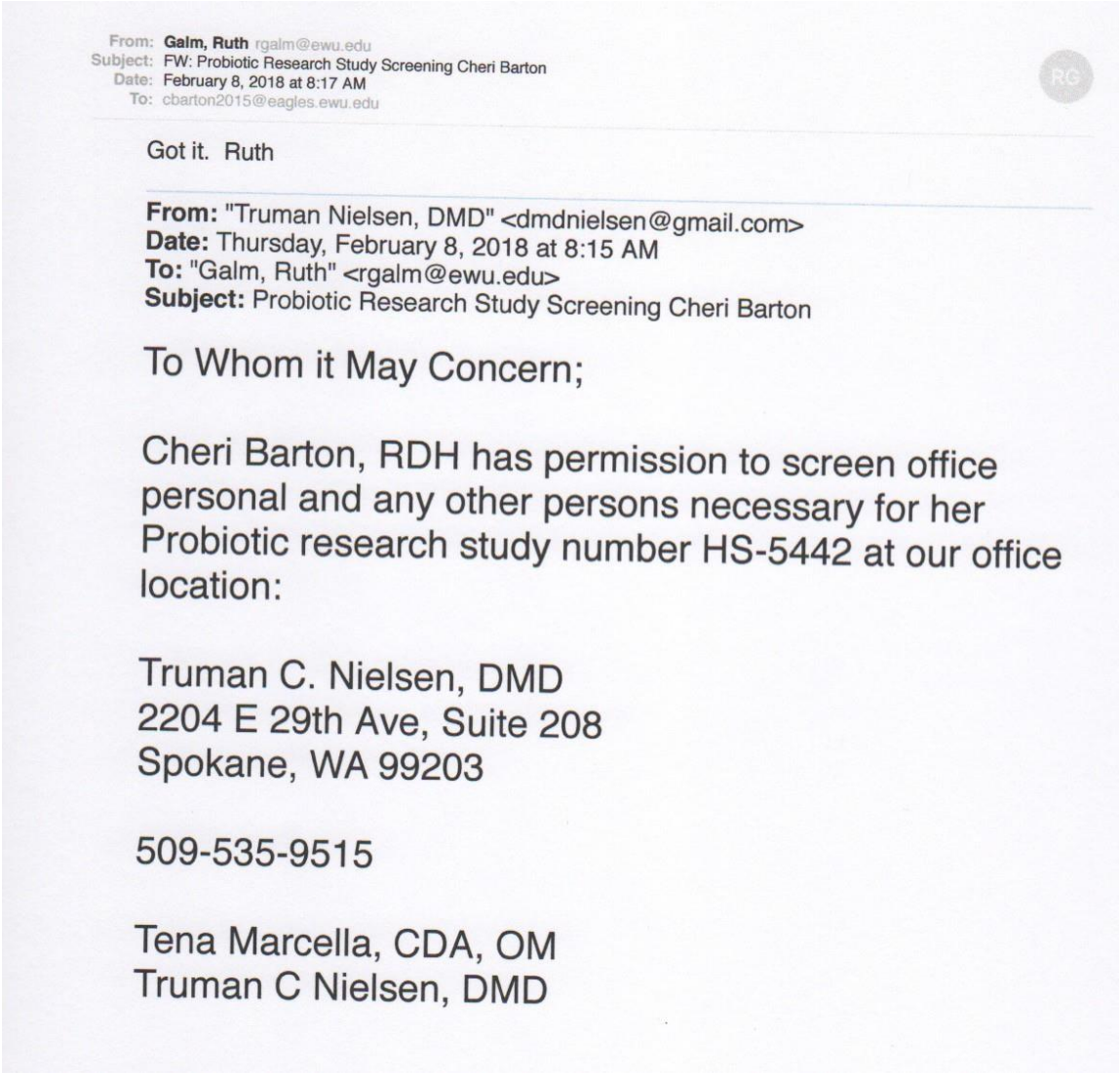
Hello Ruth,

I have 4 co-workers at my private practice dental office that would like to be included in my study. They are unable to attend the screenings that I have set up at EWU. Would it be acceptable to screen them at our office, if I follow the same protocol outlined in my IRB documents?

Thank you for your help in this matter.

Warm regards,

Cheri Barton RDH, BS, MSDH (c)



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Got it. Ruth

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Cheri Barton, RDH has permission to screen office personal and any other persons necessary for her Probiotic research study number HS-5442 at our office location:

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Abstract

Purpose: The primary aim of this study was to evaluate the efficacy of brushing with the probiotic Lactobacillus *reuteri* on plaque accumulation and gingival inflammation. This study included 34 healthy adult subjects, ages 18-65, exhibiting gingivitis. Participants were asked to participate in a randomized, double-blind, placebo controlled study testing whether brushing with L. *reuteri* probiotic drops added to toothpaste reduced the clinical parameters of gingivitis more than brushing with a placebo drop added to toothpaste.

Methods: Biological measurements of plaque accumulation were recorded using disclosing solution and an O'Leary Plaque Score (PS), and gingival inflammation was recorded using a modified Löe-Silness Gingival Index (GI). Each participant was randomly divided into one of two groups: group A or group B followed by baseline data collection of clinical parameters. Participants were assigned their study drops to add to their study toothpaste.

Results: Analysis included both descriptive and inferential statistics. Inferential statistics employed Wilcoxon Signed Rank tests, due to the small sample size and inability to assume normal distribution of the study population. Statistical significance level was set at $p < 0.05$. Variables included median differences between the probiotic and placebo groups' baseline and final data for both PS and GI. Comparisons were also made by age and gender, and to evaluate the difference between final PS for placebo versus probiotic and final GI for placebo versus probiotic. Statistically significant differences were

noticed between baseline and final data for GI of the placebo group as a whole (p = 0.001), and females of the placebo group (p = 0.004). No statistical difference was noted in PS between baseline and final data for either group.

Conclusion: Although results of this clinical study were unexpected, further research regarding the use of probiotics as a natural, healthy alternative therapy to antibiotics for the treatment and management of oral diseases should continue to be evaluated. Several research studies reviewed in the process of completing this thesis have shown probiotics have great possibilities in the oral cavity. Most researchers agree the type, application method, and quantity of probiotic needed to be effective at treating and managing oral diseases has yet to be determined.

Acknowledgements

I would like to begin by expressing my gratitude to Rebecca Stolberg RDH, MSDH, former chair of the Dental Hygiene Department at EWU. She put the idea of attaining a master's degree into my head, and encouraged me to apply and follow through with my application when I had reservations regarding my age and length of time since I had been in college. Without her encouragement, I would not be here today. I would like to thank the Dental Hygiene Department at EWU as a whole, including all faculty, staff, and students for allowing me access to the clinic to run my research study, and the many individuals who helped with data collection, sterilization, disinfection and all facets of this research study. I would like to acknowledge my thesis chair, Lisa Bilich RDH, BS, MEd, for her help implementing my research study. She stayed late, after normal school hours, to screen participants and collect data, and spent many hours reading and revising my thesis drafts. I appreciate you and all your work toward this project. I wish to thank my second and third thesis committee members Sarah Jackson RDH, MSDH and Dr. Lisa J. Woodard, PharmD, MPH for your thoughtful observations and input toward this thesis. I would also like to thank the current chair of the Dental Hygiene Department at EWU, Ann O'Kelley Wetmore, RDH, MSDH for your mentorship throughout my MSDH journey. Lastly, I would like to thank my partner in life, Scott Hammond MEd, for countless hours of emotional support, advice, and encouragement. Many others helped me along the way in my journey, and even if you are not mentioned, you are not forgotten.

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Introduction/Literature Review

Introduction to the Research Question

Probiotics are defined by the Food and Agricultural Organization (FAO) of the World Health Organization (WHO) as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Venugopalan, Shriner, & Wong-Beringer, 2010, p. 1661). Use of probiotics can be traced back in time to dates before microbes were even discovered. Pictures of Egyptian hieroglyphics depicting fermented milk products have been uncovered, and it is known Tibetan nomads used fermented yak milk to preserve their milk during long journeys (McFarland, 2015). Subcategories of probiotics include foods, food ingredients, and supplements containing live microorganisms (Venugopalan et al., 2010, p. 1661). Probiotics come in many forms, such as tablets or pills that are ingested, and often they are naturally occurring in common foods we eat daily. Sometimes they are added by food manufacturers to enhance the health claims of their products. Examples of probiotic foods and beverages include yogurt, Kefir, fermented milk products and cheese, buttermilk, miso, kimchi, sauerkraut, and many other foods and beverages.

Since the early twentieth century, beneficial micro-organisms have been used to support immune function and prevent and manage health problems. These useful bacteria, named probiotics, derived from the Greek term meaning "for life," have been proven effective in controlling chronic gastrointestinal diseases such as Crohn's disease, ulcerative colitis, and other irritable bowel disorders (Anusha, Umar, Basheer & Baroudi, 2015; Isolauri, 2001). In their systematic review on probiotics, Vuotto, Longo, and

Donelli, (2014), discuss research studies that demonstrate probiotics are effective in the treatment or prevention of acute viral gastroenteritis, pediatric post-antibiotic-associated diarrhea, certain pediatric allergic disorders, necrotizing enterocolitis in preterm infants, inflammatory bowel diseases, and post-surgical pouchitis (Vuotto et al., 2014).

With the growing concern regarding overuse of antibiotics, and the emergence of multi-resistant strains of bacteria, scientists have looked back through history to review methods for fighting diseases. This retrospection brought to light the forgotten concept of using bacteria beneficial to health to combat infectious diseases (Meurman, 2005). Similarities between the types of microbiota living in the gastrointestinal tract and oral cavity of healthy people led investigators to consider whether the beneficial effects exhibited by probiotics in the gastrointestinal tract would show comparable results in the oral cavity (Pandey, Berwal, Solanki, & Malik, 2015). Current research on probiotics in the oral cavity utilizing several different types of microbes, and methods of application, have yielded mixed results (Gupta & Gupta, 2010).

Theories for inconsistent study results have been attributed to variances in the mechanism of action of different probiotics. Known mechanisms of action include immune modulation, production of anti-microbial substances, competitive exclusion theory, hindrance of adhesion of pathogenic bacteria, and competition for nutrients (Teughels, Loozen, & Quirynen, 2011). However, most studies executed to date indicate a need for additional research to determine the efficacy of application methods employed, and mechanism of action of probiotics in the oral cavity. This study attempted to determine the efficacy of brushing with the probiotic L. *reuteri*, as an application method by evaluating the results on the common oral disease, gingivitis.

Statement of Problem

Using probiotics for the treatment of gastrointestinal ailments is well documented, demonstrating their health promoting potential (Haukioja, 2010), and good benefit-to-risk ratio (Krasse et al. 2005). Probiotics have been proven to be an effective approach to reducing symptoms related to gastrointestinal diseases (Isolauri, 2001). Research studies seeking to examine the effects of probiotics on gastrointestinal diseases have evaluated many types of bacteria. Although several different probiotic strains have been found to be beneficial, probiotics with the ability to adhere to mucus and epithelial cells have been proposed as one of the most important selection criteria for potential probiotic strains (Vuotto et al., 2014).

In the oral cavity, beneficial and pathogenic bacteria maintain a delicate balance. The accumulation of anaerobic gram-negative bacteria within the dental biofilm predisposes otherwise healthy individuals to periodontal diseases by transforming dental plaque into a difficult to treat pathogenic biofilm (Vuotto et al., 2014). Therefore, "oral cavities have been suggested as a relevant target for probiotic applications, through the use of non-pathogenic, bacteriocin-producing *Lactobacilli* and *Bifidobacteria* to restore the microbial balance and to counteract pathogenic bacteria" (Vuotto et al., 2014, p. 189). L. *reuteri* is often chosen for studies due to its historically recorded safety, and ability to produce two bacteriocin, reuterin and reutericyclin (Haukioja, 2010; Raff & Hunt, 2012).

Bacteriocin are antibacterial substances produced by certain bacteria, that kill or inhibit the growth of closely related bacteria species or other strains of the same bacteria (The American Heritage® Medical Dictionary, 2007). Reuterin and reutericyclin have the ability to inhibit the growth of several different pathogens by adhering to host tissues,

resulting in competition for pathogenic bacteria, and reducing the secretion of proinflammatory cytokines (Gupta, 2011). Cytokines are proteins secreted by cells that effect communication and interaction between cells. Pro-inflammatory cytokins are released as part of the bodies immune system. They are produced by cells during cell injury, infection, invasion, and inflammation, and have been shown to be the cause of injury related pain (Zhang & An, 2007). Given the proven benefit of probiotics, and similarities in microbiota between the gastrointestinal tract and the oral cavity, probiotics may be effective at reducing oral diseases.

Previous application methods to introduce probiotics into the mouth have yielded inconsistent results (Hallstrom et al., 2013; Harini & Anegundi, 2010; Iniesta et al., 2012; Karuppaiah et al., 2013). Various methods of application have been studied, however, limited studies exist that have evaluated brushing with probiotics as a means of direct application to the oral tissues (Hallstrom et al., 2013; Harini et al., 2010; Iniesta et al., 2012; Karuppaiah et al., 2013). The efficacy of brushing has been determined as an effective treatment for reducing gingivitis (Lang et al., 1973; Slot, Wiggelinkhuizen, Rosema, & Van der Weijden, 2012; Sowinski et al., 2008). Based on this information, it appears there is a gap in the research regarding the efficacy of brushing with probiotics, as this practice has yet to be thoroughly evaluated. In order to address this deficiency, this study sought to answer the following question:

- Will brushing with toothpaste and L. *reuteri* probiotic drops reduce clinical parameters of gingivitis in healthy adults in comparison to brushing with toothpaste and placebo drops?

Overview of Research

Gingivitis is a common chronic disease affecting the crevice (sulcus) surrounding the teeth of humans and animals, and limited to the gingiva. It is caused by an accumulation of bacteria in the form of dental plaque, also known as bacterial biofilm, around these crevices, resulting in an inflammatory response from the body's immune system (Krasse, et al. 2005) (Fig. 1).

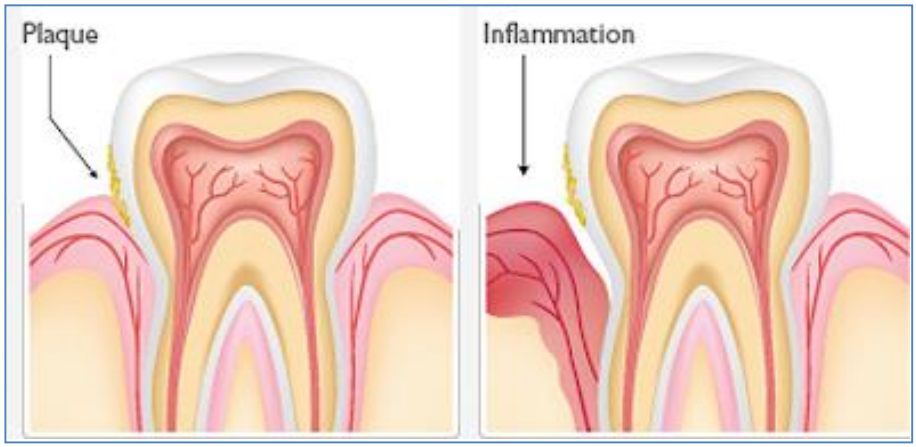

Figure 1. Gingivitis

Clinical assessments often utilized to measure gingivitis include PS, GI, periodontal probing depths (PD), and bleeding on probing (BOP) (Amižić et al., 2016; Hallstrom et al., 2013; Harini et al., 2010; Iniesta et al., 2012; Karuppaiah et al., 2013; Krasse et., al. 2005; Slawik et al., 2011; Twetman et al., 2009). Initial treatment for gingivitis usually incorporates brushing instruction to improve a patient's oral hygiene (Krasse et. al., 2005). However, clinical studies show gingivitis is a recurrent infection, despite earlier successful treatment (Krasse et al., 2005). In a study by Lang et al. (1973), they discuss information gained from previous research performed by Löe, Theilade & Jensen (1964) entitled *Experimental Gingivitis in Man*, noting "It has been shown that if oral hygiene procedures are suspended, localized gingivitis develops in only 4-11 days and generalized

gingivitis within 2-3 weeks. On the other hand, resumption of oral hygiene procedures has resulted in a dramatic improvement in gingival health" (Lang et al., 1973, p. 396).

Due to the documented beneficial effects of probiotics on inflammatory gastrointestinal diseases, and the similarities of the oral and gastrointestinal microbiota, scientist are searching for ways to test whether probiotics would be beneficial in reducing clinical and systemic parameters associated with oral inflammatory diseases. Research designs evaluating the use of probiotics in the oral cavity vary greatly. Methodology for these studies has included systemic, topical, and site specific evaluation of probiotics on the oral environment (Amižić et al., 2016; Hallstrom et al., 2013; Harini et al., 2010; Iniesta et al., 2012; Karuppaiah et al., 2013; Krasse et., al. 2005, Shimauchi, 2008; Slawik et al., 2011, Teughels et al., 2011; Teughels et al., 2013; Twetman et al., 2009).

Systemic Introduction of Probiotics

Systemic introduction through ingestion of probiotics is one of the ways probiotics have been tested for their therapeutic benefit to oral health. This method involves the consumption of probiotics through food and tablets. Evaluative methods of this design have included saliva samples, subgingival plaque samples, PS, PD, BOP, and GI (Iniesta et al., 2012; Karuppaiah et al., 2013; Slawik et al., 2011). These research designs showed some promising results, however, constraints exist with this method, such as limited contact time to oral tissues, and degradation of probiotics due to stomach acid (Lawande, 2012).

Slawik et al. (2011), determined the effects of a probiotic milk drink on clinical inflammatory parameters of the oral gingiva. The bacteria contained in the milk drink was Lactobacillus *casei* Shirota strains with concentrations of $1x10^8$ colony forming

bacterial units (CFU) per ml, and each milk drink was 65 ml. This 28-day study included healthy adults (N = 28) age 20 to 33 (16 females, 12 males), non-smokers, with no clinical signs of gingival inflammation, probing pocket depths 3mm or less, attachment loss 2 mm or less, and a GI of zero at baseline. A test group (n = 11), and a control group (n = 17), were utilized for comparison. Evaluation methods of PS, GI, gingival crevicular fluid (GCF), and bleeding on probing (BOP) were collected. The study began with a 14-day non-brushing period to induce gingivitis, and evaluated six teeth on the right side of the maxilla. Researchers concluded the data based on a controlled experimental setting indicate an anti-inflammatory effect of the tested probiotic milk drink. The inflammatory parameters of BOP and GCF were significantly lower for the test group compared to the control group (p = 0.005). There was an increase in plaque accumulation indicated by an increased PS, which was attributed to the high amount of carbohydrate in the probiotic milk drink (Slawik et al., 2011).

