

**ASSESSING THE EFFICACY OF A BIOFLAVONOID-BASED
MOUTH RINSE IN REDUCING ORAL MALODOR RELATED TO
REMOVABLE ORTHODONTIC APPLIANCES IN COMPARISON
TO CHLORHEXIDINE**

by

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Abstract

Halitosis is an unpleasant breath odor; it affects roughly 30-50% of the population. The major compounds that contribute to halitosis that originates from the mouth, typically referred to as oral malodor, are volatile sulfur compounds (VSCs), which are produced by a variety of microorganisms. It has been shown that orthodontic patients with acrylic appliances complain of oral malodor. The source of the odor is in part due to the nature of the appliance, which retains microorganisms that are not removed by mechanical cleaning so typically the addition of an antimicrobial agent, such as Chlorhexidine (CHX) to cleanse the appliances is recommended. However, CHX has some side effects so alternatives need to be explored. **Objective:** To assess the antimicrobial and the anti-malodor efficacy of a bioflavonoid mouth rinse (BFMR) compared to CHX and water on removable orthodontic appliances. **Method:** Participants between 8-20 years of age who complained of malodor from their removable orthodontic appliances from the UBC dental clinics and a private orthodontic practice were recruited and blindly randomized into groups in which different mouth rinses were used to soak the appliances for one week (BFMR, Chlorhexidine and water). Baseline and one-week follow-up data collection included the oral hygiene index (OHI), gingival index (GI), tongue coating index (TCI), VSC measurement by gas chromatography, organoleptic assessment, a microbial swab **Results:** Data was collected from 27 participants ranging in age from 8-16 years old. The baseline measurements of OHI, GI and TCI did not change significantly throughout the study. The organoleptic measurements of odor as well as the aerobic and anaerobic bacterial counts showed a significant reduction in the BFMR group, but for the CHX and water group no significant difference was detected from

baseline to follow-up. **Conclusion:** The BFMR has superior antimicrobial and anti-malodor effect when compared to CHX or to water.

Lay Summary

Orthodontic patients with acrylic appliances often complain of oral malodor, unpleasant breath, which can affect their compliance. The aim of this study is to assess the antimicrobial and the anti-malodor efficacy of a bioflavonoid mouth rinse (BFMR) on these appliances. **Method:** Patients who complained of malodor from their removable orthodontic appliances were recruited, and blindly randomized into three test groups (BFMR, CHX, and water) and instructed to soak their appliance for 15 minutes/day for one-week. Baseline and one-week follow-up data collection included oral hygiene index (OHI), gingival index (GI), tongue coating index (TCI), VSC measurements, organoleptic assessment and microbial swab. **Results:** Data was collected from 27 participants. The baseline measurements (OHI, GI, and TCI) did not change significantly throughout the study. Oral-malodor measurements and bacterial counts showed a significant reduction in the BFMR-group, but not in the CHX and water groups. **Conclusion:** The BFMR has good antimicrobial and anti-malodor effect.

Preface

This research project was designed by Dr. Hajer Alsabban under the supervision of Dr. Leeann Donnelly and the guidance of thesis committee members, Drs. Donald M. Brunette and Angelina Loo. The clinical data was collected and analyzed by Dr. Hajer Alsabban, and the microbial analysis was conducted in Dr. Ya Shen's lab by Dr. Hazuki Maezono. The scanning electron microscope (SEM) scans were performed by Dr. Gethin Owen in the Center for High-Throughput Phenogenomics at the University of British Columbia. The statistical analysis was performed in conjunction with the Statistical Consulting and Research Laboratory (SCARL). This study was approved by the University of British Columbia, Office of Research Services, Clinical Research Ethics Board (Certificate # H15-01642).

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List of Abbreviations

CHX	Chlorhexidine
CPC	Cetylpyridinium Chloride
BFMR	Bioflavonoid mouth rinse
VSC	Volatile sulfur compounds
H₂S	Hydrogen sulfide
CH₃SH	Methyl mercaptan
(CH₃)₂S	Dimethyl sulfide
GC	Gas chromatography
BHI	Brain heart infusion
OHI-S	The Simplified Oral Hygiene Index
MGI	Modified gingival index
SEM	Scanning electron microscope

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Chapter 1: Introduction

1.1 Halitosis

Halitosis is a broad term that is used to describe a foul odor emanating from the nasal or oral cavity. It is also commonly referred to as foul breath, oral malodor, or bad breath (Tonzetich and Ng 1976, Tonzetich 1977). This is an undesirable condition causing psychological and social problems, affecting a high percentage of the population with different studies reporting the prevalence of halitosis at about 30-50% (Sanz et al. 2001, Liu et al. 2006). However, the prevalence does not appear to be consistent globally; in Brazil, it has been reported much lower (15%) and it was found to be three times higher in males than in females and three times higher in people over 20 years old (Nadanovsky et al. 2007).

1.1.1 Classification of Halitosis

Halitosis can be classified as genuine halitosis when there is obvious malodor beyond a socially accepted level, pseudo halitosis when there is no obvious malodor although the patients complain of its existence and halitophobia when the patient persists in believing they have halitosis even though there is firm evidence for the absence of it (Yaegaki and Coil 2000, Scully and Greenman 2008).