In contrast to Slawik et al. (2011), Karuppaiah et al. (2013) demonstrated reduced levels of plaque when using probiotics compared to a control group. The aim of this study was to evaluate the efficacy of probiotics in plaque reduction among school children. This study consisted of healthy children age 14 to 17 (N = 216), and participants were divided into two groups. For 30 days, the test group (group A, n = 108) included curd in their diet, and the control group (group B, n = 108) excluded curd from their diet. Curd is derived from the fermentation of milk, and contains probiotics similar to yogurt (India Parenting, 2017). At the conclusion of this study, Karuppaiah et al., (2013) found both groups had a decrease in GI from the baseline, this was attributed to the prophylaxis prior to the study. Prophylaxis is defined by the American Dental

Association (ADA) as the professional removal of plaque, calculus and stains from the tooth structures in the permanent and transitional dentition. It is intended to control local irritational factors (ADA, 2017). This study did find a significant difference in PS ($p < 0.001$) in regard to the curd group (group A), showing an overall decrease in plaque accumulation as compared to the control group. Limitations for this study include the short period of the study trial. Examiners concluded the reduction in PS and GI of the probiotic group suggests probiotics may have reduced gingival disease, however further long term studies are needed to confirm this implied benefit (Karuppaiah et al., 2013).

Iniesta et al. (2012) found no significant difference in PS and GI with people who consumed tablets containing L. *reuteri* (Iniesta et al., 2012). This study ($N = 40$) utilized a double-blind, prospective, placebo-controlled, crossover randomized clinical trial with two test periods of four weeks, and an intermediate washout period of four weeks. Subjects in this study consumed one tablet per day for 28 days of either the placebo (control group, $n = 20$), or L. *reuteri* (test group, $n = 20$) containing tablets with 2×10^8 CFU per tablet. Data was taken at baseline, week four, and week eight to evaluate saliva, subgingival plaque, PS and GI. Collection of microbiological samples to evaluate colonization patterns of L. *reuteri* were collected every two weeks for fourteen weeks, and analyzed using standard qualitative polymerase chain reaction (PCR) analyses. Results of the research showed colonization of the saliva and subgingival habitat of some gingivitis patients was possible to achieve by consuming L. *reuteri* containing tablets. A discussion on the lack of clinical significance stated possible reasons for this could have been the short duration of the study, or the sample population, being primarily female dental students with above average oral hygiene, making it difficult to determine any

significant change (Iniesta et. al., 2012).

Vicario et al. (2013) used a double-blind placebo-controlled, randomized clinical trial over a 30-day test period to evaluate the clinical effects of L. *reuteri* Prodentis® in the treatment of initial to moderate periodontitis. Clinical parameters assessed PS, PD, BOP, and data was gathered at the initial onset of the study and the end of the study. Participants included healthy adults ($N = 19$), divided into one of two groups, test group ($n = 10$) or placebo group ($n = 9$). The test group ingested one tablet daily for 30 days of two different strains of L. *reuteri* Prodentis® known as Gum PerioBalance® (Sunstar, Switzerland) containing 2×10^8 CFU per tablet. The placebo group received a tablet of inert ingredients containing no L. *reuteri* Prodentis®. Results of this study show periodontal parameters of PS, PD, and BOP achieved clinically significant reductions for the test group after the 30-day trial was complete $(p < 0.05)$. The placebo groups did not show any significant changes for any of the parameters evaluated. In their conclusion, investigators of this research stated the reduction in all clinical parameters measured in this study demonstrated L. *reuteri* Prodentis® may be useful in the treatment of initial to moderate periodontitis (Vicario et al., 2013).

Shimauchi et al. (2008) found probiotics may be useful in improving periodontal health with individuals in high risk groups for periodontal disease. This study ($N = 67$) did not exclude smokers, and utilized a double-blind, randomized, placebo-controlled design over eight weeks. The probiotic used for this study was Lactobacillus *salivarius* WB-21, and the test group ($n = 34$) consumed three tablets per day containing Lactobacillus *salivarius* WB21 (2.01×10^9 CFU/day) and xylitol (840 mg/day). The control group ($n = 33$) received tablets with no probiotic, and xylitol only (840 mg/day)

and were directed to use tablets three times per day for eight weeks. Clinical parameters of BOP, PD, PS, GI, and salivary samples were collected to evaluate probiotic levels in plaque and saliva. Data collection was performed at baseline, week four and week eight. Results showed clinical parameters for both the test and placebo groups were improved after the eight-week intervention. This anomaly was attributed to attention bias (Hawthorn effect). Results did show participants who were smokers had a statistically significant improvement in PS and PD when compared to baseline $(p < 0.05)$ at eight weeks, and salivary levels of lactoferrin were significantly reduced in this group (Shimauchi et al., 2008).

Mayanagi et al. (2009) also used Lactobacillus *salivarius* WB-21 containing tablets in a research study to evaluate the effects on five different oral microbes: Aggregatibacter *actinomycetemcomitans*, Provotella *intermedia*, Porphyromonas *gingivalis*, Treponema *denticola*, and Tanerella *forsythia*. This study divided participants ($N = 66$) into two groups. The test group ($n = 34$) received tablets containing 2×10^9 CFU per day of L. salivarius WB-21 and xylitol, and were directed to ingest these tablets three times per day for eight weeks. The control group ($n = 32$) received tablets with no probiotic, but they did contain xylitol, and were directed to use tablets three times per day for eight weeks. Data collection intervals were at baseline, week four, and week eight, and supragingival and subgingival samples were collected. Results showed a suppressive effect on numerical sums of the counts of these five bacteria in subgingival plaque leading the researchers to believe oral administration of L. salivarius WB-21 could be beneficial in periodontal treatment (Mayanagi et al., 2009).

Montero et al. (2017) conducted a study using a combination of three different

types of probiotic bacteria. The aim of this study was to evaluate the effects of probiotics for treatment of gingivitis, and also the effect on subgingival microbiota. This six-week study included participants (N = 59) broken into two groups; test group and control or placebo group. The test subjects (n = 29) were instructed to ingest two tablets per day containing the probiotics Lactobacillus *plantarum*, Lactobacillus *brevis*, and Pediococcus *acidilactici*. The control group (n = 30) received the same tablet with no probiotic bacteria added. Clinical parameters assessed were GI, PS and an angulated bleeding score, performed by using a periodontal probe held at a 60-degree angle to the longitudinal axis of the tooth. Contact was made with the sulcular gingiva, gently pushing the tissue away from the tooth. Subgingival assessments were made by taking one sample from each quadrant from the area of highest gingival inflammation. Samples were collected with sterile paper points. Quantitative PCR was utilized to determine the DNA of each sample. Results showed a significant decrease in the amount of T. *forsythia* bacteria present in the test group ($p < 0.008$), but no statistically significant difference was shown for any clinical parameter between treatment groups. Conclusions stated use of a probiotic containing Lactobacillus *plantarum*, Lactobacillus *brevis*, and Pediococcus *acidilactici* induced a significant impact in the subgingival microbiota of users (Montero et al., 2017).

Topical Introduction of Probiotics

Another method of introducing probiotics into the oral environment is through topical application. Studies employing this method utilize rinses, gum, lozenges, and toothpaste containing probiotics and evaluate clinical parameters of PD, PS, GI, and BOP (Amižić et al., 2016; Hallstrom et al., 2013; Harini et al., 2010; Twetman et al., 2009;

Slawik et al., 2011). The principle aim of these research studies was to assess the efficacy of probiotics for the potential treatment of the oral diseases known as gingivitis and periodontitis, however studies exist that evaluate probiotics on other oral conditions such as caries (tooth decay), halitosis (bad breath), and Candida infections (yeast infections).

Topical studies using mouthrinses. Harini et al. (2010) compared a probiotic oral rinse with a chlorhexidine gluconate rinse. Chlorhexidine mouthrinse has long been considered one of the most effective gingivitis reducing therapies available. In a systematic review by James et al. (2017), 51 research articles regarding chlorhexidine use and its effectiveness at reducing gingival inflammation and plaque were analyzed. Conclusions noted by the investigators of this review stated there is high-quality evidence showing chlorhexidine mouthrinse reduces PS, and in mild cases of gingival inflammation, GI has also been reduced. Conversely, their research showed insufficient evidence to determine whether chlorhexidine rinse has resulted in a reduction of GI in moderate to severe cases of gingivitis (James et al., 2017). The aim of this study by Harini et al. (2010) was to evaluate the efficacy of probiotic and chlorhexidine mouth rinses in the reduction of plaque and gingival inflammation in children. This study included healthy children ($N = 45$) age six to eight years of age. The trial was 14 days and utilized a double-blind parallel group study where subjects were randomly divided into one of three groups ($n = 15$):

Group A: Control Group (mint water)

Group B: Probiotic Group

Group C: Chlorhexidine Group

Each group was instructed to rinse once daily for 60 seconds using 15 ml of the assigned solution, approximately 30 minutes after brushing, then expectorate residual rinse. Final results showed the probiotic group and the chlorhexidine group had less plaque accumulations compared to the control group, however, there were no significant differences in plaque accumulation between the probiotic and chlorhexidine groups. GI was also significantly different for the probiotic and chlorhexidine groups compared to the control group ($p < 0.001$). The probiotic group's GI was significantly reduced when compared to the chlorhexidine group ($p = 0.009$), (mean = 0.2300 and 0.6805, respectively). The results of this study suggest an anti-inflammatory benefit of probiotics similar to the systemic application studied by Slawik et al., (2011) (Harini et al., 2010; Slawik et al., 2011).

Yousuf et al. (2017) studied the effects of freeze dried powdered probiotics on gingival status and plaque inhibition in school children ($N = 33$). In this study, freeze dried probiotics containing Lactobacillus *acidophilus*, Bifidobacterium *longum*, Bifidobacterium *bifidum* and Bifidobacterium *lactis* (Prowel, Alkem Laboratories), lactic acid bacillus only (Sporolac, Sangyo), and a placebo powder calcium carbonate 250 g (Calcium Sandoz, Novartis) were assigned to two test groups and a placebo group. Each group ($n = 11$) included school children ages 12 to 15, who were instructed to mix the powder with 30 ml of water and swish once daily for three minutes, then expectorate the mouth rinse. The study period was three weeks, and clinical parameters of PS and GI were evaluated at baseline, day seven, day fourteen, and day twenty-one. Results indicated both probiotic groups had a statistically significant reduction ($p < 0.05$) in gingival status and plaque, while the placebo group showed no

significant difference in either clinical parameter. Investigators surmise the use of probiotic mouth rinses improves the oral health in children by significantly reducing the plaque accumulation and gingival inflammation (Yousuf et al., 2017).

Nadkemy et al. (2015) conducted a study to assess and compare the plaque reducing benefit and anti-inflammatory potential between mouthrinses containing either a probiotic, 0.2% chlorhexidine, or saline. The probiotics of choice for this study consist of Lactobacillus *acidophilus*, Lactobacillus *rhamnosus*, Lactobacillus *sporogenes*, Bifidobacterium *longum*, and Saccharomyces *boulardii*. Methodology was a randomized parallel group of subjects ($N = 45$) over four weeks. Group A ($n = 15$) used the probiotic mouthrinse, group B ($n = 15$) were given a 0.2% chlorhexidine rinse, and group C ($n = 15$) was the control group, and rinsed with normal saline. Pre-study oral prophylaxis was performed on all subjects, and oral hygiene instructions on brushing and flossing were given to all. Participants were instructed to rinse with 10 ml of their rinse, undiluted, for one minute twice daily 30 minutes after brushing, and expectorate. Clinical parameters of PS and GI were evaluated at baseline, week two and week four of the study. Results of this study indicate from baseline to week 4, the difference in PS between the probiotic and saline groups was clinically significant, as was the difference between the control and chlorhexidine groups (significance not noted as a *p*-value; mean values of PS for probiotics was 0.36 ± 0.14, control 1.1 ± 0.22, and chlorhexidine 0.21 ± 0.15). Similar findings were noted for GI, with both probiotic and chlorhexidine versus control showing clinical significance (significance not noted as a *p*-value; mean GI scores were 0.45 ± 1.174 for probiotic, 1.03 ± 0.142 for control, and 0.40 ± 0.124 for chlorhexidine). Conclusions from this research state probiotics could be useful in improving and

maintaining periodontal health of individuals at risk for periodontitis. Although chlorhexidine and probiotic rinse seemed equally effective, probiotic may be a better alternative for periodontal management, because there are no known or proven toxicities to use, antibiotic resistance is not a concern with use, and they are a natural, biologically friendly substance (Nadkemy, 2015).

Noordin and Kamin (2007) conducted a placebo-controlled, double-blind, crossover study consisting of two 14-day test periods to evaluate plaque and gingivitis in young adults. Participants ($N = 32$) were divided into two groups; group A and group B. At the onset of the first phase of this study, group A ($n = 16$) was given an active mouthrinse containing the bacteriocin nisin, extracted from Lactobacillus *lactis*, and group B ($n = 16$) received a placebo mouthrinse with no probiotic or bacteriocin. Each group was instructed to use their mouthrinse twice daily for 60 seconds, approximately 30 minutes after brushing, and expectorate mouthrinse after rinsing. Measurements of PS and GI were taken at baseline, and at the end of 14 days, conclusion of phase one of the study. A washout period of four weeks was initiated after phase one, and when completed, phase two of the study commenced. Phase two involved having each previous group use the alternate therapeutic and placebo mouthrinse for 14 days. Data collection followed the same interval as phase one, with data collected at baseline and day 14, end of the study. Results of this study demonstrated a clinically significant reduction in gingival inflammation (GI) in the probiotic group when compared to the placebo ($p < 0.001$), as well as a clinically significant increase in plaque accumulation (PS) in the control group when compared to the probiotic group ($p < 0.001$). This difference in PS and GI led researchers to deduce probiotic mouthrinse is effective at

reducing plaque accumulation and gingival inflammation compared to a placebo mouthrinse. Therefore, a probiotic therapeutic mouthrinse may be an effective adjunct to regular oral hygiene for treating clinical parameters of gingivitis (Noordin et al., 2007).

A study by Shah (2014) compared mouthrinses containing either probiotics, chlorhexidine, or fluoride. This 28-day study included children age six to ten years old ($N = 40$), divided into four groups ($n = 10$):

Group A: Probiotic Group (probiotic not identified in the study)

Group B: Chlorhexidine Group

Group C: Fluoride Group (concentration of fluoride not identified in the study)

Group D: Control Group

Participants were instructed to rinse twice daily with 15 ml of their assigned rinse for 60 seconds after brushing teeth. Rinse was then expectorated, and subjects were advised not to eat, drink or rinse their mouth for 30 minutes. Data was collected for PS and GI at baseline and day 28, conclusion of the study. Results showed a statistically significant difference in PS for all three therapeutic rinse groups when compared with the control group, with similar findings for all three groups compared ($p < 0.001$). No notable difference for GI was determined between the probiotic, chlorhexidine and fluoride group when these three groups were compared to each other, however, there was a significant decrease in GI between the control group and these three therapeutic groups ($p < 0.001$). These results led investigators to determine probiotic mouthrinse is effective at reducing plaque accumulation and gingival inflammation in children, and given the concern with side-effects associated with available therapeutic mouthrinses, probiotic mouthrinse could be a good alternative antiplaque agent (Shah, 2014).

Topical Studies Using Gum. Krasse et al. (2005) was one of the first research studies to investigate the use of probiotics related to gingivitis, and set the stage for future research in this domain. Methodology of this two-week study was a double-blind placebo controlled prospective randomized study, (N = 59) with moderate to severe gingivitis. Participants of this study were randomly divided into three groups and received one of two different L. *reuteri* chewing gums, or placebo chewing gum. Group one (n = 20) (LR-1) and group two (n = 20) (LR-2) received one of two different L. *reuteri* formulations, both of human origin, containing 1 x 10^8 colony forming units (CFU) of live bacteria. Group 3 (n = 39) received a placebo. All participants of this study received a professional teeth cleaning and instruction on brushing and flossing technique prior to beginning the study. Participants were instructed to chew one piece of their study gum each morning and evening after brushing and flossing the teeth. Each participant used a total of 28 study gums during the 14-day clinical trial. Results showed a clinically significant difference PS for the L. *reuteri* groups LR-1 *($p < 0.05$)* and LR-2 *($P < 0.01$)* when compared to the placebo group. This led investigators to conclude L. *reuteri* was effective at reducing both gingivitis and plaque in patients with moderate to severe gingivitis (Krasse et al., 2005).

Twetman et al. (2009) conducted a two-week study using chewing gum containing two strains of L. *reuteri*, 1 x 10^8 CFU per gum, in order to investigate the effects on gingival inflammation and specific inflammatory mediators in gingival crevicular fluid (GCF). This study was a randomized, double-blind, parallel, placebo controlled study using healthy adults (N = 42) with moderate levels of gingival inflammation. Three groups were included in the study, group A/P was given one active

and one placebo gum daily, group A/A received two active gums, and group P/P received two placebo gums per day. Participants were instructed to chew two study gums per day for 10 minutes each time. Results showed in all groups BOP and GCF had decreased during the chewing period, but results were only statistically significant ($p = 0.05$) for the A/P and A/A groups. The levels of TNF-alpha and IL-8 decreased significantly ($p = 0.05$) in group A/A, but significant decreases on other inflammatory mediators were not observed. Research concluded the reduction of pro-inflammatory cytokines in GCF may prove the principle for a probiotic approach to reducing inflammation in the oral cavity (Twetman et al., 2009).

Sinkiewicz et al. (2013) conducted a randomized double-blind placebo controlled trial ($N = 23$) to investigate the effects of L. *reuteri* on saliva after supplementation, and the probiotic effects of L. *reuteri* on PS and supragingival and subgingival microbiota. Participants were divided into two groups; test group ($n = 11$) and placebo group ($n = 12$). The test group was given chewing gum developed by BioGaia AB, Sweden, containing L. *reuteri* 2×10^8 CFU per gum. The placebo group received chewing gum being identical in size, shape, and taste, containing inert ingredients and no L. *reuteri*. Participants were instructed to chew two study gums per day for a minimum of 10 minutes each for 12-weeks. Data gathered at baseline (visit 1), week 12 (visit 2), and week 16 (follow-up). Results of this study show no significant difference in PS for the test group when comparing visit one and visit two data. The control group, however, had a statistically significant increase in PS from visit one to visit two ($p = 0.0023$). From this information, the investigators of this study suggest L. *reuteri* may inhibit plaque accumulation, as shown by the previous research of Krasse et al. (2005) (Krasse et al.,

2005; Sinkiewicz et al., 2013).

Topical Studies Using Lozenges. Research by Hallstrom et al. (2013) used probiotic lozenges containing L. *reuteri* to test the effect on the inflammatory response and composition of supragingival plaque in healthy adult females ($N = 18$). This three-week study utilized a double-blind, randomized, placebo-controlled, cross-over design. The experimentation site chosen was the buccal surface of first molars, and during the test period, a mouth-guard was worn during brushing to cover the teeth from the first premolar to the second molar to prevent accidental cleaning. Lozenges containing the probiotic or a placebo were taken twice daily, and data was collected for PS, GI, BOP and GCF (Hallstrom et al., 2013). The study concluded daily intake of probiotic lozenges did not significantly affect plaque accumulation or inflammatory mediators during experimental gingivitis. One limitation to consider is the short duration of time this study utilized (Hallstrom et al., 2013).

Toiviainen et al. (2015) used lozenges containing Lactobacillus *rhamnosus* GG (LGG) and Bifidobacterium *animalis* subsp. *lactis* BB-12 (BB-12) to evaluate levels of salivary mutans streptococci (MS) and gingival inflammation in young adults ($N = 60$) in a randomized, controlled, double-blind trail. This four-week study included a test group ($n = 29$) and a control group ($n = 31$), and utilized a run-in procedure where subjects were instructed to use one chewing gum containing 42% xylitol, 18% sorbitol, and 5% maltitol four times per day for two weeks prior to the beginning of the study. After the run-in period was completed, subjects were broken into one of two groups; control/placebo, and test groups. The test group used lozenges containing 50% xylitol, 46% sorbitol, and probiotics LGG and BB-12 at levels of 2×10^9 cells of each type of probiotic. The

control group received lozenges containing no probiotics, but the same polyol composition as the test lozenges. A Silness-Löe (1964) PS and Löe-Silness (1963) GI were utilized to evaluate plaque levels and gingival inflammation. Salivary samples and supragingival plaque samples were collected from 15 randomly chosen subjects per group, and cultured for microbe analysis. Results of this study show mean PS and GI values for the probiotic group significantly decreased ($p = 0.016$ for PS and $p = 0.012$ for GI), whereas no change was observed for the control group. Statistically significant changes in microbial composition were not demonstrated for either group. This information led investigators to surmise immune modulation could be a possible explanation for the improved PS and GI of the probiotic group. Therefore, taking probiotics topically may improve the periodontal health of healthy individuals (Toiviainen et al., 2015).

In a randomized, placebo-controlled, double-blind, split-mouth designed study, Vivekananda et al. (2010) used lozenges containing L. *reuteri* 1×10^8 CFU (Prodentis®, BioGaia AB, Stockholm, Sweden) or a placebo lozenge, to evaluate the effects of L. *reuteri* alone, and in combination with scaling and root planning. Healthy adults ($N = 30$) with chronic periodontitis were included in this split-mouth study design. Each participant receiving scaling and root planning (SRP) on two quadrants, and the other two quadrants were left untreated. At day 21 after SRP, participants were divided into two groups ($n = 15$) and given lozenges, identical in appearance, except one group received lozenges with probiotics added, and the other group received placebo lozenges. Participants were instructed to use lozenges twice daily from day 21 to day 42 of the study. Data for clinical parameters of PS, GI, PD, BOP, and clinical attachment level

(CAL), and microbial parameters for Aggregibacter *actinomycetemcomitans* (Aa), Porphyromonas *gingivalis* (Pg), and Provotella *intermedia* (Pi) were collected at baseline, day 21, and day 42 of the study. Results of this study show both SRP plus probiotics and probiotics alone had the highest reduction of the three microbes assessed ($p < 0.01$, and $p < 0.01$ respectively). SRP plus placebo did not significantly reduce the evaluated pathogens. Clinical parameters of PS, GI, BOP exhibited clinically significant improvement for all treatment modalities. CAL and PD were most improved in the SRP plus probiotic group ($p < 0.001$, and $p < 0.001$ respectively). Based on the results, these researchers concluded this study confirms the plaque inhibition, anti-inflammatory, and antimicrobial effectiveness of L. *reuteri* containing lozenges (Prodentis®, BioGaia AB, Stockholm, Sweden), and recommended the addition of this therapeutic agent along with mechanical debridement, and during maintenance phases of periodontal treatment (Vivekananda et al., 2010). The lozenges tested during this study were provided by the company who developed them, BioGaia, AB, Stockholm, Sweden, and the researchers also received a publication grant from this company. Although the researchers in this study claim there is no conflict of interest involved with this study, this could be a limitation (Vivekananda et al., 2010).

In a study by Ince et al. (2015) lozenges containing L. *reuteri* 1×10^8 CFU (Prodentis®, BioGaia, Lund, Sweden) were used during initial periodontal therapy on patients with chronic periodontitis to evaluate the effects on clinical and biochemical parameters. This study was a randomized, double-blind, placebo-controlled, comparative study ($N = 30$). All participants were divided into two groups ($n = 15$) and received SRP and either lozenges containing the probiotic L. *reuteri*, or a placebo. Participants were

given their assigned lozenges at the beginning of initial SRP therapy, and instructed to take them twice daily for three weeks. Clinical parameters of PS, GI, BOP, and PD were assessed, along with biochemical parameters of gingival crevicular fluid (GCF), and pro-inflammatory and anti-inflammatory cytokines MMP-8 and TIMP-1 levels. Data was collected at baseline, day 21, day 90, day 180, and day 360. Results of this study revealed statistically significant differences in PS, GI, BOP and PD for the test group when compared to control ($p < 0.05$) for all clinical parameters measured, and across all data collection intervals. Biochemical parameters showed a clinically significant decrease in GCF MMP-8 and an increase in TIMP-1 levels for the test group at 180 days ($p < 0.05$). Researchers concluded L. *reuteri* containing lozenges may be a useful adjunct in the treatment of chronic periodontitis, and decreased MMP-8 and increased TIMP-1 at day 180 ($p < 0.05$) may demonstrate the anti-inflammatory benefit of this topical treatment. One limitation to consider in this study is the lozenges tested were provided by the company who developed them, BioGaia, AB, Lund, Sweden, although the researchers in this study claim there is no conflict of interest involved with this study, as the company was not involved in data management (Ince et al., 2015).

In a very similar study conducted by Tekce et al. (2015), L. *reuteri* 1 x 10^8 CFU (Prodentis®, BioGaia, Lund, Sweden) containing lozenges were also used during initial periodontal therapy to evaluate L. *reuteri* as an adjunctive therapy for the treatment of chronic periodontitis, and to detect levels of L. *reuteri* colonization in periodontal pockets. This study was a randomized, parallel, placebo-controlled, double-blind clinical trial lasting 360 days. Patients with chronic periodontitis ($N = 40$) participated in this study evaluating clinical parameters of PS, GI, BOP, and PD. Both the placebo ($n = 20$)

and probiotic group (n = 20) were given lozenges at the beginning of initial therapy, and instructed to take assigned lozenges twice daily for three weeks. Microbiological assessments included evaluation of total volume of colonizing bacteria (TVC) measured as CFU/ml., and obligate anaerobic bacteria, calculated by taking TVC minus the total colony counts on plates incubated in 10% CO_2 (expressed as a percentage of TVC). Data was collected at baseline, day 21, day 90, day 180, and day 360 for both groups. Results of this study show similar results to the study by Ince et al. (2015), where the probiotic group had statistically significant changes in all clinical parameters and across all data collection intervals ($p < 0.05$). Similar evaluations were noticed on the probiotic group for TVC and obligate anaerobes, and across all data collection intervals except day 360. Researchers concluded L. *reuteri* containing lozenges may be a useful adjunctive therapy to SRP, to slow the recolonization of periodontal pockets (Tekce, 2015). Similar to the other studies using this product, the lozenges tested were provided by the company who makes them, BioGaia, AB, Lund, Sweden, although the researchers in this study claim there is no conflict of interest involved with this study, as the company was not involved in data management, but this could be a limitation of this research (Tekce et al., 2015).

Topical Study Using Toothpaste. To date, studies involving the use of probiotic toothpastes are few, as this research methodology has not been well investigated, and only one study was found to discuss during this literature review. Results of a study by Amižić et al. (2016), showed brushing with probiotics as an application method is superior to mouthrinses at inhibiting common oral microbes, and set the stage for additional research regarding brushing with probiotics. Amižić et al. (2016) conducted a comparative study using two mouthrinses and two toothpastes. Although this study had

limitations including a complicated research design, after the results were revealed, the researchers concluded "Probiotic toothpaste, as a relatively new concept in the prevention of oral infectious diseases such as caries and periodontal disease, can contribute to the prevention of oral infectious diseases" (Amižić et al., 2016, p. 142). The aim of this study was to compare two toothpastes containing different strains of probiotics (one containing L. *paracasei*, and one containing L. *acidophilus*) and one toothpaste without probiotics separately, and in combination with two different mouthrinses (one containing essential oils, and the other containing Hexetidine). Hexetidine is a bactericidal and fungicidal antiseptic used as a 0.1% mouthwash for treating localized infections and oral hygiene (Aoun, Saadeh, & Berberi, 2015; National Center for Biotechnology Information, n.d). This study did not specify the number of participants involved in the study, or the length of the test period. According to the investigators, results of this study demonstrate probiotic toothpastes exhibited inhibition of Candida *albicans* ($p = 0.043$) and Streptococcus *salivarius* ($p = 0.043$) to a higher degree than toothpaste without probiotic. Across all the cases, toothpastes had stronger inhibition potential than mouthrinses ($p < 0.05$). A stated limitation for this study by the investigators include the fact only a few specific bacteria were tested against agents in their pure form. The researchers suggest future studies should use toothpaste and mouthrinses with varying concentrations of agents on different microorganisms. This study may have additional limitations due to the unreported participant number and study length (Amižić et al., 2016).

Site Specific Introduction of Probiotics

Research studies involving site specific introduction of probiotics into the oral

cavity are limited. Currently, no studies exist that have used humans as participants. A four-week study conducted by Teughels et al. (2007) used male Beagle dogs to test guided periodontal pocket recolonization ($N = 8$) and incorporated a split-mouth, double-blind, randomized method. The study design included four different treatments including negative control (Nc) = no treatment; positive control (Rp) = subgingival scaling and root planning; $(Rp)_{single}$ = root planning and a single application of bacterial mixture at baseline; $(Rp)_{multi}$ = root planning and repeated application of the bacterial mixture. Data was collected to evaluate subgingival plaque, probe depths, and BOP at baseline, weeks one, two, and four. Each dog received one of four treatments in each randomly chosen quadrant. Results of this research showed $(Rp)_{multi}$ was superior to all three other groups at reducing anaerobic species and black pigmented bacteria; $(Rp)_{multi}$ versus (RP) results were ($p = 0.002$ and $p < 0.001$), and $(RP)_{multi}$ when compared to $(RP)_{single}$ was ($p = 0.03$ and $p < 0.001$). Conclusions of this study state due to the significant difference in the multiple treatment group, the research supports the concept that application of beneficial bacteria can lead to a more host-compatible periodontal environment, and in conjunction with scaling and root planning, may be a valid non-antibiotic approach to periodontal treatment (Teughels, 2007). Limitations of this study included concerns about beneficial bacteria being transferred to the control site, and the possibility that the application of probiotics induced an immunological response that interfered with the recolonization of the control sites (Teughels, 2007).

Systematic Reviews and Meta-Analysis

In the systematic review by Yanine et al. (2013), the stated objective was to analyze the available research on the effects of probiotics on periodontal diseases. After

eliminating several articles that did not meet stringent standards for inclusions criteria, the researchers were left with four articles, one using a topical approach with gum, and three using a systemic approach by means of a probiotic milk drink, and a tablet containing probiotics. The focus question was to determine the clinical impact on probiotic therapy compared to conventional and placebo treatments. Conclusions of this review state current research demonstrates probiotics have shown a slight benefit on PS and GI. They also determined more research is necessary to evaluate efficacy of probiotics utilizing correct methodology, larger population sizes, and longer trial periods (Yanine et al., 2013).

In a review of the role of probiotics in oral health and the effects they induce on periodontal disease, Gupta & Gupta (2010) discuss methods of application of previous studies and limitations of these past study designs. In their analysis of available research, Gupta et al. (2010) point out the fact ingestion of probiotics does not provide prolonged contact time with oral tissues, facilitating probiotic adhesion to saliva coated surfaces, and recommend more research utilizing topical applications with prolonged contact be conducted (Gupta et al., 2010). In order to address these concerns, this study incorporated subjects brushing their teeth with a dentifrice with probiotic drops added. This method provided longer contact time, and brushing the gums with probiotics aided in adhesion, which these researchers have stated is a limitation in previous studies.

Another review of current research by Teughels et al. (2011) examines studies on probiotics in the prevention and treatment of periodontal diseases. Their conclusions also discuss methodological limitations of previous studies and recommend an evaluation of the bacteria to be included in the probiotics used for a study. They recommend including

beneficial bacteria that are indigenous to the oral environment because they are perfectly adjusted to the oral ecology (Teughels et al. 2011). In this research study, the PI used probiotic drops containing L. *reuteri*, a probiotic bacteria common to the oral cavity, and commonly utilized in comparative research studies, in order to acknowledge this limitation (Iniesta, et al., 2012; Krasse et al., 2005; Sinkiewicz et al., 2010; Twetman et al., 2009; Vicario et al., 2013).

Summary

Probiotics are a beneficial remedy for several gastrointestinal aliments, and show encouraging results when applied to oral diseases (Amižić et al., 2016; Hallstrom et al., 2013; Harini et al., 2010; Iniesta, et al., 2012; Karuppaiah et al., 2013; Krasse et al., 2005; Sinkiewicz et al., 2010; Slawik et al., 2011; Teughels, Newman et al., 2007; Twetman et al., 2009; Vicario et al., 2013; Yanine et al., 2013). One of the complications with evaluating the efficacy of probiotics for oral disease is the variety of application methods available for implementation. The oral environment is accessible for topical and site specific application, as well as systemic methods, whereas, the gastrointestinal tract can only utilize a systemic approach. Therefore, topical, systemic, and site specific applications have been used in research studies involving the oral cavity. This presents a dilemma for researchers when trying to ascertain the best methodology to incorporate, and has left many questions unanswered regarding application methods.

This study attempted to determine the efficacy of brushing with probiotics as a means of application to the oral environment by evaluating the benefit of this method on a common oral disease, gingivitis. Research parameters evaluated included PS and GI (Amižić et al., 2016; Hallstrom et al., 2013; Harini et al., 2010; Krasse, et al., 2005;

Nadkemy et al., 2015; Noordin et al., 2007; Shah, 2014; Slawik et al., 2011; Sinkiewicz et al., 2013; Twetman et al., 2009; Yousuf et al., 2017). Previous research limitations such as study length and population demographics were addressed by implementing a longer study than many previous topical trials, and following a research design that employed a larger study population, increasing the chance for a more diverse population.

Methodology

Research Method or Design

This study was a randomized, placebo-controlled, double-blind design utilizing a convenience sample of participants from the population of students, faculty, and staff at the Spokane campus of Eastern Washington University (EWU), and friends and family members of the Principal Investigator (PI). The study period was three weeks in length, and had two data collection intervals; baseline, and week three (end of the study). Comparable research studies regarding topical application of probiotics have utilized treatment intervals of two to twelve weeks (Amižić et al., 2016; Hallstrom et al., 2013; Harini et al., 2010; Ince et. al., 2015; Krasse et al., 2005; Nadkemy et al., 2015; Noordin et al., 2007; Shah, 2014; Sinkiewicz et al., 2013; Tekce et al., 2015; Toiviainen et al., 2015; Twetman et al., 2009; Vivekananda et al., 2010; Yousuf et al., 2017). Biological measurements of plaque accumulation were recorded using disclosing solution and an O'Leary PS, and gingival inflammation was recorded using a modified Löe-Silness GI (Karuppaiah et al., 2013; Slawik et al., 2011). Biological parameters in comparable studies have used these, and similar indices to evaluate gingivitis (Hallstrom et al., 2013; Harini et al., 2010; Iniesta et al., 2012; Karuppaiah et al., 2013; Slawik, et al., 2011).

Procedures

Human subjects protection/informed consent. In order to ensure protection of human subjects, the PI submitted a completed non-exempt application to the EWU Internal Review Board (IRB) prior to beginning the study. After IRB approval was obtained, the PI began recruiting participants for the study. During the screening process,

a detailed informed consent document (Appendix M) was given to each potential participant, and a signed copy was collected from each participant of this study. Participants were asked to be part of a 3-week clinical trial and agreed to the use either probiotic drops containing L. *reuteri*, or a placebo drop consisting of sunflower oil, added to a plain fluoride dentifrice. Participation was voluntary, and participants were informed they could drop out of the study at any time. Participants were also told if problems occurred during the study, they should bring them to the PI's attention, and contact information for the PI was given to all participants. Confidentiality was maintained by assigning each participant a chart number, and all data collected was held by the PI in individual paper charts. HIPAA regulations regarding personal and health information were considered prior to beginning the study. Any identifiable information regarding participants of this study was blocked once participants had been accepted into the study, and a chart number was assigned to each participant. The PI kept all paper charts in a locked briefcase for transportation to and from the EWU dental hygiene clinic, where data collection took place. The locked briefcase was held at the PI's personal residence, and remained locked when the PI was not using the information contained inside for study purposes. Data for statistical analysis was held in the PI's personal computer, which is password protected.

Informed consent (Appendix M) for this study considered all possible risks to the study participants, although the possibility of side effects or adverse reactions was considered low in view of the fact the probiotic drops used contained no common allergens such as milk protein or lactose, nuts, peanuts, soy, corn, gluten, wheat, eggs, fish, shellfish, artificial ingredients or flavors, and the administration of the probiotic

drops was topical.

This study followed the guidelines established by the U. S. Food and Drug Administration (FDA) regarding dietary supplements. The FDA defines dietary supplements as products taken by mouth that contain a dietary ingredient, such as vitamins, minerals, amino acids, herbs, or botanicals, intended to supplement the diet (FDA, 2017). The FDA does not require regulation of dietary supplements, as they are deemed safe for use throughout the general public. The WHO describes probiotics as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (Venugopalan et al., 2010, p. 1661). The manufacturer of the probiotic drop does not make any health claims beyond the promotion of digestive and immune health, and as described by the WHO, probiotics have been determined to confer health benefits to the user.