Genuine halitosis can be further classified as either physiologic (transient with no known disease or disorder) or pathologic (persistent with an identifiable disease or disorder). Physiologic halitosis is commonly associated with oral malodor on awaking that is mainly due to a decrease in saliva flow at night and the production of volatile sulfur compounds (VSCs) from commensal bacteria that inhabit the tongue coating of

periodontally and systemically healthy people (Yaegaki and Coil 2000, Porter and Scully 2006). Physiologic malodor can be rectified by eating, tooth brushing, tongue brushing or scraping, and rinsing the mouth with fresh water (Faveri et al. 2006). Other causes of transient malodor might be a consequence of certain foods such as garlic, onion, cabbage, spices, cauliflower, and radish all of which contain sulfur compounds (Suarez et al. 1999). Lifestyle habits such as smoking tobacco also contribute to this form of halitosis because tobacco also contains sulfur compounds, and can increase the risk of periodontal disease and hypo-salivation, which can both contribute to odor production (Stedman 1968).

1.1.2 Causes of Pathologic Halitosis

Pathologic halitosis (persistent) can originate from either extra-oral or intra-oral sources. It has been shown that 85% of persistent genuine halitosis originates from the mouth (Delanghe et al. 1997, Scully and Greenman 2008). Tongue coating and periodontal disease can cause halitosis. One study showed that periodontal disease accounted for 60% of the oral factors, while the remaining 40% were related to tongue coating (Armstrong et al. 2010, Rosing and Loesche 2011).

Intra-oral halitosis, most commonly referred to as oral malodor, is caused by the proteolytic activity of gram-negative bacteria, which cause microbial degradation of S-amino acids into VSC's. However, some researchers have suggested that gram-positive oral bacteria produce β -galactosidase, which removes the carbohydrate side-chains of salivary glycoproteins and contribute to VSC production by gram-negative oral bacteria (Suzuki 2012). Furthermore, β -galactosidase activity in the saliva has been shown to be related to the VSC concentration in periodontally healthy patients complaining of

halitosis (Masuo et al. 2012).

The VSC's that typically contribute to oral malodor are hydrogen sulfide (H₂S), methyl mercaptan (CH₃SH), and dimethyl sulfide ((CH₃)₂S) (Tonzetich 1977). However, methylamine, dimethylamine, butyric acid, indole, propionic acid, scatole, and cadaverine have also been reported to be associated with oral malodor, but more commonly from extra-oral sources. Hydrogen sulfide (H₂S) and methyl mercaptan (CH₃SH) are the main gases related to oral malodor while dimethyl sulfide ((CH₃)₂S) has been mainly associated with extra-oral malodor (Tangerman and Winkel 2007).

Gram-negative oral microorganisms in particular, *P. gingivalis*, *T. forsythensis*, *A. actinomycetemcomitans*, and *P. intermedia* are responsible for most cases of oral malodor (McNamara et al. 1972, Solis-Gaffar et al. 1975, Awano et al. 2002). While these species are typically isolated from patients with oral malodor, it has been suggested that the amount of microbes is more important than the specific type (Scully and Greenman, 2008).

The tongue is the most populated area with bacterial biofilm due to its high number of crypts, which protect the organisms from mechanical abrasion and provide them with nutrients. The anaerobic bacteria that populate these crypts have an abundance of nutrients, such as desquamated cells, food debris, and saliva, which help them to grow on the tongue surface and produce VSC's (Kazor et al. 2003, Roldan et al. 2003, Scully and Greenman 2008). One study has shown that tongue coating was associated with halitosis in more than 60% of 2000 patients of a breath clinic (Quirynen et al. 2009).

Periodontal disease is also associated with oral malodor, and several studies have shown a relation to both gingivitis and periodontitis (Miyazaki et al. 1995, Delanghe et

al. 1997, Bornstein et al. 2009, Bornstein et al. 2009, Quirynen et al. 2009). Acute necrotizing ulcerative gingivitis or periodontitis cause the most notable halitosis, but are not commonly seen in healthy adults (Porter and Scully 2006). Several other oral factors also increase the risk of oral malodor such as a decrease in saliva quality and quantity from medication, radiotherapy or Sjogren's syndrome. Other related factors such as wearing dentures, bone diseases (osteonecrosis, malignancy), wound healing, fixed orthodontic appliances, peri-implantitis and cleft lip and/or palate have also been described (Scully and Greenman 2008, Suzuki et al. 2008, Al-Zahrani et al. 2011, Zurfluh et al. 2013).

Extra oral sources are less often associated with oral malodor, with dimethyl sulfide as the main contributor (Tangerman and Winkel 2007, Scully and Greenman 2008). Sources of extra-oral halitosis can be from metabolic disorders, anxiety, upper and lower respiratory disorders, liver and kidney dysfunction, and cancer (Calil and Marcondes 2006, Mariona Monfort-Codinach 2014). Gastrointestinal problems can also be associated with halitosis as they produce odiferous gases that can be expelled from the mouth and nose (Moshkowitz et al. 2007, Kinberg et al. 2010). The side effect of medications containing a dimethyl sulfide structure can also be detectible in breath air and contribute to halitosis (Murata et al. 2003). The genetic metabolic disorder trimethylaminuria (fish odor syndrome) is the principal cause of undiagnosed body odor (Mitchell 2005).