Informed consent included side effects or adverse reactions possible from engagement in the study as noted below:

- Gastrointestinal upset, gas, bloating, diarrhea, nausea
- Allergic reaction to fluoride or the plain fluoridated study toothpaste
- Allergic reaction to sunflower oil, L. *reuteri*, or any component of the study or placebo drops

Participants were advised how signs and symptoms of an allergic reaction may present as described below:

- Gastrointestinal upset, gas, bloating, diarrhea, nausea
- Oral conditions of the gums or oral tissues such as increased swelling, bleeding, tenderness, appearance of sores

- Burning sensation of oral tissues, tongue, or gums
- Difficulty breathing, feeling of swelling of the throat/airway, wheezing, shortness of breath
- Appearance of rash or hives

Participants were advised of recommendations regarding intervention or treatment should any adverse reaction or side effect occur as detailed below:

- Stop all study drops and toothpaste immediately if you experience any unusual signs or symptoms
- Call the PI on her cell phone as soon as you notice any unusual symptoms or reactions
- If you experience difficulty breathing, swelling of the throat/airway, wheezing, shortness of breath, rash or hives, call **911** immediately. As soon as possible, inform the PI of this study about the reaction, and discontinue use of the drops and study toothpaste.

As an incentive to become a study participant and complete the full study term, all participants who completed the study received a $5 Starbucks gift card. Additionally, each person who completed the study was entered into a drawing for the chance to win a Sonicare electric toothbrush, donated by Jennifer Workman, Phillips Sonicare Senior Field Sales Representative for Spokane and Boise, or professional teeth whitening, including custom whitening trays and two syringes of whitening gel, courtesy of Dr. Truman Nielsen DMD at Fawson Dentistry. Participants who withdrew from the study early, and did not complete the full study term, were not eligible for these incentives.

Criteria for sample selection. Inclusion criteria included healthy adults between

the ages of 18 and 65 with no systemic diseases or chronic illnesses (see Appendix D), willing to provide detailed information regarding their health, medications and known allergies, and ability to complete the entire study. Comparable studies where L. *reuteri* has been ingested have incorporated a four-week (30 day) washout period to ensure no residual benefits from the ingested probiotics remain (Iniesta et al., 2012). Therefore, for one month prior to the beginning of the study, and during the study, subjects could not be taking any antibiotics or using probiotic tablets. Additionally, participants were asked to consume only limited amounts (3 times per week or less) of foods and beverages containing probiotics (Iniesta et al., 2012; Vicario et al. 2013) (Appendix I). Inclusion criteria also called for subjects to have at least 20 evaluable teeth (Nadkemy et. al., 2015) and exhibit gingivitis.

Participants were assessed through a screening process prior to beginning the study to determine whether they had gingivitis. Participant screenings took place in the dental hygiene clinic at EWU or the PI's private practice office, located in Spokane, Washington. Clinical parameters of plaque accumulation and gingival inflammation were assessed with a PS (Appendix C) and GI (Appendix B). Exclusion criteria dismissed people who were using nicotine gum or lozenges, electronic cigarettes or vaping devices, chewing tobacco, smoking tobacco in any form; cigars, cigarettes, pipes, smoking marijuana or hookah, or using marijuana sublingual drops, and anyone using chlorhexidine oral rinse solutions (Sinkiewicz et al., 2010). Use of edible and non-smoking forms of marijuana were permissible for the study, as was the use of nicotine patches (Appendix I).

Description of the setting. This study was conducted in the dental hygiene clinic

at EWU, Spokane, Washington. This location was selected for pragmatic purposes, as the PI is affiliated with EWU and it had facilities to support screening and data collection of study subjects. All screenings and data collection were scheduled during times when the EWU clinic was not in use for academic purposes, and participation by any current faculty, staff, or students of EWU was voluntary, and took place outside of the volunteers' regular work hours.

Source. A convenience sample of participants was obtained for this study drawing from the faculty, students, and staff at the EWU Spokane campus, and co-workers, friends and family members of the PI. This sample provided the variety in age, ethnicity, gender, and demographics required for application to a larger population. Sample selection for this study consisted of individuals responding to a flyer posted at various sites on the Spokane campus of EWU (Appendix J), and through social media blasts entered onto the PI's personal social media accounts to alert the PI's co-workers, friends, and family of the study. The flyer used was approved for display by EWU prior to recruitment, and the social media blast was placed on Facebook, using a modified version of the recruitment flyer (Appendix J).

Plan. The design of this study was modeled after comparable clinical trials, and participants were evaluated at the beginning of the study (baseline data collection) and at end of week three (final data collection) (Vicario et al., 2013). Each participant was randomly divided into one of two groups: Group A or Group B. During the screening process, the research coordinator (RC) randomly assigned a drop to each participant, alternating the dispensing of Drop A and Drop B to ensure equal distribution of each type of drop. Participants given Drop A were said to be in Group A, and participants given

Drop B were said to be in Group B. The study began with a screening for each individual wanting to be a participant, and baseline data was collected at this time for the clinical parameters being evaluated. After baseline data was recorded, participants were assigned their study drops to add to their study toothpaste, and given a schedule of the study (Appendix G) to track their daily usage of toothpaste with drops. Participants were asked to check off each day of use on the schedule. The schedule also included designated days for data collection, to remind participants when to return for clinical assessments (Appendix G). The PI requested participants bring the schedule to the final data collection at the end of week three to confirm participation. Each participant was given a laminated study reminder card (Appendix L) outlining important information about the use of the drops and toothpaste. This card also served to help them remember to use their drops and toothpaste once a day.

Subjects were asked to undergo a screening process to confirm they met inclusion criteria for study enrollment. The screening process include filling out a printed version of the current health history form used in the EWU Dental Hygiene Clinic, containing questions on demographic and health information (Appendix E). This health history is part of Eaglesoft©, the electronic health record (EHR) software utilized at EWU. Participants were also asked to fill out a survey on recent usage of probiotics, antibiotics, and known allergies (Appendix F). An evaluation of the mouth to assess oral health status and existence of gingivitis was performed by using a modified Löe-Silness GI (Appendix B) to assess gingival inflammation, and an O'Leary PS (Appendix C) to assess plaque levels.

During the duration of the study, participants were instructed to use limited

amounts (3 or less times per week) of any food or beverage on the excluded foods list (Appendix I), and not to use any medications, over the counter remedies such as probiotics, or engage in the use of any excluded habits or recreational substances (Appendix D) including:

- Chewing tobacco, and Smoking tobacco products such as cigarettes, cigars, pipes
- Nicotine products used orally such as gum or lozenges
- Electronic cigarettes or vaping devices
- Hookah
- Smoking marijuana
- Marijuana sublingual drops

Participants of this study visited the EWU Dental Hygiene clinic a total of two times for initial screening and data collection, and all participants were given a schedule with data collection dates, times, and detailed information on when to use drops (Appendix G).

Participants of the study were given a plain, fluoridated toothpaste to use during the study period. The plain toothpaste provided during the study contained no triclosan, xylitol, whitening or tartar control additives. Other comparable studies have used fluoride mouthwash, and xylitol, in conjunction with the probiotic treatment remedies without concern for bioavailability of the probiotics, therefore, use of a plain fluoridated toothpaste in this study was not contraindicated (Amižić et al., 2016; Shah, 2014; Toiviainen et al., 2015). Along with the assigned toothpaste, participants were given a vial of liquid containing either probiotics, or organic sunflower oil (placebo), the main component of the probiotic drop used during the study. The probiotic drops used for this study were obtained through the online shopping website Amazon.com. These drops are

manufactured in Canada by Bioamicus Laboratories® and contain 2 x 10^8 CFU of the probiotic L. *reuteri* per 5 drops, and contain no milk protein or lactose, nuts, peanuts, soy, corn, gluten, wheat, eggs, fish, shellfish, artificial ingredients or flavors. The original use of this drop is as an oral drop to promote digestive and immune health for infants and children. Dosage is described as:

> *Infants and children age 0 – 3, use 5 drops once per day*
>
> *Adults, adolescence, and children age 4 or older, use 5 drops one to three times per day*

Comparable research utilized L. *reuteri*, with similar dosages as used in this study (Vicario et al., 2013).

Participants were instructed not to alter their normal homecare routine, and use only the assigned study toothpaste when brushing (Sinkiewicz et al., 2010; Vicario et al., 2013). Additionally, they were instructed to add 5 drops of their assigned drops to their toothbrush, then add the desired amount of study toothpaste, and brush per their normal routine. It was important the vial of drops be refrigerated to maintain the live active probiotic cultures contained in the drops, so participants were instructed to keep the vial of drops refrigerated when not being used, and to shake the drops well before use to ensure no separation of ingredients.

Due to the number of participants (N = 34), and the complexity of this research study, the PI enlisted volunteers. The source for these volunteers were the PI's colleagues, and fellow graduate and undergraduate students from the EWU Dental Hygiene Department. Participation was voluntary, and no compensation was given to volunteers. Volunteers helped outside of their regular work hours, and were able to

attend all screenings and data collection dates, in order to maintain consistency in data collection. The PI enlisted help from two research assistants, three clinical assistants, and a research coordinator to help implement this clinical trial.

Research Assistants (RA) were registered dental hygienists (RDH) holding an active license in the state of Washington and had an active professional liability insurance policy. The RAs and the PI, conducted the clinical assessments and data collection during screenings. To ensure interrater reliability, RAs received calibration and training materials, an orientation on the use of study forms, and a brief question and answer session prior to any screening or data collection. (Appendix K). Research assistants were in charge of administering informed consent to study participants, and obtaining a signed informed consent document from them. Therefore, RAs completed the Collaborative Institutional Training Initiative (CITI) prior to beginning the research study to comply with IRB regulations regarding the handling of informed consent for research subjects.

Clinical Assistants (CA) helped with recording data collection, cleaning and sterilization of instruments, and dental unit set-up, break-down, and disinfection. CAs had a brief training session prior to screening and data collection to ensure interrater reliability (Appendix K). The Research Coordinator (RC) was responsible for randomly assigning the study drops to the participants, coordinating the CAs and helping CAs with their duties when needed, and handing out study packets to participants, containing study drops, toothpaste, schedule, and reminder card.

The PI enlisted a person outside the study, called the study contractor (SC) to prepare the study drops. The SC prepared both the probiotic and placebo drops by using sterile pipettes to transfer either the Bioamicus probiotic drops, or organic sunflower oil,

into sterile, identically appearing pharmaceutical vials with self sealing caps. The SC randomly numbered each drop, recording the numbers into a log, identifying the group the drop fell into, and maintained this information in a separate password protected computer from the PI's computer throughout the study period. When the study was complete, the SC released the information to the PI regarding the drop numbers included in the probiotic (test) or placebo (control) groups. This anonymous random design kept the participants, RAs, CAs, RC, and PI from knowing which groups were test or control, ensuring the study followed a randomized, double-blind design.

Size. This study had 34 participants ($N = 34$) consisting of 11 males ($n = 11$) and 23 ($n = 23$) females. Comparative research utilized between 18 and 45 subjects, with predominately female participants (Hallstrom et al., 2013; Harini et al., 2010; Iniesta et al., 2012; Slawik et al., 2011; Twetman et al., 2009). According to research conducted by Chan (2003), when paired samples are being analyzed using the pre and post mean difference of two treatment groups, a simple formula can be used to determine the statistically significant sample size: Total sample size = $c/\delta 2 + 2$ (Chan, 2003). For this study to have 80% power with a 95% confidence level, 34 participants were needed (Chan, 2003).

Variables. This study evaluated the dependent variables of plaque levels and gingival inflammation for each participant (Krasse et al., 2005). Plaque levels were determined by using a PS (Appendix C). Inflammation of the gingival tissues was assessed through use of a GI (Appendix B) (Karuppaiah et al., 2013; Shah, 2014; Slawik, et al., 2011). Independent variables for this study were placebo and probiotic drops. The PI examined the outcome of baseline versus final data on these variables to ascertain if

brushing with probiotics added to toothpaste significantly reduced PS and GI more than brushing with a placebo added to toothpaste.

Instruments. Quantitative data included a PS using the O'Leary PS (Appendix C) (Conn, Warren-Morris, Prihoda, Hicks, & Hernandez, 2017) and a GI using a modified Löe-Silness GI (Appendix B) (Karuppaiah et al., 2013; Slawik, et al., 2011). To address concerns with reliability and validity of PS assessments, 2Tone™ brand disclosing solution was used to expose participant's plaque. This disclosing solution helps to differentiate plaque accumulation by staining new plaque pink and mature plaque purple. RAs were instructed to only record the purple, or mature plaque, for plaque scores. In order to ensure validity and reliability of the GI index used, the PI utilized a modified GI. Modification included changing a traditional Löe-Silness gingival index with a scale of 0, 1, 2, 3 to a 1, 2, 3 scale, thus changing the scale to a bleed or no bleed evaluation. The modified scale incorporates information and suggestions from Dr. Aldredge regarding bleeding on probing defined (Aldredge, 2012). Original Löe-Silness index parameters have number 0 and 1 as no bleeding, and instead examine differences in color, consistency, and contour of gingival tissue to determine a score of 0 or 1. The PI was concerned with reliability of data collected with the original index, as this evaluation can be subjective and vary between examiners. The modified index assigned a 1 for no bleeding, and bleeding was divided into a score of 2, equaling slight bleeding, or 3, equaling moderate to heavy bleeding (Appendix A).

Equipment. The PI requested and obtained permission from the Chair of the Dental Hygiene Department at EWU to use the dental hygiene clinic for this research study. Equipment and use of the dental hygiene clinic at EWU was borrowed or donated

through an in-kind donation by the EWU Dental Hygiene Department including use of clinic/office printers, paper, laminating machine, dental chairs, dental units, disposable barriers, disposable personal protective equipment (masks, gloves), disposable sundries (paper towels, gauze, cups, cotton tip applicators), and basic instrument sets: including a mirror, air/water syringe tip, and periodontal probe. The PI supplied disclosing solution, individual vials of placebo drops or probiotic drops for study participants, instructional material for participants, informed consent for participants, forms to record parameters of plaque (Appendix C) and gingival inflammation (Appendix B).

Steps to implementation.

1. Applied for IRB research study approval
2. Arranged screening dates with faculty and staff of the EWU Dental Hygiene Department to screen for study participants
3. Arranged data collection dates with faculty and staff of the EWU Dental Hygiene Department
4. Printed recruitment flyers for study participants
5. Hung recruitment flyers around the EWU Campuses
6. Sent out a Facebook social media blast to PI's friend's and family seeking participants for the study and using a similar recruitment flyer as demonstrated in Appendix J
7. Recruited registered dental hygienists to be research assistants to help gather data
8. Recruited a research coordinator
9. Recruited clinical assistants

10. Calibrated all RAs and CAs (Appendix K).
11. Held screenings to choose study participants
12. Prepared all vials of probiotic and placebo drops
13. Prepared labels for probiotic and placebo drops
14. Had the SC assign numbered labels to drops and record the numbers, and groups, into an Excel© 2015 spreadsheet, and hold this information confidential until the end of study
15. Implemented the study on first selected data collection date

Summary

Probiotics present an exciting new approach for dentistry in the management of oral disease. Current research shows promising results using probiotics administered through several different mediums in the oral cavity. Results from these studies has been inconclusive and call for further research to determine the best means of application to the oral environment (Gupta et al., 2010; Hallstrom et al., 2013; Harini et al., 2010; Iniesta et al., 2012; Karuppaiah et al., 2013; Meurman, 2005; Yanine et al., 2013). Most researchers agree future studies involving probiotics for the treatment and prevention of oral diseases should strive to identify proper strains for specific diseases, application methods, dosages, and duration of use (Gupta et al., 2010; Meurman, 2005; McFarland, 2015; Teughels et al., 2011; Yanine et al., 2013). This study sought answers to these questions by utilizing toothbrushing as the application method, as few studies exist incorporating brushing as an application method.

Results

Description of Sample

This study had 34 participants (*N* = 34) consisting of 11 males and 23 females (Fig. 2). Comparative research utilized between 18 and 45 subjects, with predominately female participants (Hallstrom et al., 2013; Harini et al., 2010; Iniesta et al., 2012; Slawik et al., 2011; Twetman et al., 2009). According to research conducted by Chan (2003), when paired samples are being analyzed using the pre and post mean difference of two treatment groups, a simple formula can be used to determine the statistically significant sample size: Total sample size = $c/\delta 2 + 2$ (Chan, 2003).

For this study to have 80% power with a 95% confidence level, 34 participants were needed (Chan, 2003).

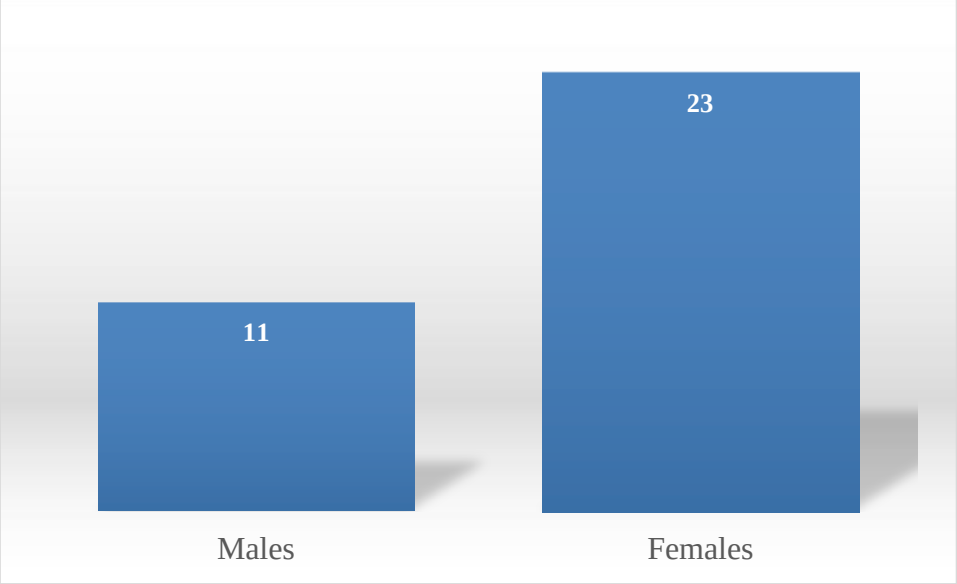

Figure 2. Gender (*N* = 34)

Diversity among different ethnic groups include three different ethnicities, although numbers were small for some groups.