1.1.3 Diagnosis of Oral Malodor

The first step in the diagnosis of oral malodor is a thorough medical and dental history, including an oral examination to try to determine if the origin is an intra-oral or extra-oral source. There are different measurement methods for oral malodor, starting with organoleptic measurements, which demonstrate a good level of reproducibility for odor measurements, and are considered as the gold standard because in the end objectionable odor is determined by the human nose. In addition, the human nose can detect pleasant and unpleasant smells, not only VSC's (Porter and Scully 2006, Scully and Greenman 2008, Armstrong et al. 2010). Assessment depends on the olfactory response of a trained clinician under standard conditions who sniffs the air exhaled from the mouth and nose to determine the presence of halitosis and rates the strength of the odor using a scoring system. The most widely used is the organoleptic score developed by Rosenberg and McCulloch, which scores malodor on a scale of 0-5 as shown in Table 1 (Rosenberg and McCulloch 1992, Donaldson et al. 2007).

Table 1.1 Organoleptic Scoring Scale (Rosenberg and McCulloch, 1992)

Rosenberg & McCulloch Scale	Description
0	No detectable odor
1	Hardly detectable odor
2	Light odor
3	Moderate odor
4	Strong odor
5	Extremely strong odor

Gas chromatography (GC) is considered the most reliable, objective and reproducible method, and is the preferable measurement for determining the concentration of specific VSCs. Measurements can be taken from breath, saliva and tongue debris, and can determine the concentration of each VSC (Suzuki et al. 2008). However, standard gas chromatographs are not easily used on a daily basis because of their complexity, high cost and the need for highly trained personnel to operate and interpret results (Solis-Gaffar et al. 1975, Tonzetich 1977, Persson 1992, Murata et al. 2002, Bollen and Beikler 2012). They are also quite large and cumbersome, making them difficult to use in a clinical dental setting. Therefore, portable, less complex GC devices are now available and in use such as the OralChroma™ and the Twin Breasor (Yoneda M, 2014).

The OralChroma™, is a sensitive device capable of detecting VSCs and can differentiate between H₂S, CH₃SH and (CH₃)₂S. Its ability to detect dimethyl sulfide allows for the potential to detect extra-oral sources of oral malodor. Similar to other more traditional GCs, it is an expensive apparatus and requires training to appropriately operate it and interpret results (Tangerman and Winkel 2008).

The Halimeter® is another portable and reproducible monitor to detect VSCs, but it cannot differentiate between the types of sulfides (Armstrong et al. 2010, Bollen and Beikler 2012). The B/B Checker® is a portable device capable of detecting several kinds of gases including VSC, ethanol, hydrogen, ammonia, acetone, and butylate. It detects the malodor level in oral, exhaled, and nasal gases independently in a short period of time (Tamaki et al. 2011).

Other surrogate measurement methods that do not measure odor directly are also

used such as the BANA (benzoyl–arginine–naphthyl–amide) test, the salivary incubation test, and polymerase chain reaction to identify microbes associated with the production of VSC's. Still other methods focus on the identification and quantification of bacterial bi-products such as β -galactosidase activity and ammonia (van den Broek et al. 2008, Rosing and Loesche 2011).

1.1.4 Treatment of Oral Malodor

Malodor originating from the mouth is due to certain oral microorganisms. Therefore, treatment is primarily directed to the reduction of these microorganisms and their available nutrients by mechanical or chemical approaches (Suzuki 2012). Good oral hygiene practices such as brushing teeth, flossing and cleaning of the tongue can help prevent oral malodor. Tongue cleaning has been suggested as the most important strategy for the prevention of oral malodor (Pham et al. 2011). The reduction of intra-oral nutrients and microorganisms that cause pathogenic oral malodor has also been achieved by treating periodontitis, gingivitis, inadequate restorations, deep caries, endodontic lesions, and dry mouth (Tanaka et al. 2003).

Periodontitis is one of the main causes of oral malodor, and professional periodontal therapy should be considered, as several studies have shown that performing periodontal treatment reduces VSCs and organoleptic scores (Delanghe et al. 1997, Quirynen et al. 2009, Silveira et al. 2012). Furthermore, the combination of scaling and root planning with chlorhexidine, has shown a significant microbiological improvement for up to two months and an improvement in organoleptic scores (Bollen et al. 1996).

If oral hygiene is improved and oral malodor persists, the tongue may be the

likely source of odor and approaches directed to tongue coating should be applied (van den Broek et al. 2008). Regular tongue cleaning, as posterior as possible, is recommended since the largest amount of coating is typically found on the posterior dorsal section of the tongue surface. Tongue cleaning reduces the available nutrients and microorganisms and can be accomplished with either a tongue scraper or a toothbrush and cold water. While there is weak evidence showing a superior method over the other, the brush is less aggressive on the soft tissue (Menon and Coykendall 1994).

Chemical approaches using mouth rinses for the treatment of oral malodor act by reducing either the bacterial load or the associated odoriferous compounds. The antimicrobial properties of the mouth rinses can reduce oral malodor by reducing the number of microorganisms. Chlorhexidine Gluconate (CHX) and Cetylpyridinium Chloride (CPC) are two active ingredients successfully used in odor reducing products (Cortelli et al. 2008). Essential oils have also been tested, but found to provide only a short-term and restricted effect (25% reduction) for three hours (Pitts et al. 1983). Triclosan, commonly used in mouth rinses and toothpastes has both an antimicrobial effect and a direct action on VCSs and has shown an 84% reduction of VSCs after three hours (Porter and Scully 2006). Although CHX is the gold standard in treating malodor it has an unpleasant taste, can give rise to a burning sensation of the oral mucosa if used too frequently, and can cause (reversible) staining of the teeth (Porter and Scully 2006).

Other chemical agents can reduce oral malodor by chemically neutralizing VSCs. The active ingredients in these products are most commonly zinc and chlorine dioxide (Roldan et al. 2003). Metal ions with a high affinity for sulfur invert volatile fragrant gases into non-volatile components (Waler 1997, Young et al. 2001). A combination of

chemical agents can markedly reduce VSC concentrations (Winkel et al. 2003). In particular, the combination of low concentrations of zinc and CHX has been effective in neutralizing VSCs that cause bad breath (Thrane et al. 2007). As well, the combination of a rinse containing 0.05% CHX, 0.05% CPC and 0.14% zinc lactate is also effective at reducing VSCs, more so than CHX alone, with the added benefit of reduced adverse effects (Young et al. 2003).

Masking of the malodor is another approach, though it only has a temporary effect or improvement (Rosing and Loesche 2011). Chewing gum, rinsing products, parsley, cloves, fennel seeds and mint tablets all have a short-term masking effect mainly due to increased saliva production (Sterer and Rubinstein 2006).

Biological therapeutic approaches such as the probiotics *Streptococcus salivarius* (K12), *Lactobacillus salivarius* or *Weissella cibaria* have also been utilized with the aim of preventing the re-establishment of the bacteria responsible for malodor (Bollen and Beikler 2012). Bovine lactoferrin and lactoperoxidase have also been shown, in healthy volunteers, to reduce CH₃SH levels after 10 minutes (Shin et al. 2011). Furthermore, a trial using tablets containing *Lactobacillus salivarius* WB21 reported that VSC levels were significantly lower at two weeks in subjects with halitosis (Iwamoto et al. 2010).

If an oral approach for treating malodor is not successful the patient should be referred to a general physician and possibly a specialist such as an otorhinolaryngologist, pulmonologist, gastroenterologist or endocrinologist. However, if halitosis is not detected and the patients persist in their belief that they have a malodor a psychological aspect should be considered. In these instances they may be suffering from either pseudo-halitosis or halitophobia, which is more often associated with depression or other mental

health disorders (Suzuki et al. 2008). Both are difficult to treat, as patients tend to insist on the presence of halitosis and will seek different opinion from multiple specialists without addressing the true underlying psychological issue (Suzuki et al. 2008, Bollen and Beikler 2012).

1.2 Mouth Rinses and Oral Malodor

Mouth rinses have been shown to be effective in the treatment of malodor either on their own or in combination with mechanical measures. The ideal mouthwash would be an antimicrobial agent with long lasting efficacy that has a therapeutic effect on oral malodor either by antimicrobial action and/or the inhibition of VSCs such as CHX, chlorine dioxide, zinc chloride, CPC and triclosan (Pitts et al. 1981, Kozlovsky et al. 1996, Quirynen et al. 1998, Grootveld et al. 2001, Young et al. 2002). While chlorine dioxide, and zinc chloride, which contain a metal ion, can reduce VSC production through their affinity to react with VSC precursors, they do not exert an antimicrobial effect (Waler 1997, Grootveld et al. 2001, Young et al. 2001, Young et al. 2002, Shinada et al. 2010).

Chlorhexidine (CHX), an oral antiseptic, is used in many studies for treating oral malodor. One study compared different CHX-based commercial mouth rinses in regard to their antimicrobial activity and anti-halitosis effect and found that different formulations of CHX have different effects (Roldan et al. 2004). Furthermore, another study showed that a formulation containing CHX (0.05%), CPC (0.05%) and zinc lactate (0.14%) to be most effective in reducing bacteria related to oral malodor and improvement in organoleptic and VSC scores (Roldan et al. 2003).

Another study evaluated the efficacy of CHX (0.12% and 0.2%) on 16 subjects to control oral malodor for 7 days. The results showed that the mean whole-mouth odor and VSC scores were reduced by 73.3% and 68.6%, respectively (De Boever and Loesche 1995). Moreover, another study done by Bosy et al., evaluated the efficacy of CHX (0.12% and 0.2%) on 127 subjects to control oral malodor for 7 days. The results showed a significant reduction of VSC levels, as well as a reduction of anaerobic periodontal pathogens on the tongue (Bosy et al. 1994).

1.3 Microbiological Changes with Orthodontic Appliances

The normal flora of the human mouth is a mixture of organisms consisting of more than 200 species of bacteria including lactobacilli, streptococci, staphylococci and corynebacteria, with a great number of anaerobes, especially bacteroides (Todar 2002). The composition of the normal flora changes with age and increase in complexity; at birth it is a sterile environment that then becomes colonized with *Streptococcus salivarius*. As the teeth erupt, further changes occur in the flora as the bacteria require non-epithelial surfaces to colonize and may now include *Streptococcus mutans* and *Streptococcus sanguis* (Todar 2002). Removable orthodontic appliances act as a reservoir and retentive site for microorganisms that can further modify the environmental condition of the oral cavity (Lessa et al. 2007). When the oral microbiological status of 45 healthy children (6-10 years old), with and without removable orthodontic appliances, were assessed by collecting biofilm samples from the hard palate and the dorsum of the tongue, those with the removable orthodontic appliances had an increase in *Lactobacilli*, *Acinetobacter*, *Entrobacter*, *E. coli*, and *Alpha-hemolytic Streptococcus* (Jabur 2008).

Another study that measured the prevalence of biofilms and the types of microorganisms on removable orthodontic appliances of 25 healthy children (10-14 years old) found a higher prevalence of Enterobacteriaceae (Pathak and Sharma 2013).