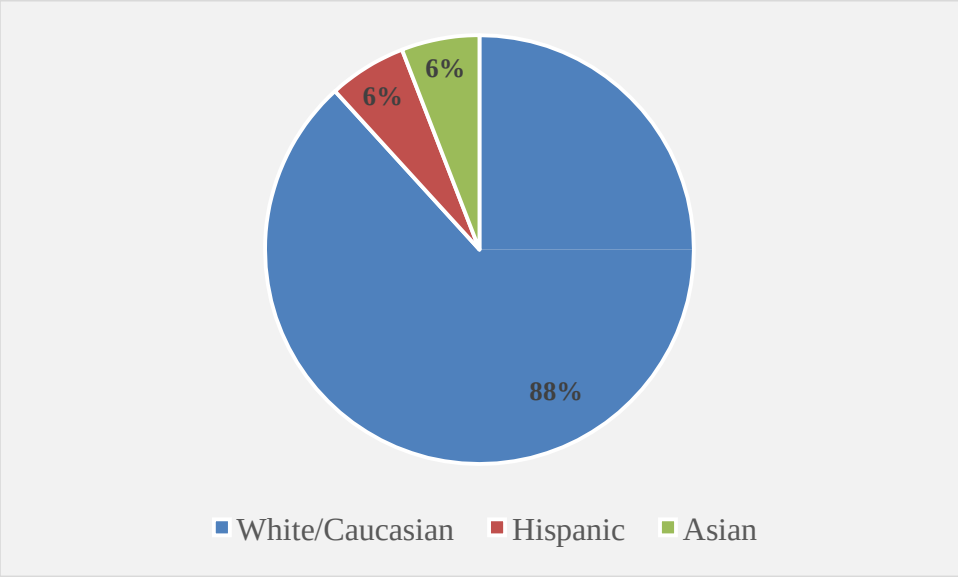

Figure 3. Ethnicity (*N* = 34)

This study did have a wide distribution of ages represented in the sample of participants, and included individuals from age 20 to 64. Ages were divided into four age ranges.

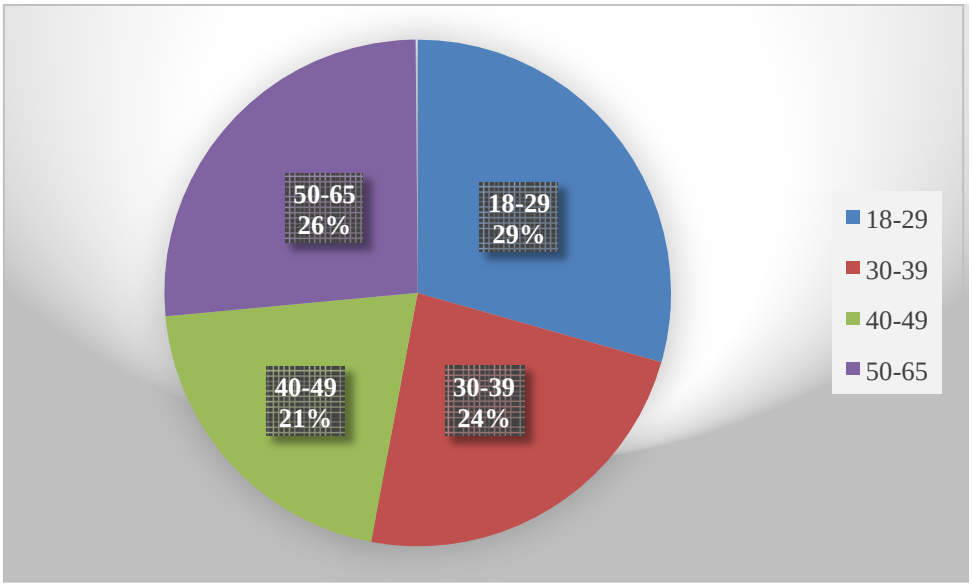

Figure 4. Age Ranges (*N* = 34)

Participant compliance for this study was 100% with no included participants dropping out of the study. Figure 5 shows the percentage of participants who used their drops every day of the study, in comparison to participants who missed one to four days, and five or more days during the study period.

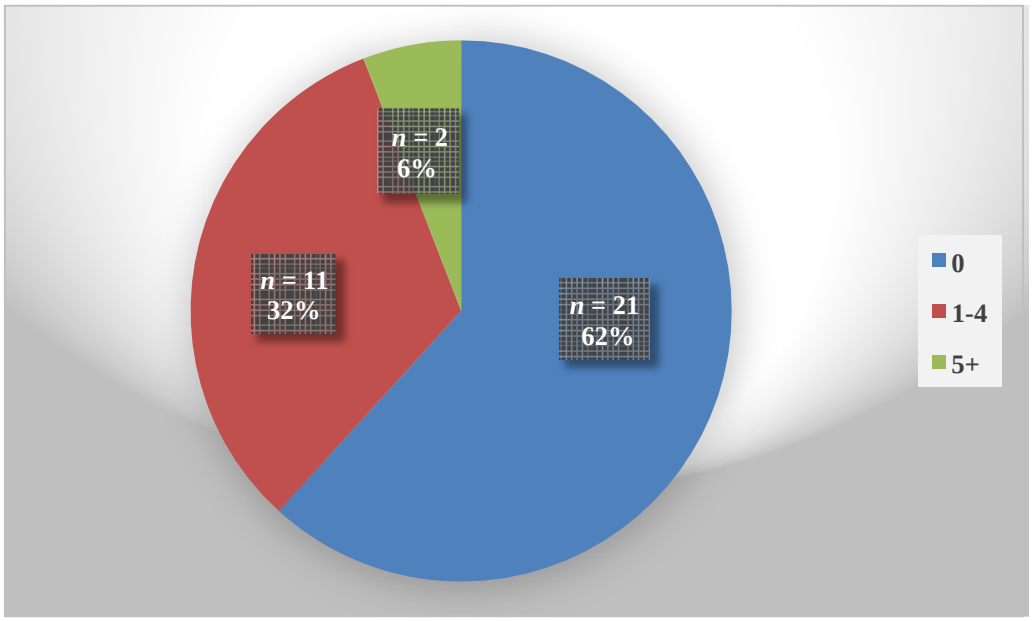

Figure 5. Participation Compliance Rate. Number of missed days. (*N* = 34)

Statistical Analysis

The study hypothesis claimed brushing with L. *reuteri* probiotic drops added to a plain fluoride dentifrice would result in a statistically significant decrease in the clinical parameters of gingivitis compared to brushing with a placebo drop added to the same type of toothpaste. All study participants exhibited some stage of gingivitis, either localized or generalized. Clinical parameters of gingivitis were observed and measured through the use of a GI to assess gingival inflammation, and PS, to evaluated plaque accumulation.

The PI entered all data into Excel© 2015 and transfer it to IBM® SPSS® (Statistical Package for Social Sciences) Version 24 for analysis (Karuppaiah et al., 2013,

Vicario et al., 2013). Analysis included both descriptive and inferential statistics. Inferential statistics employed Wilcoxon Signed Rank tests, due to the small sample size and inability to assume normal distribution of the study population. Statistical significance level was set at $p < 0.05$ (Imam, Mohammed, & Abanyam, 2014). Variables included median differences between the probiotic and placebo groups' baseline and final data for both PS and GI (Table 1, Table 2). Comparisons were also made by age and gender (Table 3, Table 4, Table 6, Table 7), and to evaluate the difference between final PS for placebo versus probiotic (Table 5) and final GI for placebo versus probiotic (Table 5). Table 8 evaluates difference in the number of participants with dental affiliations based on gender, and Table 9 evaluates differences in dental affiliations between the probiotic and placebo groups.

Table 1 depicts the placebo group's significance level between the beginning of the study (baseline) and week three (final) data. As noted in the table, differences in PS were not significant, although differences in GI did show a statistical significance.

Table 1
Placebo Group Baseline vs Final Data

	Null Hypothesis	Test	Sig.	Decision
PS	The median of differences between Baseline PS and Final PS equals 0	Related samples Wilcoxon Signed Rank Test	.394	Retain the null hypothesis
GI	The median of differences between Baseline GI and Final GI equals 0	Related samples Wilcoxon Signed Rank Test	.001	**Reject the null hypothesis**

Note. Sig. = significance. PS= Plaque Score. GI= Gingival Index.
Asymptomatic significances are displayed
The significance level is $p < 0.05$

Table 2 depicts the probiotic group's significance level between the beginning of the study (baseline) and week three (final) data. As noted in the table, differences in both PS

and GI were not significant.

Table 2
Probiotic Group Baseline vs Final Data

	Null Hypothesis	Test	Sig.	Decision
PS	The median of differences between Baseline PS and Final PS equals 0	Related samples Wilcoxon Signed Rank Test	.352	Retain the null hypothesis
GI	The median of differences between Baseline GI and Final GI equals 0	Related samples Wilcoxon Signed Rank Test	.485	Retain the null hypothesis

Note. Sig. = significance. PS= Plaque Score. GI= Gingival Index.
Asymptomatic significances are displayed
The significance level is $p < 0.05$

Table 3 shows the placebo group's significance level between the beginning of the study (baseline) and week three (final) data by age group. No statistically notable differences in PS or GI were demonstrated for any age group.

Table 3
Placebo Group Baseline vs Final Data by Age

	Null Hypothesis	Test	Age	Sig.	Decision
PS	The median of differences between Baseline PS and Final PS equals 0	Related samples Wilcoxon Signed Rank Test	18-29 30-39 40-49 50-65	.465 .225 .593 .225	Retain the null hypothesis
GI	The median of differences between Baseline GI and Final GI equals 0	Related samples Wilcoxon Signed Rank Test	18-29 30-39 40-49 50-65	.068 .080 .109 .068	Retain the null hypothesis

Note. Sig. = significance. PS= Plaque Score. GI= Gingival Index.
Asymptomatic significances are displayed
The significance level is $p < 0.05$

Table 4 shows the probiotic group's significance level between the beginning of the study (baseline) and week three (final) data by age group. No statistically notable differences in PS or GI were demonstrated for any age group.

Table 4
Probiotic Group Baseline vs Final Data by Age

	Null Hypothesis	Test	Age	Sig.	Decision
PS	The median of differences between Baseline PS and Final PS equals 0	Related samples Wilcoxon Signed Rank Test	18-29 30-39 40-49 50-65	.197 .068 .655 .249	Retain the null hypothesis
GI	The median of differences between Baseline GI and Final GI equals 0	Related samples Wilcoxon Signed Rank Test	18-29 30-39 40-49 50-65	.893 .593 .465 .465	Retain the null hypothesis

Note. Sig. = significance. PS= Plaque Score. GI= Gingival Index.
Asymptomatic significances are displayed
The significance level is $p < 0.05$

Table 5 compares differences between the final data of the placebo versus probiotic group. As seen in the table, no significant differences were observed.

Table 5
Probiotic vs Placebo Final Data

	Null Hypothesis	Test	Sig.	Decision
PS	The median of differences between Baseline PS and Final PS equals 0	Related samples Wilcoxon Signed Rank Test	.586	Retain the null hypothesis
GI	The median of differences between Baseline GI and Final GI equals 0	Related samples Wilcoxon Signed Rank Test	.177	Retain the null hypothesis

Note. Sig. = significance. PS= Plaque Score. GI= Gingival Index.
Asymptomatic significances are displayed
The significance level is $p < 0.05$

Table 6 shows the placebo group's significance level between the beginning of the study (baseline) and week three (final) data by gender. No statistically notable differences in PS or GI were demonstrated for males, or for females in regard to PS, but females did show a statistical difference in GI.

Table 6
Placebo Baseline vs Final Data by Gender

	Null Hypothesis	Test	Gender	Sig.	Decision
PS	The median of differences between Baseline PS and Final PS equals 0	Related samples Wilcoxon Signed Rank Test	Male	.686	Retain the null hypothesis
			Female	.505	Retain the null hypothesis
GI	The median of differences between Baseline GI and Final GI equals 0	Related samples Wilcoxon Signed Rank Test	Male	.068	Retain the null hypothesis
			Female	.004	**Reject the null hypothesis**

Note. Sig. = significance. PS= Plaque Score. GI= Gingival Index.
Asymptomatic significances are displayed
The significance level is $p < 0.05$

Table 7 shows the probiotic group's significance level between the beginning of the study (baseline) and week three (final) data by gender. No statistically notable differences in PS or GI were demonstrated for either gender.

Table 7
Probiotic Baseline vs Final Data by Gender

	Null Hypothesis	Test	Gender	Sig.	Decision
PS	The median of differences between Baseline PS and Final PS equals 0	Related samples Wilcoxon Signed Rank Test	Male	.753	Retain the null hypothesis
			Female	.445	Retain the null hypothesis
GI	The median of differences between Baseline GI and Final GI equals 0	Related samples Wilcoxon Signed Rank Test	Male	.463	Retain the null hypothesis
			Female	.721	Retain the null hypothesis

Note. Sig. = significance. PS= Plaque Score. GI= Gingival Index.
Asymptomatic significances are displayed
The significance level is $p < 0.05$

Table 8 illustrates the percentage of participants in the placebo group working or studying in the dental field by gender versus those without any dental affiliations.

Table 8
Placebo Group Females vs Males Dental Affiliation

	Dental Affiliation	Number	Percent of Total	%With Aff	% Diff bet Groups
Females	Student	4	33.33%	67% of group had dental affiliations	Females had a 27% greater number of participants with dental affiliations
	Works in dentistry	4	33.33%		
	No Affiliation	4	33.33%		
Males	Student	0	0.00%	40% of group had dental affiliations	
	Works in dentistry	2	40%		
	No Affiliation	3	60%		

Note. % With Aff = the total percentage of the group with dental affiliations. %Diff bet Groups = difference in percentage of participants with no dental affiliation between females and males.

Table 9 illustrates the percentage of participants in the placebo and probiotic group who work or study in the dental field versus those without any dental affiliations. The table indicates the difference, in percentage, between these groups of individuals with dental

affiliations.

Table 9
Placebo vs Probiotic Group Dental Affiliation

	Dental Affiliation	Number	Percent of Total	%With Aff	% Diff bet Groups
Placebo	Student	4	23.53%	59% of group had dental affiliations	Placebo group had a 12% greater number of participants with dental affiliations
	Works in dentistry	6	35.29%		
	No Affiliation	7	41.18%		
Probiotic	Student	3	17.65%	47% of group had dental affiliations	
	Works in dentistry	5	29.41%		
	No Affiliation	9	52.94%		

Note. % With Aff = the total percentage of the group with dental affiliations. %Diff bet Groups = difference in percentage of participants with no dental affiliation between placebo and probiotic groups.

Discussion

Summary of Major Findings

During this study, median differences between the probiotic and placebo groups' baseline and final data for both PS and GI were analyzed to answer the research question:

> Will brushing with toothpaste and L. *reuteri* probiotic drops reduce clinical parameters of gingivitis in healthy adults in comparison to brushing with toothpaste and placebo drops?

Data collection showed individual response to the treatment was inconclusive, with some participant's plaque accumulation and gingival inflammation exhibiting reductions, whereas others increased. Statistical analysis revealed the only statistically significant differences were the GI of the placebo group as a whole ($p = 0.001$) and females from the placebo group ($p = 0.004$) (Table 1 and Table 6).

Additional comparisons were made by age and gender (Table 3, Table 4, Table 6, Table 7), and to evaluate the difference between final PS for placebo versus probiotic (Table 5) and final GI for placebo versus probiotic (Table 5), all showing no statistically significant differences between beginning to end of study data. Evaluation was also performed to compare differences in the number of participants with dental affiliations based on gender (Table 8), and differences in dental affiliations between the probiotic and placebo group (Table 9).

Discussion

Plaque score results. Results from this study showed comparable findings to research conducted by Iniesta et al. (2012) where no statistically significant difference

was observed in plaque accumulation between baseline and end of study data for both the test (probiotic) and control (placebo) groups. This result is also consistent with comparable research conducted by Krasse et al. (2005), Sinkiewicz et al. (2013), and Slawik et al. (2011), showing PS between visit one and visit two of the test group to be non-significant (Iniesta et al., 2012; Krasse et al., 2005; Sinkiewicz et al., 2013; Slawik et al., 2011). PS results from this current study actually showed increased plaque accumulation in both the test and control groups, similar to the L. *reuteri* lozenge study conducted by Hallstrom et al. (2013) where all subjects at the end of the study presented with an increase in plaque accumulation and gingival inflammation (Hallstrom et al., 2013). Prior to the beginning of this study, the PI assumed PS would not decrease as a result of brushing with probiotics, as plaque accumulation seemed to be non-significant, or increase, in most of the probiotic studies reviewed (Hallstrom et al., 2013; Iniesta et al., 2012; Krasse et al., 2005; Sinkiewicz et al., 2013; Slawik et al., 2011).

The PI of this study surmises one reason for this outcome could be the shorter duration of time for this study, as this study lasted only three weeks. Longer clinical studies have resulted in PS that did show statistically significant differences between test and control groups from beginning to end of study (Noordin et al., 2007; Shah, 2014; Tekce et al., 2015; Toiviainen et al., 2015; Vivekananda et al., 2010). Another possible reason for this result could have been the lower concentration of probiotic used in this study, the frequency of use each day of the probiotic in comparison to similar studies, and participant compliance to the treatment regimen (Krasse et al., 2005; Sinkiewicz et al., 2013; Tekce et al., 2015; Toiviainen et al., 2015; Vivekananda et al., 2010). This study utilized either placebo or probiotic drops added to the participant's toothbrush prior to

toothpaste once per day. The probiotic used in this study was L. *reuteri* with a probiotic concentration of 1 x 10^8 CFU per dose. Compliance with the treatment regimen of once per day use for this study by participants was 62% (n = 21). Iniesta et al. (2012) utilized a once per day treatment regimen for their systemic probiotic study with 100% compliance for the treatment regimen. Participants (N = 40) in Iniesta et al. (2012) were asked to take a tablet once per day for four week containing either a placebo, or two different strains of L. *reuteri* with probiotic levels of 2 x10^8 CFU per tablet. These researchers observed comparable results to this study, with no significant change in PS between baseline and final data (Iniesta et al., 2012). They attribute this to the short duration of the study, and sample population being dental students.