1.4 Mouth Rinses and Removable Orthodontic Appliances

Removable orthodontic appliances are considered in active treatment and in the retention of orthodontically treated patients, however they tend to be difficult to clean with routine oral hygiene measures and retain microorganisms (Lessa et al. 2007). Therefore, it is of clinical importance to find reliable strategies beyond mechanical measures to control oral biofilm colonization on removable appliances.

Lessa and colleagues 2007, used a microbial culture technique and scanning electron microscopy (SEM) to assess the contamination of acrylic baseplates of removable orthodontic appliances of 17 patients of both sexes (6-12 years old) to evaluate the efficacy of a 0.05% CPC solution (Cepacol[®]) and a 0.12% CHX solution (Periogard[®]) and water as a control. Subjects were assigned to a three-stage changeover system with a one-week interval between each stage. They found that acrylic baseplates of removable orthodontic appliances were contaminated by *Streptococcus mutans* colonies/biofilms but that those appliances sprayed with CHX showed significantly greater efficacy in reducing *Streptococcus mutans* colonies/biofilms on the acrylic surfaces and CPC had better results than sterile tap water (Lessa et al. 2007).

Others have assessed the efficacy of different disinfection protocols with 0.12% CHX sprayed either once (at day 7) or twice (at days 4 and 7) on acrylic baseplates of removable orthodontic appliances and found that baseplates sprayed either once or twice

a week reduced the *Streptococcus mutans* contamination significantly on the acrylic surfaces in vivo with no differences between the two protocols (Peixoto et al. 2011).

Collares 2014, studied the in vitro effect of immersing orthodontic acrylic resin in five different mouthwashes (Classic and alcohol free Plax[®], Periogard[®] with/without alcohol and Listerine[®]) for different times on the hardness, roughness and color and found that after 7 days all the mouthwashes softened the acrylic resin. After 12 hours of immersion, Plax[®] and Listerine increased the roughness and Listerine[®] caused color variation. In conclusion, immersion of orthodontic acrylic resin in mouthwashes influences the acrylic resin hardness, roughness and color (Collares 2014).

1.5 Flavonoids

Flavonoids are natural compounds found in fruits, vegetables, seeds, nuts and flowers (Cushnie and Lamb 2005). The basic structure consists of two benzene rings linked via a heterocyclic pyrane ring, an example of which is found in Figure 1.2 (Kumar and Pandey 2013). There are a variety of flavonoid classes such as flavones (e.g., flavone, luteolin and apigenin,), flavonols (e.g., quercetin, myricetin, and fisetin), and flavanones (e.g., flavanone, hesperetin, and naringenin) (Kumar and Pandey 2013).

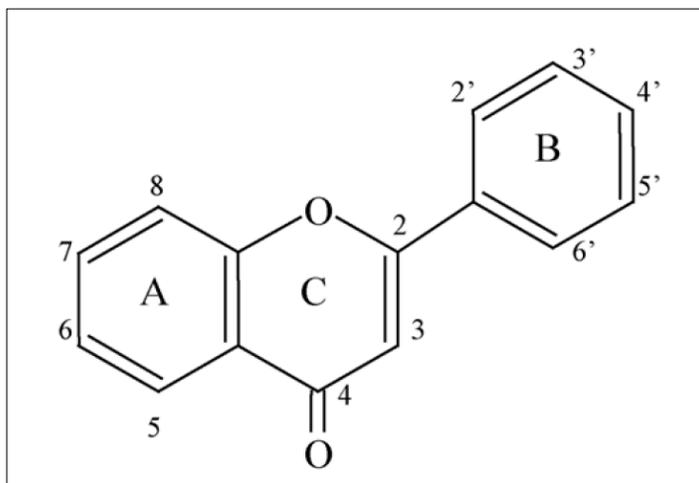


Figure 1.1 The structure of a class of Flavonoids (Kumar and Pandey 2013)

Several biological activities of flavonoids have been reported such as antioxidant activity, hepatoprotective activity, anti-inflammatory as well as vascular activity, cytotoxic antitumour activity, and most importantly antibacterial activity (Kumar and Pandey 2013). Several flavonoids including galangin, flavone and flavonol glycosides, isoflavones, flavanones, and chalcones have shown good antibacterial activity (Kumar and Pandey 2013). In particular flavonoids have the ability to inhibit the growth of *Streptococcus mutans* and *Streptococcus sobrinus* (Cushnie and Lamb 2005).

Studies indicate different compounds within each class target different components and functions of the bacterial cell rather than one specific site of action (Cushnie and Lamb 2005). Some of the antibacterial mechanisms of action are inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane function and inhibition of energy metabolism (Cushnie and Lamb 2005).

1.6 The Bioflavonoid Mouth Rinse (BFMR)

Flavonoids can be utilized in many forms, one of them being an oral rinse. One such commercially available rinse is Citrarinse, a certified organic natural oral rinse, which is produced from a combination of bioflavonoid compounds. The product's active ingredient is a proprietary product, trademarked as Citrozine™, which is a registered organic ingredient.

Lab tests done in Analytical microlab, Australia on this product have confirmed its antimicrobial activity to be broad-spectrum with the ability to kill bacteria such as *Escherichia Coli* and *Staphylococcus aureus*. It has been claimed to be non-carcinogenic, non-mutagenic with no harmful side effects and to have an anti-inflammatory potential that might be related to the bioflavonoid content.

1.7 Rationale and Purpose

Malodor has been associated with intraoral removable appliances, which may affect a patient's cooperation and compliance of wearing the appliance. Biofilms on removable orthodontic appliances act as a reservoir for microorganisms, which are capable of producing VSCs and modifying the environment of the oral cavity. These biofilms are difficult to remove with routine hygiene measures due to the porosity of the appliance resin and the retentive nature of the metal components. Some oral rinses, especially CHX, have been shown to have good efficacy in reducing microbial colonies/biofilms on acrylic surfaces. However, some studies have shown that CHX is less effective on biofilms compared to microorganisms in their free form (Bonez et al. 2013). The biofilm is a multicellular community of bacteria, which is held together by an

extracellular matrix (Branda et al. 2005). The biofilm makes the bacteria more resistant to antimicrobial agents and protects against the host defense (Mah and O'Toole 2001). Furthermore, the use of a non-prescription rinse that has similar antimicrobial properties may be beneficial and easier for patients to use, with less adverse effects.

The purpose of this study was therefore to assess the antimicrobial and anti-malodor effect of a BFMR (Citrarinse) compared to CHX on removable orthodontic appliances.

1.8 Research Questions

- ❑ What is the antimicrobial effect of the BFMR on removable orthodontic appliance biofilm?
- ❑ How effective is the BFMR at reducing malodor from removable orthodontic appliances?

1.9 The Null Hypothesis

The BFMR will be no more effective than CHX at reducing biofilm and malodor associated with removable orthodontic appliances.

Chapter 2: Materials and Methods

2.1 In-Vitro

The antimicrobial effect of the BFMR compared to water as the control was tested by collecting a subgingival biofilm swab which was then soaked in the bioflavonoid mouth rinse for 30 seconds, one minute and three minutes and compared to the water control. The biofilm was cultured and remaining bacterial colonies counted.

Using a sterile loop, bacteria from the biofilm subculture were picked up and suspended in 2 mL of BHI (brain heart infusion). A pipette was used to mix the suspension up and down a few times until the suspension appeared homogeneous. For both groups (test/control) three rows of Eppendorf tubes were set-up with six tubes in each row for a dilution series. Three parallel series of five dilutions were arranged by pipetting 900 μ L of BHI medium into tubes two to five of all three rows. For the control plate, a pipette of 900 μ L of sterile water was placed into the first tube of all three rows then a 100 μ L of bacterial suspension was added to the first tube in each row. The dilution series was completed by pipetting 100 μ L from the first tube and adding it to the second tube in the row, and continuing this dilution series until tube five of the row. These steps were repeated for the parallel rows two and three. For the test plate: 900 μ L of the BFMR was pipetted into tube one of each parallel. Then 100 μ L of the bacteria suspension and inoculate was pipetted into tube one while starting the timer (30 s, 1 min and 3 min). When the incubation time was up, 100 μ L was pipetted from tube one and dispensed into tube two. This dilution series was continued until tube five was reached. The dilutions were plated in the same manner as mentioned before. 20 μ L from each tube was pipetted onto each test plate. Each plate was incubated upside down in a plastic bag

overnight at 37°C. After 24 hours of incubation the number of bacterial colonies on the plates were counted to calculate the killing effect (killing%).

A protocol for measuring the malodor from a removable orthodontic appliance was developed. A three-week-old biofilm from a periodontal pocket, anaerobically cultured, was swabbed onto a retainer. The retainer was placed in a sealed Teflon bag and an air sample was taken using a syringe from a valve on the bag at 1, 3 and 5 minutes and then injected in the OralChroma™ to determine the best time interval in which the gases reached the peak concentration. This was determined to be 1 minute, because after 1 minute the concentration of the gasses was reduced, that might be due to either leakage or absorption of the gases by the Teflon bag.

For the organoleptic scoring, the investigator was first tested for the smell function using The Smell Identification Test which include four enveloped-sized booklets each containing ten scratch and sniff odorants; above each odorant strip is a multiple question with alternative responses. The investigator was then trained, which included sniffing pure solutions of known odorants across a wide range of dilutions to ensure accuracy of detecting odor. The investigator then sniffed and scored a series of breath sample dilutions with varying degrees of hydrogen sulfide and methylmercaptan. This enabled the investigator to become familiar and internally calibrated to varying levels of the oral gases.

2.2 In-Vivo: Pilot Clinical Trial

The Human Ethics Board of the University of British Columbia approved the proposed Randomized Clinical Trial (Certificate # H15-01642) in September 2015.

2.2.1 Study Design and Recruitment of Patients

This clinical pilot study was designed as a double-blinded, randomized clinical trial. Three study groups were included and consisted of an experimental group, where participants used the BFMR (Citrarinse), a negative control group, where participants used water (H₂O), and a positive control group, where participants used 0.12% CHX (PERIDEX[®]) to soak their removable orthodontic appliance. The participants were recruited from the undergraduate and graduate orthodontic clinics at the University of British Columbia (UBC) and a private Orthodontic practice in British Columbia (B.C).

A meeting was held with the Clinical Trial Manager, of the Frontier Clinical Research Center at the University of British Columbia and a recruitment plan was established including how to contact the potential participants and how reminders would be provided to the students.

Initially an email (Appendix A.1) was sent to the undergraduate students and their instructors, including the Study Invitation Letter (Appendix A.2), explaining the study and the inclusion criteria. As well, a weekly reminder was provided verbally to the students on the day of each clinic session and students and instructors were asked to contact the investigator if they identified any potential participants.

A study invitation letter (Appendix A.1, A.2) was also sent to a private Orthodontic practice. After the agreement by the Orthodontist at the office, another letter was sent to explain the study in more detail and the inclusion and exclusion criteria were attached to help the Orthodontist in identifying potential participants.