Probiotic studies utilizing similar or higher concentrations of probiotics, with usage two or more times per day, demonstrated improved PS in the treatment group at the end of the study. In the topical probiotic study by Krasse et al. (2005), probiotic gum was chewed two times each day by the subjects, with a concentration of 1 x 10^8 CFU for each piece of gum. A similar study by Sinkiewicz et al. (2013) had subjects using chewing gum with 2 x 10^8 CFU per gum twice daily. Lozenge studies with similar probiotic concentration to Krasse et al. (2005) and Sinkiewicz et al. (2013) had subjects using the lozenges two to three times a day (Tekce et al., 2015; Toiviainen et al., 2015; Vivekananda et al., 2010). This study may have seen an equivalent reduction in PS had a higher concentration of probiotic drop been used, and the frequency were more than one time per day (Krasse et al., 2005; Sinkiewicz et al., 2013; Tekce et al., 2015; Toiviainen et al., 2015; Vivekananda et al., 2010).

Gingival inflammation results. Prior to the beginning of this study, the PI hoped brushing with probiotics added to toothpaste would promote application of probiotics into the oral environment. Gupta et al. (2010) discussed the relevance of application method and contact time in their systematic review, and recommended more research utilizing topical applications with prolonged contact. In their analysis of available research, Gupta et al. (2010) suggest ingestion of probiotics does not provide prolonged contact with oral tissues, facilitating probiotic adhesion to saliva coated surfaces (Gupta et al., 2010). In a topical study by Amižić et al. (2016), comparisons were made between two different toothpastes containing probiotics, plain toothpaste, and two different antimicrobial mouthrinses. Results of this study revealed probiotic toothpaste to have better inhibitory effects than toothpaste without probiotics. Additionally, this study showed toothpaste to have stronger inhibition properties than mouthrinses (Amižić et al., 2016). Therefore, the PI theorized the action of brushing might improve adherence to the existing mature biofilm, and prolong the contact time of probiotics to oral tissues. The expected outcome was a decrease in gingival inflammation due to improved adherence and application time. Actual results of this study did not corroborate this theory. As previously mentioned, the PI attributes this to frequency of treatment application, thereby resulting in a therapeutic level of the test agent not being achieved, and possibly participant compliance, as some participants did not brush daily with their assigned drops. This lack of compliance would have resulted in even less therapeutic agent being administered to these individuals throughout the study period.

Results of this study comparing GI baseline versus final data showed no significant difference for the probiotic (test) group ($p = 0.485$) (Table 2). Conversely, the

placebo group (control) did show statistically significant differences from the beginning to final scores ($p = 0.001$) (Table 1). This was an unexpected result the PI attributes to participation bias. The placebo group had 59% of the participants with dental affiliations, where participants were dental hygiene students, or worked in the dental field. The probiotic group had 47% of participants with similar dental affiliations. Having dental affiliations would afford these participants a higher level of dental knowledge, and may have made these participants more aware of their oral health status when baseline data was collected. The PI surmises this may have led to an unintentional improvement in these participants previous homecare, resulting in improvements in their GI when comparing baseline versus final data. The results for females in the placebo group (Table 6) were statistically different *(P = 0.004)* as compared to males in this group. The female population of the placebo group had a 27 % greater number of participants with dental affiliations. Therefore, the females in the placebo group had a perceived greater knowledge of the data collection process, and were more likely to exhibit an unintentional participation bias. This difference may have been enough to account for the statistically relevant difference between females and males GI scores in the placebo group.

Observer bias may have also effected the outcome of the placebo group's GI. Research assistants collecting data may have been expecting to see improved results in participants, and overestimated their oral health when collecting final data, causing lower GI scores to be recorded for some participants. In order to produce a statistically significant difference in GI when comparing baseline to final data, it is possible a combination of both participation and observer bias may have contributed to the placebo

group's results. Shimauchi et al. (2008) also postulates participation bias may have altered the findings of their 2008 study, since the only statistically significant difference in clinical parameters for this study were in a small subgroup of participants who were smokers. This subgroup noticed a decrease in PS as compared to all other groups (Shimauchi et al., 2008).

Research studies seeking to determine changes in the clinical parameter of gingival inflammation, have had mixed outcomes. Several systemic and topical application studies have been able to demonstrate statistically significant differences in GI (Harini et al., 2010; Nadkemy, 2015; Noordin et al., 2007; Karuppaiah et al., 2011; Tekce et al., 2015; Toiviainen et al., 2015; Vivekananda et al., 2010; Yousuf et al., 2007). Although findings of this study were not as originally expected, and no significant differences in GI could be noted, other studies evaluated in the literature review for this study have similar conclusions. Systemic probiotic studies by Iniesta et al. (2012), Montero et al. (2017), and Shimauchi et. al. (2008) all had no significant change in GI between baseline and final data. Studies utilizing topical introduction of probiotics have also had some unexpected results. Research by Shah (2014), and Hallstrom et al. (2013) also presented discouraging outcomes due to results not demonstrating significant differences in the placebo or probiotic groups GI (Hallstrom et al., 2013; Shah, 2014). The PI of this study speculates the study limitations of a short study duration, in conjunction with the lower overall amount of therapeutic agent applied, may have contributed to GI results not being statistically significant. Participation compliance was less than 60% for this study, and the study design of using the drops once per day was less than many similar studies that used therapy application two to three times per day.

Limitations

As with all research, the question of attention or participation bias, also known as the Hawthorne Effect, is a notable possible limitation. This phenomenon occurs when it appears changes in behavior of research participants may be due to the knowledge they are being evaluated. The main concern with this limitation is the possibility of an increased false negative result due to participation awareness (McCambridge, Witton, & Elbourne, 2014).

Observer bias is also a limitation to any clinical research study. The expectation of seeing a certain result, or improvement, in participants can lead research observers to determine results to be more favorable. Comparable studies have tried to limit observer bias by starting all participants on the same day, rather than multiple days, and assessments were conducted by the same observer (Krasse et al. 2005). Due to the challenges of obtaining research subjects, the PI of this study found it necessary to start participants on multiple days, and to use two research assistants, as well as herself, to collect data. Although effort was made to provide calibration among research observers, the PI acknowledged the need for additional calibration for future research studies.

Participant compliance to study protocol is another limitation, increasing the possibility of outliers and inconsistent results. Participants in this study were asked to brush once a day with their study drops, but refrigeration of the drops was necessary to preserve the live, active cultures in the probiotic drops. Remembering to retrieve the drops from the refrigerator before brushing was a concern during the study design. In order to help decrease this limitation, the PI gave each participant a laminated study reminder card highlighting the important details of the study. Additional concerns noted

by the participants regarding compliance were forgetting to use the drops because of travel for work or pleasure, the drops were not part of their regular oral health regimen, and difficulty getting the drops out of the bottle due to the slow dropper and cold liquid. Also, the amount or dosage used for each application may not have been equal because of the difficulty of getting the drops to come out of the vial. Participants were advised 5 drops per application was the recommended study dose. A few participants ran out of study drops before the end of the study, and had to contact the PI to obtain more, and some participants returned to final data collection with left over drops in excess of what was expected if 5 drops were used each day for the 3-week study period.

One final limitation of this study is the frequency of probiotic application per day in comparison to similar studies. This study had participants use the probiotic and placebo mediums once per day. All other comparative research had subjects using the probiotic and placebo two or more times per day (Krasse et al., 2005; Nadkemy et al., 2015; Noordin et al., 2007; Sinkiewicz et al., 2013; Tekce et al., 2015; Toiviainen et al., 2015; Vivekananda et al., 2010). The lack of statistically significant results in regard to the probiotic medium in this study could be attributed to the frequency of use, and therefore the overall amount to treatment received by the participants. Once a day use may not have been enough to see any result, as the study results indicates. One of the methodology concerns the PI had when designing this study was not wanting to alter the participant's home care routine, causing an inadvertent improvement in PS and GI. Therefore, the PI choose the lowest acceptable level of brushing each participant should have already been performing as the amount of frequency for the probiotic drop application, as each participant was brushing with the study therapy. Participants were

allowed to brush additional times per day, if that was already part of their pre-study oral hygiene regimen. Consequently, the test and control therapies were only administered one time per day.

Recommendations/Suggestions for Future Research

Recommendations that might improve future research were noted during the implementation of this study. Initially, utilizing three test groups rather than two may have been advisable to compare brushing with toothpaste alone versus toothpaste plus placebo drops, and toothpaste plus probiotic drops. There is no research to know how brushing with the drops containing sunflower oil would impact oral tissues. Additionally, a crossover study design might have been beneficial, as results from using both the test drop and the placebo drop could have been compared on each participant (Iniesta et al., 2012). Comparisons from a crossover study would potentially have more reliability of results then the traditional pre and post treatment comparisons performed in this study (Iniesta et al., 2012). The PI initially designed this study to utilize a cross-over method with an initial 3-week phase, a 2-week wash out period, and a second 3-week phase where participants would use each drop. For pragmatic reasons of time and cost, the PI choose to reduce the study to a 3-week clinical trial, and eliminate the wash out and secondary cross-over phase of the study.

Future studies may consider utilizing a larger sample size, and a longer study period, where participants could return to the test site daily to brush with the test or placebo toothpaste under supervision. This would ensure equity of the amount of toothpaste used, and length of time brushing, etc. For this study, the PI requested participants not change anything about their current homecare routine, except to use the

study toothpaste and add the assigned test or placebo drop prior to brushing. Rationale for this design being whatever the study subjects were doing prior to the study resulted in their current oral health condition. The PI did not want to alter their regimen, as this might cause an improvement in oral health, therefore making it difficult to determine if improvements resulted from the study drops or the changes in participant's oral hygiene. Making participation compliance easier in future studies could be achieved by compounding a toothpaste with a stabilized form of L. *reuteri* that does not need to be refrigerated or added to anything. This would help to confirm the dosage or amount of probiotic being administered was the same for each use, and for all participants.

Comparable research studies have implemented a prophylaxis prior to the beginning of the study (Karuppaiah et al.,2013; Nadkemy et al., 2015). This methodology takes advantage of the competitive exclusion principle in regard to probiotic mechanism of action. This principle suggests two species competing for the same resources cannot steadily co-exist (Teughels et al., 2011). Providing a teeth cleaning prior to beginning a topical probiotic research study may improve the opportunity for adherence of probiotic bacteria to the teeth and oral tissues, due to the elimination of competition for adhesion sites and nutrients (Teughels et al., 2011). Another reason for a prophylaxis prior to beginning a topical study would be for the removal of calculus. The PI noticed on participants with calculus present, bleeding and GI were always higher, and bleeding was present at baseline and final data collection. Removing this known local irritant would help to eliminate a variable that cannot be predicted or controlled without elimination. Some studies implementing a prophylaxis prior to the beginning of the study also incorporated and waiting period of two to three

weeks before beginning the study, to allow for the participant's normal oral conditions to return. Baseline data was then collected without the presence of any other local irritants besides plaque accumulation (Karuppaiah et al.,2013; Nadkemy et al., 2015).

Future research should also consider the frequency and amount of probiotic being administered. This study utilized a once a day, topical, application of the probiotic L. *reuteri* by adding drops containing the probiotic to a toothbrush. Participants then added the study toothpaste, and brushed following their usual oral hygiene regimen. This method had pros and cons to its design. Positive aspects of this method were by not giving home care instructions, or making any changes to the participant's oral hygiene, improvements to PS and GI due to intervention were minimized. Conversely, not standardizing participant's oral hygiene may have produced a situation where probiotic application was not administered long enough, effectively, or in large enough quantities to produce a desired result, or any result. Therefore, future research designs should consider requesting participants brush more than one time per day, and give oral hygiene instruction for the recommended brushing and flossing technique to be used during the study. Having participants use the probiotic or placebo therapy two or more times per day would help ensure adequate dosage of therapeutic agents (Krasse et al., 2005; Nadkemy et al., 2015; Noordin et al., 2007; Sinkiewicz et al., 2013; Tekce et al., 2015; Toiviainen et al., 2015; Vivekananda et al., 2010). Standardizing participant's oral hygiene for the study period would help to ensure consistent application time and equal amounts of probiotic or placebo agents are administered. Participants should be interviewed at the beginning and end of the study about their current homecare regimen, and any changes during the study should be noted for evaluation during statistical

analysis. These suggestions should help to eliminate outliers and unintentional outcomes to future research.

Conclusions

Although results of this clinical study were unexpected, further research regarding the use of probiotics as a natural, healthy alternative therapy to antibiotics for the treatment and management of oral diseases should continue to be evaluated. The only statistically significant differences observed in this study were the GI of the placebo group as a whole ($p = 0.001$), and females from the placebo group ($p = 0.004$). Several research studies reviewed in the process of completing this thesis have shown probiotics have great possibilities in the oral cavity. Most researchers agree the type, application method, and quantity of probiotic needed to be effective at treating and managing oral diseases has yet to be determined. This study confirms frequency of administration of probiotics, and therefore overall quantity of probiotics administered, is a considerable factor in order to achieve a desired effect. Once a day application is not adequate to obtain a dosage potent enough to observe a statistically significant response. Future research should focus on developing the foundation of knowledge already established, to increase understanding and ability to translate this knowledge into clinical practice.

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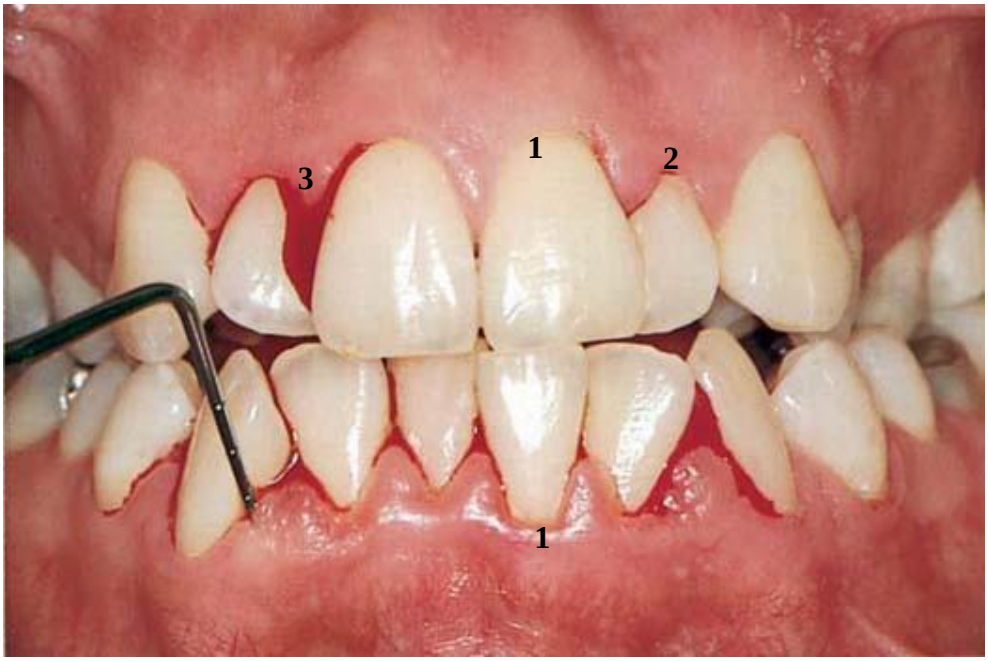
Appendix A
Instruction form for modified gingival index

Modified Gingival Index: (Löe-Silness, 1963)
(Adapted from Quizlet Inc. 2017). Retrieved from https://quizlet.com/32081334/community-health-dental-indices-scoring-methods-flash-cards/

- Score assigned to each of the four areas by inserting probe (based on color, consistency & bleeding)
- Scores facial, lingual, mesial, distal (mesial and distal evaluated from buccal side of teeth) (Lang et al., 1973)

SCORE	CRITERIA
1	Normal gingiva or slight color change, mild edema, slight texture change; **no bleeding**
2	Redness, hypertrophy, edema, glazing; **slight bleeding**
3	Marked redness, hypertrophy, edema, ulceration; **moderate to heavy bleeding**

Method of evaluation:
Insert probe into sulcus 1-2 mm, gently press against gingiva to determine firmness
Move probe circumferentially in horizontal stroke along soft tissue side of pocket
Scoring: give each tooth assessed a score of 1-3, and divide score of each tooth by 4



Appendix B
Modified Löe-Silness gingival index form

Participant/Drop Number: Group Assigned: (circle one) Group A Group B

Collection Interval: (circle one) Baseline Week 3

Date:

Tooth #	2	3	4	5	6	7	8		9	10	11	12	13	14	15
Distal								Mesial							
Facial								Facial							
Mesial								Distal							
Lingual								Lingual							
Total Score / 4															

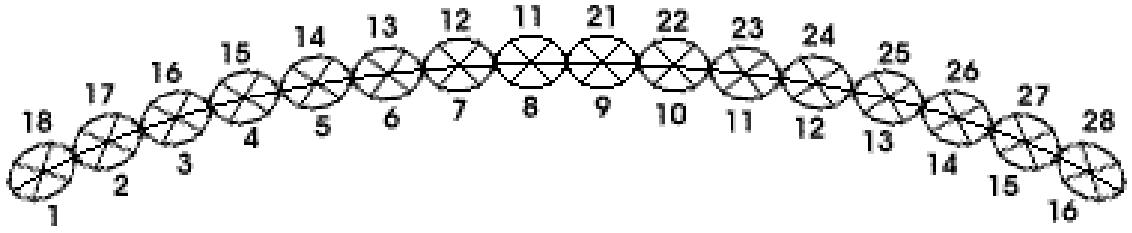
Tooth #	31	30	29	28	27	26	25		24	23	22	21	20	19	18
Distal								Mesial							
Facial								Facial							
Mesial								Distal							
Lingual								Lingual							
Total Score / 4															

Appendix C
O'Leary plaque score form

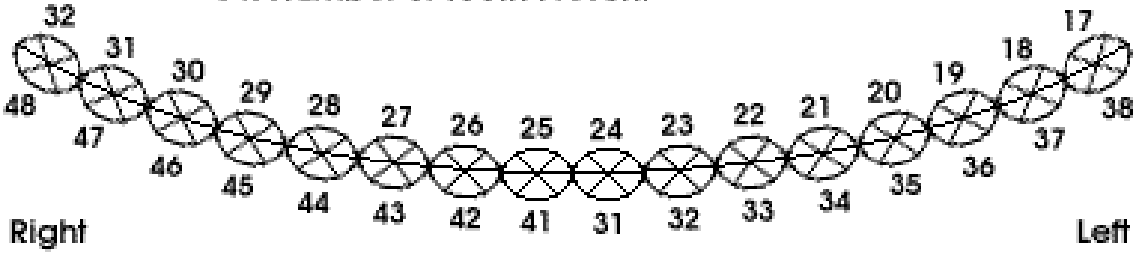
Participant/Drop Number: Group Assigned: (circle one) Group A Group B

Collection Interval: (circle one) Baseline Week 3

Date:



$$\frac{\text{Total Number Tooth Parts With Plaque}}{6 \times \text{Number of Teeth Present}} \times 100 = \_\_\_\_\_\%$$



Appendix D
Inclusion and exclusion criteria form

Inclusion Criteria (Appendix H, Appendix I)

- Healthy Adults between the ages of 18 and 65
- At least 20 evaluable teeth
- Willing to complete the full 8-week study and attend screening and data collection dates
- Habit of brushing teeth at least 1 time per day
- Participant needs to exhibit gingivitis
- Participants must be able to read and write the English language at an eighth grade level or higher

Exclusion Criteria (Appendix H, Appendix I)

- Individuals currently using, or with previous use, of antibiotics for 30 days prior to the beginning of the study. Anyone requiring antibiotic treatment after the beginning, and during the study, will need to be excluded at that time.
- Individuals who regularly use (more than 3 times per week) probiotics, or food containing probiotics, for 30 days prior to the beginning of the study. (see entire list in Appendix I)
- Anyone needing to use probiotics for health reasons, or foods containing probiotics, on a regular basis (3 or more times per week) during the 3-week study
- Individuals with a systemic disease or chronic illnesses from the list below: Robinson, J. (2016). What are Autoimmune Disorders? Retrieved from https://www.webmd.com/a-toz-guides/autoimmune-diseases?