Eligible participants were identified by the investigator at the UBC orthodontic undergraduate and graduate clinic and by an orthodontist at the private practice. An email

containing the invitation letter was sent to the potential participants with all the information of the study and the consent forms. Any participant who expressed interest in the study was contacted by the investigator for more details.

2.2.2 Inclusion Criteria

Males and females between 8-20 years of age who presented to the clinics with a removable orthodontic appliance that had an obvious odor as assessed by the investigator, were invited to participate. In the initial planning of the study, additional inclusion criteria included, confirmation of a VSC level of >1.5 mg/10ml for hydrogen sulfide (H₂S) or 0.5mg/10ml for methyl mercaptan (CH₃SH) using the OralChroma™. However, during recruitment it became apparent that it was difficult for participants to come for an extra appointment to utilize this inclusion criteria. Therefore, to improve recruitment, this additional criteria was excluded and all participants that complained of malodor of the retainers were included.

2.2.3 Exclusion Criteria

Potential participants with an allergy to citrus fruit or CHX were excluded because citrus is an active ingredient in the BFMR and CHX is the mouth rinse used in the positive control group. Potential participants with active caries or who had used antibiotics in the previous month were also excluded due to the potential effect on the microbial nature and count in the mouth that might affect oral malodor.

2.2.4 Randomization of Participants

A randomization list of 30 participants was generated using a computer-based research randomizer. Patients who agreed to participate in the study were assigned randomly to one of the three study groups.

A set of 30 bottles were labeled with numbers from 1- 30 and filled with the rinses according to the randomization list, then the full randomization list was held in the principle investigator's office for future reference.

2.2.5 Pre-Assessment Protocol

Participants who agreed to participate were contacted by the investigator who explained the study in more detail and answered any questions. Consent forms (Appendix A.3) were signed by parents and a Child or Adolescent Assent form (Appendix A.4, A.5) was signed by the participants. Appointments for clinical assessments and measurements were booked to coincide with the participant's regular orthodontic recall visits to lessen the burden on the participants in the study.

The parents were contacted a few days before the clinical assessment by email or by phone to confirm the appointment and to give them written instructions (Appendix A.6) for the participants to follow, which included avoidance of spicy foods, garlic and onions for two days prior to examination and to refrain from oral activities such as brushing, flossing, rinsing, tongue cleaning, eating and drinking for at least three hours before the appointment time.

2.3 Participants and Data Collection

Sixty potential participants were contacted from the private practice, and seven potential participants were identified from the UBC undergraduate and graduate clinics. Of the 67 potential participants, 27 participants were suitable and enrolled in the study.

At the first appointment for clinical assessment and measurements, the investigator ensured that all the consent/assent forms were signed, instructions were followed, and answered any further questions regarding the study; a checklist was used by the investigator to make sure all the steps of the examination were performed in order (Appendix A.7) as well as an examination form (Appendix A.8). The medical history was then completed to ensure there were no allergies or contraindications to the clinical assessments and to ensure that no medications, especially antibiotic use in the past month that would exclude them from the study.

2.3.1 Oral Examination

The clinical assessment started with an oral breath sample taken using the OralChroma™ (Figure 2.1) to measure the concentration of H_2S , CH_3SH and $(\text{CH}_3)_2\text{S}$ without the appliance. The participant was seated upright, and a syringe was inserted into the oral cavity until the flange reached the lip. The participant was instructed to softly bite on the syringe to stabilize it and to breathe through the nose while keeping the lips sealed for 30 seconds, and to avoid touching the tip of the syringe with the tongue. After 30 seconds measured using a stopwatch, the piston of the syringe was pulled and filled with oral breath, then pushed back and returned the gas to the oral cavity. The piston was then again pulled to fill the syringe completely, at this time the syringe was removed. The

saliva was cleaned from the tip with tissue paper, and the plunger pushed to the 1 cc (ml) position and within one minute the breath sample was injected at a right angle to the inlet of the Oral Chroma™, as shown in Figure 2.2. Analysis took approximately 4 minutes and results were then recorded, an example of one of the readouts can be seen in Figure 2.3.



Figure 2.1 Image of an OralChroma™ Device



Figure 2.2 Image of the Process of the OralChroma™ The tip of the syringe injected at a right angle to the inlet of the OralChroma™

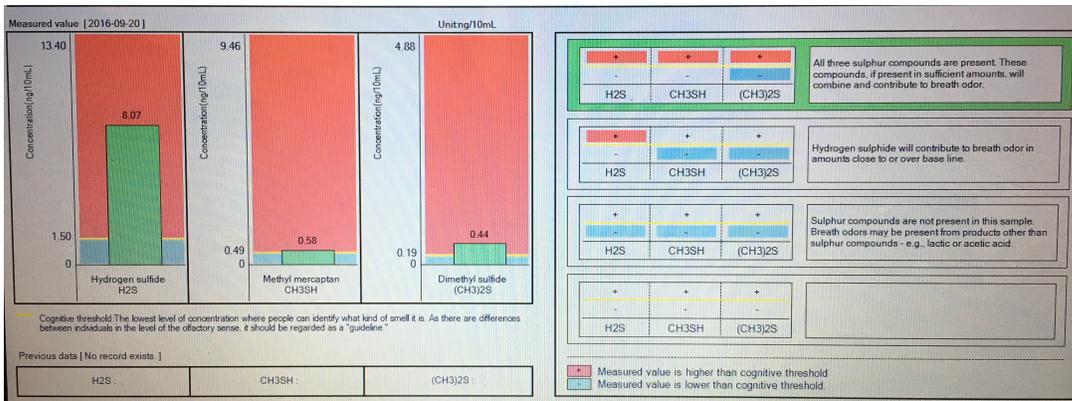


Figure 2.3 Example of an OralChroma™ Reading showing the concentration of hydrogen sulfide, methyl mercaptan and dimethyl sulfide

The following clinical data were also collected: plaque level, gingival condition, and tongue coating using a periodontal probe and a mirror while the patient was seated in a dental chair.