∗ Note: Participants with well controlled diabetes, and/or taking diabetes medication, with an A1C of 7 or less may participate in the study. Also, individuals with systemic illnesses in remission or well controlled with medication, exhibiting no signs or symptoms of the disease, may be in the study).

  - Patients diagnosed with Type I or Type II Diabetes, with an A1C over 7
  - Patients with autoimmune diseases, not in remission or controlled by medication, such as:
    a) Rheumatoid Arthritis
    b) Systemic Lupus Erythematosus (Lupus)
    c) Multiple Sclerosis (MS)
    d) Inflammatory Bowel Disease (IBS), Crohn's Disease, Ulcerative Colitis
    e) Guillain-Barre Syndrome
    f) Chronic Inflammatory Demyelinating Polyneuropathy
    g) Psoriasis

    h) Graves' Disease
    i) Hashimoto's Thyroiditis
    j) Myasthenia Gravis
    k) Vasculitis
  - Patients with Liver disease, Hepatitis B or C or elevated liver enzymes
  - Patients that are immune compromised, on immunosuppressive medications, have AIDS, or HIV
  - Pulmonary Disease such as Chronic Obstructive Pulmonary Disease (COPD), Emphysema, or asthma requiring the use of inhalers or nebulizers one or more times each day.
  - Patients using the following medications on a daily basis:
    a) Calcium Channel blockers
    b) Blood thinners/anti-coagulants
    c) Asthma inhalers
    d) Dilantin
    e) Prednisone
    f) Anti-inflammatory
    g) Rheumatoid Arthritis
    h) Antibiotics
    i) Probiotics
    j) Cyclosporine
- Individuals with an allergy or adverse reactions to sunflower oil, Regular flavor Colgate toothpaste, fluoride, or the probiotic L. *reuteri*
- Individuals needing antibiotic pre-medication or condition requiring antibiotic premedication prior to dental treatment
- Current users of: (see entire list in Appendix I)
  - Smoking tobacco products such as cigarettes, cigars, pipes
  - Nicotine products used orally such as gum or lozenges
  - Electronic cigarettes or vaping devices
  - Hookah
  - Smoking marijuana
  - Marijuana sublingual drops
- Anyone currently using chlorhexidine oral solutions
- Women who are pregnancy, lactating, or breastfeeding
- Individuals cannot have had a dental prophylaxis or nonsurgical periodontal therapy for 6 weeks prior to the beginning of the study.

Appendix E
Medical history

Time 3:03 PM E.W.U. Dental Hygiene Date 1/8/2018

Eaglesoft Medical History - Adult Age 14 and over

Patient Name: Birth Date: Date Created:

Although dental personnel primarily treat the area in and around your mouth, your mouth is a part of your entire body. Health problems that you may have, or medication that you may be taking, c receive. Students are required to obtain a detailed medical and dental history prior to starting dental hygiene procedures. Information is confidential and is considered essential for complete care.

Dental Questions

Are you having dental pain or discomfort at this time?	○ Yes ○ No	If yes	
Have you had any complications associated with previous dental treatment?	○ Yes ○ No	If yes	
Date of your last dental visit:		Comment	
Dentist's Name / Address / Phone:		Comment	
Do you have any swelling or sores in your mouth?	○ Yes ○ No	If yes	
Do you have a dry mouth?	○ Yes ○ No	If yes	

How stressful are dental appointments for you? (Please select one of the following):

☐ None ☐ Slight ☐ Moderate ☐ Extreme

Dental Anxiety Comments:		Comment	

Medical Questions

Are you under a physician's care now or within the last 2 years?	○ Yes ○ No	If yes	
Date of your last physical examination?		Comment	
Doctor's Name / Address / Phone:	○ Yes ○ No	If yes	
Have you ever been hospitalized or had a major operation?	○ Yes ○ No	If yes	
Have you ever had a serious head or neck injury?	○ Yes ○ No	If yes	
Have you ever taken Phen-Fen or Redux?	○ Yes ○ No	If yes	
Have you ever taken Fosamax, Boniva, Actonel or any other medications containing bisphosphonates?	○ Yes ○ No	If yes	
Are you on a special diet?	○ Yes ○ No	If yes	
Do you eat a well balanced diet?		Comment	
Have you experienced any recent weight changes?	○ Yes ○ No	If yes	
Do you have issues with frequent urination?	○ Yes ○ No	If yes	
Do you have swollen glands in your neck?	○ Yes ○ No	If yes	
Do you use tobacco?	○ Yes ○ No	If yes	
Do you use marijuana?	○ Yes ○ No	If yes	
Do you use controlled substances?	○ Yes ○ No	If yes	
Is there anything you feel may casue you to be more at risk for infectious diseases?	○ Yes ○ No	If yes	

Women

Women: Are you...

☐ Pregnant ☐ Trying to get pregnant ☐ Nursing

☐ Taking Oral Contraceptive ☐ Using Topical Contraceptive ☐ Post Menopause

Allergies

Are you allergic (Itching, Rash, Swelling) or made sick by any of the following?

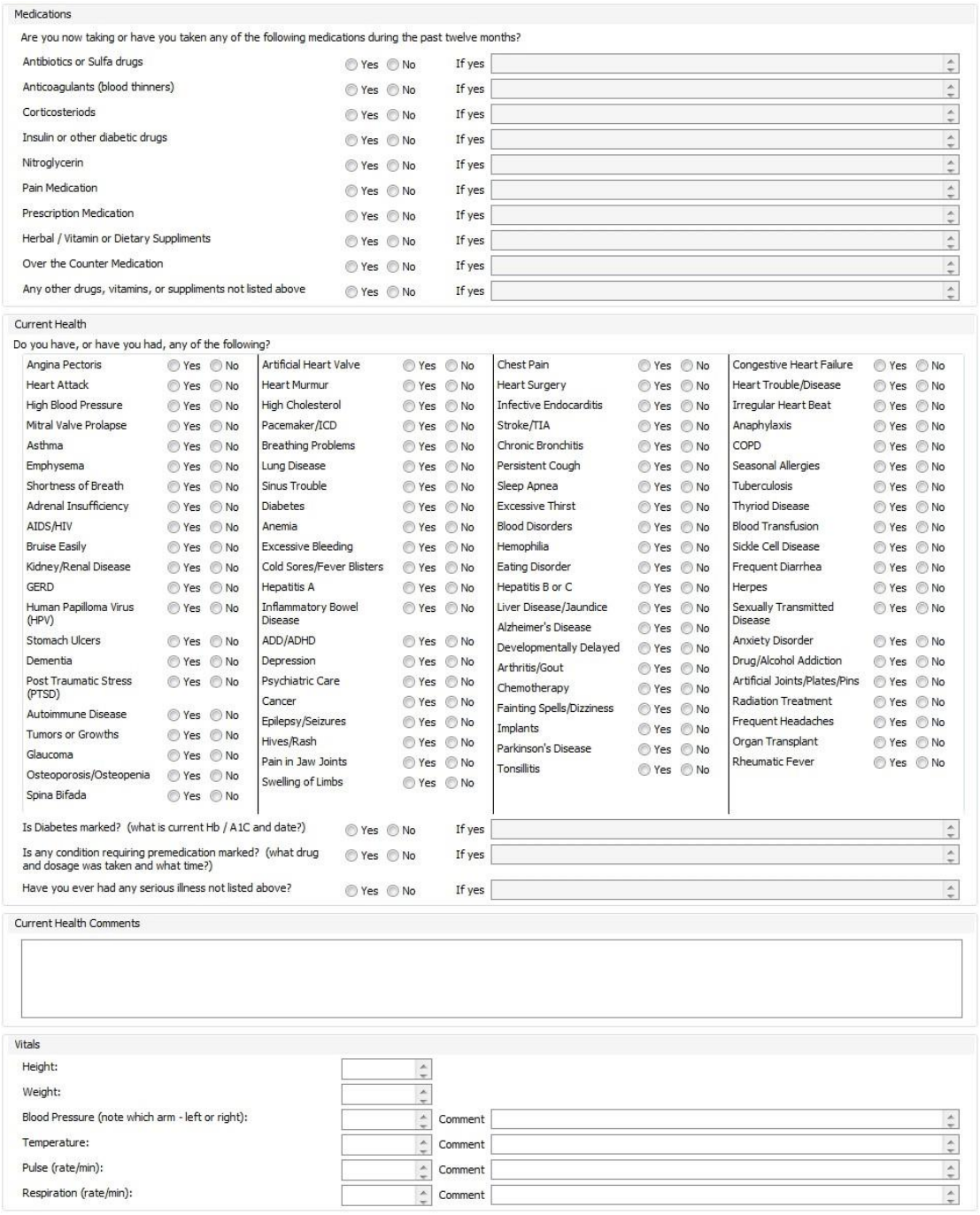
☐ Acrylic ☐ Aspirin ☐ Barbituates, Sedatives, or Sleeping Pill ☐ Codeine

☐ Fluoride ☐ Food or Nuts ☐ Gluten ☐ Latex

☐ Local Anesthetics ☐ Metal ☐ Penicillin ☐ Sulfa Drugs

☐ No Allergies

Any other allergies not listed above?	○ Yes ○ No	If yes	



Medications

Are you now taking or have you taken any of the following medications during the past twelve months?

Antibiotics or Sulfa drugs	Yes No	If yes	
Anticoagulants (blood thinners)	Yes No	If yes	
Corticosteriods	Yes No	If yes	
Insulin or other diabetic drugs	Yes No	If yes	
Nitroglycerin	Yes No	If yes	
Pain Medication	Yes No	If yes	
Prescription Medication	Yes No	If yes	
Herbal / Vitamin or Dietary Suppliments	Yes No	If yes	
Over the Counter Medication	Yes No	If yes	
Any other drugs, vitamins, or suppliments not listed above	Yes No	If yes	

Current Health

Do you have, or have you had, any of the following?

Angina Pectoris Yes No	Artificial Heart Valve Yes No	Chest Pain Yes No	Congestive Heart Failure Yes No
Heart Attack Yes No	Heart Murmur Yes No	Heart Surgery Yes No	Heart Trouble/Disease Yes No
High Blood Pressure Yes No	High Cholesterol Yes No	Infective Endocarditis Yes No	Irregular Heart Beat Yes No
Mitral Valve Prolapse Yes No	Pacemaker/ICD Yes No	Stroke/TIA Yes No	Anaphylaxis Yes No
Asthma Yes No	Breathing Problems Yes No	Chronic Bronchitis Yes No	COPD Yes No
Emphysema Yes No	Lung Disease Yes No	Persistent Cough Yes No	Seasonal Allergies Yes No
Shortness of Breath Yes No	Sinus Trouble Yes No	Sleep Apnea Yes No	Tuberculosis Yes No
Adrenal Insufficiency Yes No	Diabetes Yes No	Excessive Thirst Yes No	Thyriod Disease Yes No
AIDS/HIV Yes No	Anemia Yes No	Blood Disorders Yes No	Blood Transfusion Yes No
Bruise Easily Yes No	Excessive Bleeding Yes No	Hemophilia Yes No	Sickle Cell Disease Yes No
Kidney/Renal Disease Yes No	Cold Sores/Fever Blisters Yes No	Eating Disorder Yes No	Frequent Diarrhea Yes No
GERD Yes No	Hepatitis A Yes No	Hepatitis B or C Yes No	Herpes Yes No
Human Papilloma Virus (HPV) Yes No	Inflammatory Bowel Disease Yes No	Liver Disease/Jaundice Yes No	Sexually Transmitted Disease Yes No
Stomach Ulcers Yes No	ADD/ADHD Yes No	Alzheimer's Disease Yes No	Anxiety Disorder Yes No
Dementia Yes No	Depression Yes No	Developmentally Delayed Yes No	Drug/Alcohol Addiction Yes No
Post Traumatic Stress (PTSD) Yes No	Psychiatric Care Yes No	Arthritis/Gout Yes No	Artificial Joints/Plates/Pins Yes No
Autoimmune Disease Yes No	Cancer Yes No	Chemotherapy Yes No	Radiation Treatment Yes No
Tumors or Growths Yes No	Epilepsy/Seizures Yes No	Fainting Spells/Dizziness Yes No	Frequent Headaches Yes No
Glaucoma Yes No	Hives/Rash Yes No	Implants Yes No	Organ Transplant Yes No
Osteoporosis/Osteopenia Yes No	Pain in Jaw Joints Yes No	Parkinson's Disease Yes No	Rheumatic Fever Yes No
Spina Bifada Yes No	Swelling of Limbs Yes No	Tonsillitis Yes No	

Is Diabetes marked? (what is current Hb / A1C and date?)	Yes No	If yes	
Is any condition requiring premedication marked? (what drug and dosage was taken and what time?)	Yes No	If yes	
Have you ever had any serious illness not listed above?	Yes No	If yes	

Current Health Comments

Vitals

Height:			
Weight:			
Blood Pressure (note which arm - left or right):		Comment	
Temperature:		Comment	
Pulse (rate/min):		Comment	
Respiration (rate/min):		Comment	

Patient ASA

Emergency Contact

Emergency Contact Name / Phone: Comment

Signature - Patient

To the best of my knowledge, the questions on this form have been accurately answered. I understand that providing incorrect information can be dangerous to my (or patient's) health. It is my responsibility to inform the dental office of any changes in medical status.

Signature of Patient, Parent or Guardian:

X Date:_____

Signature - Student

To the best of my ability, I have reviewed this medical history for accuracy.

Signature of Student:

X Date:_____

Signature - Faculty

To the best of my ability, I have reviewed this medical history for accuracy.

Signature of Faculty:

X Date:_____

Appendix F
Brushing with probiotics research study questionnaire

1. Have you use any antibiotics or probiotic supplements within the last 30 days?
   YES_____ NO_____. If YES, please give details below.

   Name of antibiotic or type of probiotic?

   Length of time used?

   Date of last use?

   Do you foresee a need to use this product again during the 3-week research study?
   YES_____ NO_____.

2. Do you regularly (3 or more times per week) eat food, or drink beverages, which contain probiotics or live active bacterial cultures such as yogurt, Kombucha, salad dressings made with yogurt or live active cultures, etc.
   YES_____ NO_____. If YES, please give details below.

   Is usage for a medical purpose or condition?

   Type of food or beverage?

   If probiotic, what type of bacteria does this item contain?

   Amount of food or beverage consumed during use?

   Are you willing /able to discontinue use for 30 days prior to the beginning of the study, and during the 3-week study period, as part of this research study?
   YES_____ NO_____.

3. Do you have any known allergies or adverse reactions to Colgate Cavity Protection regular flavor toothpaste?
   YES_____ NO_____. If YES, please give details below.

4. Do you have any known allergies or adverse reactions to sunflower oil?
   YES_____ NO_____. If YES, please give details below.

5. Do you have any known allergies or adverse reaction to the probiotic Lactobacillus *reuteri*?
   YES_____ NO_____. If YES, please give details below.

6. Do you use any tobacco, nicotine, electronic cigarettes, hookah, or marijuana products?
   YES_____ NO_____. If YES, please give details below.

7. How often do you brush your teeth?

8. Do you use dental floss, oral irrigators such as a Waterpik™, or any other adjunctive teeth cleaning items?
   YES_____ NO_____. If YES, please give details below.

   Item used.

   How often?

Complete this section after finishing the study

9. Have you changed anything about your homecare routine since starting the study?
   YES_____ NO_____. If YES, please give details below.

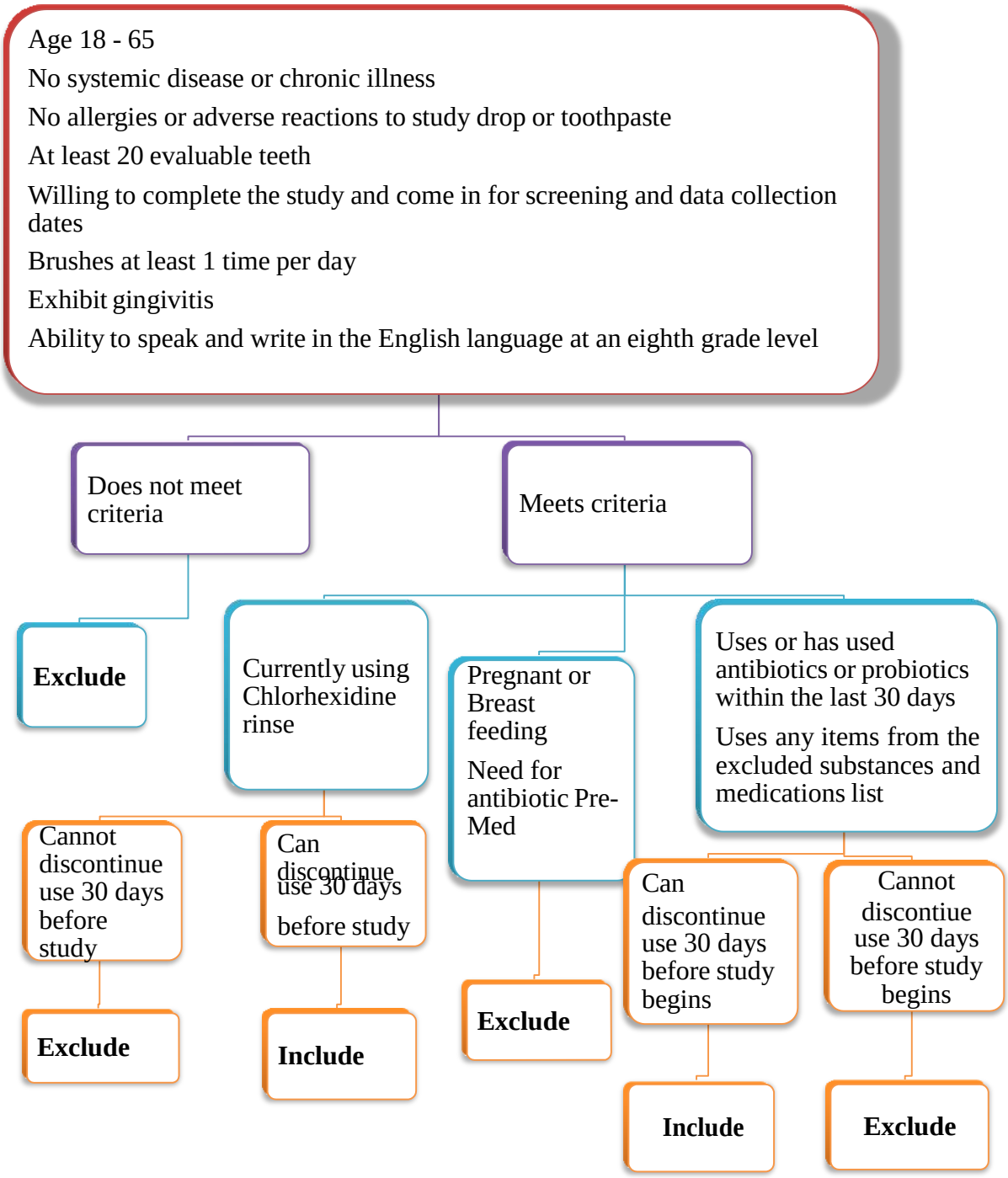
10. Have you used any of the medications, substances, or food items on the exclusion list since beginning the study?
    YES_____ NO_____. If YES, please give details below.

Appendix G
Research study schedule

(Sample with a Thursday start date)

FEBRUARY							
	Sun.	**Mon.**	**Tues.**	**Wed.**	**Thurs.**	**Fri.**	**Sat.**
Begin Study					**1** **Collect Baseline Data** Use Drops and Tooth paste	**2** Use Drops and Tooth paste	**3** Use Drops and Tooth paste
Week 1	**4** Use Drops and Tooth paste	**5** Use Drops and Tooth paste	**6** Use Drops and Tooth paste	**7** Use Drops and Tooth paste	**8** Use Drops and Tooth paste	**9** Use Drops and Tooth paste	**10** Use Drops and Tooth paste
Week 2	**11** Use Drops and Tooth paste	**12** Use Drops and Tooth paste	**13** Use Drops and Tooth paste	**14** Use Drops and Tooth paste	**15** Use Drops and Tooth paste	**16** Use Drops and Tooth paste	**17** Use Drops and Tooth paste
Week 3	**18** Use Drops and Tooth paste	**19** Use Drops and Tooth paste	**20** Use Drops and Tooth paste	**21** Use Drops and Tooth paste	**22** **Collect Data – study complete**		

Appendix H
Decision tree



Appendix I
Excluded substances, medications, and foods

Probiotics of any form:

Gum, Lozenges, Mouthrinses, Tablets, Capsules

Medications on the list below:

Calcium Channel blockers such as: Amlodipine, Caduet, Lotrel, Exforge, Twynsta, Tribenzor, Prestalia, Azor, Cardizem/Diltiazem, Clevidipine/Cleviprex, Felodipine/Cardene, Nisoldipine/Sular, Verapimil/Calan, Nimodipine, Nicardipine, Isradipine

Blood thinners/anti-coagulants such as: Coumadin, Warfin, Plavix, Aspirin

Diabetes medications such as: Insulin, Metformin, Glucophage, Lantis, etc.

Asthma inhalers or other oral inhalers such as Advair, ProAir, Flovent, etc.

Dilantin or other seizure medications

Prednisone or other steroids

Anti-inflammatory medications such as Naproxen, Ketoprofen, Ibuprofen, Indomethicin, Sulindac, Meclofenamate, Toradol, Feldene, Diclofenac, Celebrex, Mobic/Meloxicam

Rheumatoid Arthritis medications such as Methotrexate, Celebrex, Plaquenil, Enbrel, Remicade, Humira, Sulfasalizine, Arava, etc.

Antibiotics – all

Probiotics – all

Cyclosporine or other immunosuppressive drugs

Foods containing probiotics, natural live active cultures, or added live active cultures (Probiotics Now, n.d.):

Yogurt
Kombucha
Salad dressings made from yogurt
Curd
Buttermilk or products made from buttermilk
Kefir
Cultured cottage cheese
Miso
Sauerkraut
Kimchi
Pickles and olives made with traditional methods

Appendix J

Recruitment flyer

GOT TEETH?

If you are age 18 or older, you could be a participant in an exciting research study testing toothbrushing with probiotics! This study will take place at Eastern Washington University in the Dental Hygiene Department, Spokane Campus, as part of a master's thesis study.

Each person who completes the study will receive a $5 Starbucks gift card, and will be entered into a drawing for a chance to win a Sonicare Diamond Clean electric toothbrush or professional teeth whitening.

For more information, contact:

Cheri Barton RDH, BS, MSDH (c)
Text: (208) 964-5868
Email: cbarton2015@eagles.ewu.edu

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Appendix K
Informational meeting and hand-on training lesson plan

TITLE: Efficacy of Brushing with Probiotics Information and Hands-On Training
Facilitator: Cheri Barton RDH, BS, MSDH (c)

ESTIMATED LENGTH: 45 minutes

INSTRUCTIONAL METHOD:
Facilitated discussion and hands-on practice using study forms and data collection methods

EDUCATIONAL GOAL:
To introduce thesis study to volunteers, demonstrate use of study forms, and practice data collection methods.

INSTRUCTIONAL OBJECTIVES:
- Explain thesis study (Appendix G, M, K)
- Discuss screening process
  - Medical History and Questionnaire (Appendix E, F)
  - Inclusion and Exclusion Criteria (Appendix D, H, I, J)
- Review data collection forms and methods (Appendix A, B, C)
- Practice use of the O'Leary Plaque Score and Löe-Silness Gingival Index on partners – RA's
- Practice using the PS and GI forms by recording data for RA partners – CA's

INSTRUCTIONAL MATERIALS:
- Dental operatory chair, clinician chair
- Armamentarium including mirror, probe, air water syringe tip, saliva ejector, gauze, cotton tip applicators, disclosing solution, Vaseline
- PPE including gloves, mask, safety glasses/loupes
- Copies of study forms and appendices

LEARNING ACTIVITY: (40 minutes)
Volunteers of the study will value the implementation process through actively engaging in a discussion on screening and data collection protocol. The PI, RA's and CA's of the study will practice using the study forms and designated PS and GI methods to collect data on a partner.

QUESTION AND ANSWER SESSION: (5 minutes)
Discuss questions regarding the study and implementation.

Appendix L
Study reminder card

STUDY REMINDERS
• KEEP DROPS REFRIDGERATED WHEN NOT USING
• SHAKE DROPS WELL BEFORE EACH USE
• USE DROPS ONLY ONE TIME PER DAY, EVEN IF YOU BRUSH MORE OFTEN
• USE ONLY 5 DROPS – ADD TO TOOTHBRUSH FIRST, THEN ADD TOOTHPASTE
• USE ONLY THE STUDY TOOTHPASTE WHEN BRUSHING
• DO NOT RINSE, EAT, OR DRINK AFTER BRUSHING FOR 30 MINUTES
• DO NOT CHANGE YOUR REGULAR ORAL HYGIENE ROUTINE
• CHECK OFF THE STUDY SCHEDULE EACH DAY THAT YOU USE THE DROPS
• BRING STUDY SCHEDULE TO EACH DATA COLLECTION

Appendix M
Informed consent document

Consent Form

Efficacy of brushing with probiotics for the reduction of gingivitis

Principal Investigator:
Cheri L. Barton RDH, BS, MSDH (c)
MSDH Student/Part-time Clinical Professor
Eastern Washington University
310 N. Riverpoint Blvd. Box E
Spokane, WA 99202
(208)964-5868 (cell)
(509) 828-1300 (Dental Hygiene Dept. office)

Responsible Project Investigator:
Lisa Bilich RDH, MSEd, CHSE
Professor of Dental Hygiene
Eastern Washington University
310 N. Riverpoint Blvd. Box E
Spokane, WA 99202
(509)828-1295

Investigator's Statement

This study will attempt to determine the effectiveness of brushing with the probiotic Lactobacillus *reuteri*, as an application method by evaluating the results on a common oral disease, gingivitis.

Purpose and Benefits

The aim of this study is to evaluate the response adding probiotics to toothpaste prior to brushing will have on gingivitis. Gingivitis is an inflammation of the gum tissue that surrounds the teeth, and is caused by an accumulation of plaque. Plaque is a sticky biofilm made up of the natural fluid found in the mouth called saliva, sugars from the foods we eat and drink, and bacteria that are normal to the oral environment. Probiotics are healthy bacteria that are found naturally in many foods and beverages, and can also be added to a person's diet as a supplement, similar to vitamins and minerals. This clinical study is being conducted as part of a master's thesis for partial completion of the requirements needed to receive a master's of dental hygiene degree from Eastern Washington University (EWU).

Procedures

This study will be a 3-week clinical trial using either probiotic drops containing L. *reuteri*, or a placebo drop consisting of sunflower oil, added to a plain fluoride toothpaste. If admitted into the study, you will need to come to the EWU dental hygiene clinic two (2) times for data collection, for approximately one hour each. The schedule for this will be at the beginning of the study, and after three weeks has passed, at the end of the study. Participation is voluntary, and you may drop out of the study at any time, if you choose.

In order to qualify for this study, PI requests that you undergo a screening process to assess your eligibility. The screening process will take approximately 2 hours. During the screening, you will need to fill out a health history which will include demographic information such address, phone number, and health information such as current and past illnesses, medications, alcohol use, and drug use. You will also be asked to fill out a questionnaire at the beginning and the end of the study regarding your use of probiotics and antibiotics. You may choose not to answer any question you find objectionable. Below is a sample question asked on the questionnaire:

11. Have you use any antibiotics or probiotic supplements (tablets or pills) within the last 30 days? YES_____ NO_____. If YES, please give details below.

The screening process will follow this model:

Screening process:

Phase One: A group presentation will be conducted by the PI to introduced and explain the study. Questions regarding the study will be answered, and if you are interested in being a study participant, you will be given the informed consent document to read and sign.

Phase Two: After you have signed the informed consent document, you will be asked to fill out a medical history and questionnaire.

Phase Three: If not excluded from the study in phase two, the PI, or one of her research assistants, will conduct a clinical screening on you to ensure you qualify for the study. The clinical screening will consist of a plaque score, evaluating visible plaque preset on your teeth, and a gingival inflammation score, evaluating gum areas that appear inflamed. After acceptance into the study, you will be given other forms and information regarding the beginning of the study.

Risk, Stress or Discomfort

Any supplement has the possibility of producing an unwanted response from the body or an adverse reaction. It is also possible to be allergic, or become allergic, to a substance at any time. Risk level for this study has been determined to be minimal.

The probiotic drops to be used for this study contain no milk protein or lactose, nuts, peanuts, soy, corn, gluten, wheat, eggs, fish, shellfish, artificial ingredients or flavors. The placebo drop will contain only sunflower oil, a common oil used for cooking purposes, and also the primary ingredient in the probiotic drops being used for this study.

While possible side effects or allergic reactions for this study are rare, they include:

- Gastrointestinal upset, gas, bloating, diarrhea, nausea
- Allergic reaction to fluoride or the plain fluoridated study toothpaste
- Allergic reaction to sunflower oil, L. *reuteri*, or any component of the study or placebo drops

Possible signs and symptoms of an allergic reaction may present as described below:

- Gastrointestinal upset, gas, bloating, diarrhea, nausea
- Oral conditions of the gums or oral tissues such as increased swelling, bleeding, tenderness, appearance of sores
- Burning sensation of oral tissues, tongue, or gums
- Difficulty breathing, feeling of swelling of the throat/airway, wheezing, shortness of breath
- Appearance of rash or hives

In the event you have an adverse reaction or side effect, you should:

- Stop all study drops and toothpaste immediately if you experience any unusual signs or symptoms
- Call the PI on her cell phone as soon as you notice any unusual symptoms or reactions
- Call **911** immediately if you experience difficulty breathing, swelling of the throat/airway, wheezing, shortness of breath, rash or hives. As soon as possible, inform the PI of this study about the reaction, and discontinue use of the drops and study toothpaste.

Other Information

Your identity will remain confidential. Upon admittance into the study, you will be given an identification number, and your identifiable information will be removed from all documents. Participation in this study is voluntary, and you are free to withdraw from the study at any time.

As an incentive to become a study participant, and upon completing the full study term, you will receive a $5 Starbucks gift card. Additionally, when you complete the study, you will be entered into a drawing for the chance to win a Sonicare Diamond Clean electric toothbrush, or professional teeth whitening, including

custom whitening trays and two syringes of whitening gel. If you withdraw from the study early, and do not complete the full study term, you will not be eligible for these incentives.

Alternative procedures to participation in this study which have been shown to improve oral health and reduce gingivitis include professional teeth cleaning by a registered dental hygienist, and dental examination by a licensed dentist.

Signature of Principal Investigator Date

Subject's Statement

The study described above has been explained to me, and I voluntarily consent to participate in this study. I have had an opportunity to ask questions, and my questions have been answered to my satisfaction. I understand that by signing this form, I am not waiving my legal rights. I understand that I will receive a signed copy of this form.

Signature of Subject Date

If you have any concerns about your rights as a participant in this research or any complaints you wish to make, you may contact Ruth Galm, Human Protections Administrator, at (509) 359-7971 or rgalm@ewu.edu.

Curriculum Vita

CHERI BARTON RDH, BS
Clinical Instructor
Department of Dental Hygiene
Eastern Washington University

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Rathdrum, ID 83858
208.964.5868
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Eastern Washington University
EWU Dental Hygiene Clinic
Health Science Building
310 N. Riverpoint Blvd.
Spokane, WA 99202
cbarton@ewu.edu

Education

2018 Master of Science in Dental Hygiene *(pending)*
Eastern Washington University
Spokane, WA

1990 Bachelor of Science in Dental Hygiene
Oregon Health Science University
Portland, OR

1987 Associate of Arts in Pre-Dental Hygiene
North Idaho College
Coeur d'Alene, ID

Academic Appointments

2016-2017 Graduate Service Appointment
2015-2016 Eastern Washington University
Spokane, WA

Teaching Experience

2014-Present Eastern Washington University
DNHY 450, 451, 452, 453
Senior Year Dental Hygiene Clinic

2014-Present Eastern Washington University
DNHY 350, 351, 352
Junior Year Dental Hygiene Clinic 2014-

Present Eastern Washington University
Medical Emergency Simulation Lab
Junior and Senior Dental Hygiene Students

2017 Eastern Washington University
DNHY 484S Principles of Advocacy and Ethics
Practicum for MSDH degree

2016, 2017 Eastern Washington University
DNHY 380S Radiology Lab
2008-2009 Eastern Washington University
DNHY 452, 453
Third Year Dental Hygiene Clinic
2008-2009 Eastern Washington University
DNHY 350
Second Year Dental Hygiene Clinic
2006-2007 Eastern Washington University
DNHY 250, 251, 252
First Year Dental Hygiene Clinic

Presentations

2018 Patterson Dental Supply Co.
Young Dental Vendor Presentation
2018, 2017, 2016 Eastern Washington University
Young Dental Lunch and Learn for Dental Hygiene Students
2017, 2016 Eastern Washington University
Guest Lecturer DNHY 446S Advanced Periodontal Elective
2015-2017 Burkhart Dental Supply Co.
Young Dental Vendor Presentation
2016, 2015 Inland Northwest Dental Conference
Young Dental Vendor Presentation
2015 Eastern Washington University
Faculty In-service Probing Re-calibration
2015 Eastern Washington University
Faculty In-service Calculus Detection Re-calibration
2015 Student American Dental Hygiene Association Meeting (SADHA)
Young Dental Product presentation

Mentoring

2017-2018 Eastern Washington University
Academic Mentor for 10 dental hygiene students
2016-2017 Eastern Washington University
Academic Mentor for 8 dental hygiene students

Professional Experience

2017-Present Truman Nielsen DMD
Spokane, WA
Dental Hygienist
2014-Present Young Innovations Inc.
Algonquin, Il
Independent Clinical Representative
2006-Present Eastern Washington University
Spokane, WA
Clinical Instructor

2015-2017	Avenue Dental Care Spokane Valley, WA Dental Hygienist
2013-2014	Post Falls Periodontics Post Falls, ID Dental Hygienist
2005-2013	Dr. Robert R. Shaw DMD Spokane, WA Dental Hygienist
2003-2005	Dr. Vincent A. Rossi DDS, PA Hayden, ID Dental Hygienist
2003	Dr. Lauralee Nygaard DDS, MS Spokane Valley, WA Dental Hygienist
1991-2003	Dr. Kenneth E. Gibson Jr. DDS, Pullman, WA Dental Hygienist

Licenses

1990 – Present	State of Idaho, Active Dental Hygiene License
1991 - Present	State of Washington, Active Dental Hygiene License
2001 – Present	State of Washington School Sealant and Fluoride Varnish Endorsement
1990-Present	American Heart Association Healthcare Provider CPR and AED Certification

Professional Affiliations

2015-Present	American Dental Education Association
2014-Present	Washington State Dental Hygienists' Association
2014-Present	Eastern Washington Dental Hygiene Society
2006-Present	American Dental Hygienists' Association
2015	Organization for Safety, Asepsis and Prevention
2006-2014	Idaho State Dental Hygienists' Association

Community Involvement

2011-2018	Inland Northwest Blood Center Coeur d' Alene, ID Regular Blood Donor
2014-2018	Smiles for Veterans Eastern Washington University, Spokane, WA Volunteer
2015-2017	Recovering Smiles Eastern Washington University, Spokane, WA Coordinator for participant screenings
2012-2013	Post Falls High School

Post Falls, ID
Volunteer

2006-2010 Boy Scouts of America, Boy Scout Troop 206
Post Falls, ID
Fundraising Chairperson

2003-2006 Boy Scouts of America, Cub Scout Pack 206
Post Falls, ID
Den Leader