The Simplified Oral Hygiene Index (OHI-S) was utilized to score plaque and

calculus levels (Greene and Vermillion 1964). Six surfaces from four posterior and two anterior teeth were examined, a visual representation of which can be found in Figure 2.4. The buccal surfaces of the selected upper first molars and the lingual surfaces of the selected lower first molars were inspected. In the anterior portion of the mouth, the labial surfaces of the upper right and the lower left central incisors were scored. In the absence of either of these anterior teeth, the central incisor on the opposite side of the midline was scored.

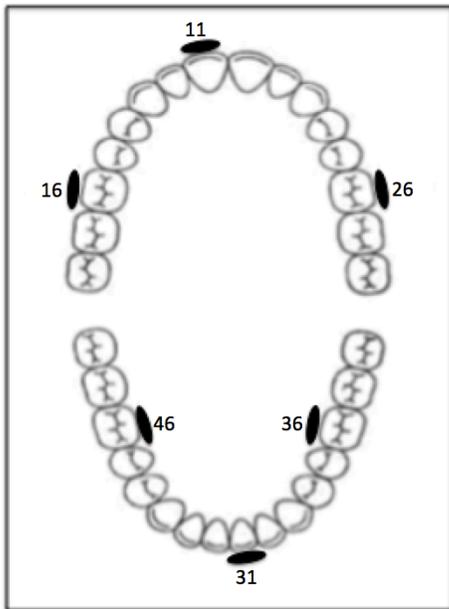


Figure 2.4 The Teeth Examined for OHI-S (Greene and Vermillion 1964)

If no debris or stain was present a score of 0 was given. Soft debris covering not more than one third of the tooth surface, or presence of extrinsic stains without other debris regardless of the surface area covered was given a score of 1. Soft debris covering more than one third, but not more than two thirds, of the exposed tooth surface was given a score of 2. Soft debris covering more than two third of the exposed tooth surface was

given a score of 3. The Calculus index was recorded by giving score 0 if there was no calculus. Supragingival calculus covering not more than third of the exposed tooth surface was given score of 1. Supragingival calculus covering more than one third but not more than two thirds of the exposed tooth surface or the presence of individual flecks of subgingival calculus around the cervical portion of the tooth was given score of 2. Supragingival calculus covering more than two-thirds of the exposed tooth surface or a continuous heavy band of subgingival calculus around the cervical portion of the tooth was given score of 3. Then the total OHI was calculated (Figure 2.5, Table 2.1) (Greene and Vermillion 1964).

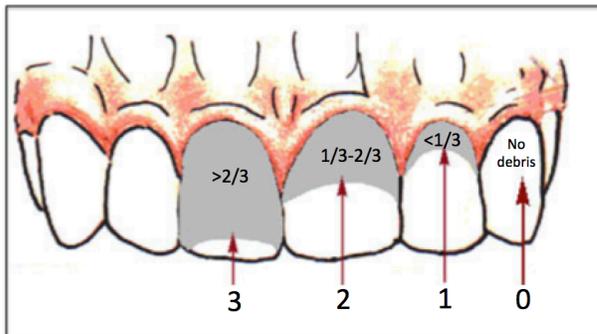


Figure 2.5 Score of OHI-S (Greene and Vermillion 1964)

Table 2.1 Simplified Oral Hygiene Index (OHI.S) (Greene and Vermillion 1964)

Debris		Calculus	
0	No soft debris or stain	0	No calculus
1	Less than 1/3 of surface covered	1	Supra calculus over less than 1/3 of tooth
2	1/3 to 2/3 of surface covered	2	Supra calculus cover 1/3 to 2/3 of tooth
3	More than 2/3 of surface covered	3	Supra calculus over More than 2/3 of tooth

OHI-S = Total debris score divided by the number of teeth scored	
0	Excellent
0.1-0.6	Good
0.7-1.8	Fair
1.9-3.0	Poor

The gingival condition was assessed according to Lobene et al 1986; this modified gingival index (MGI) permits non-invasive evaluation of early visual changes in severity and extent of gingivitis. Absence of inflammation was scored 0. Mild inflammation; slight change in color, little change in texture of any portion of the marginal or papillary gingival unit was scored 1. Mild inflammation; criteria as above but involving the entire marginal or papillary gingival unit was scored 2. Moderate inflammation: glazing, redness, edema, and/or hypertrophy of the marginal or papillary gingival unit was scored 3. Severe inflammation; marked redness, edema and/or hypertrophy of the marginal or papillary gingival unit, spontaneous bleeding, congestion, or ulceration was scored 4. This index was done on the same teeth that were selected for the OHI-S (Table 2.2) (Lobene et al. 1986).

Table 2.2 Modified Gingival Index (MGI) (Lobene, Weatherford et al. 1986)

Modified Gingival Index (MGI)	Description
0	Normal, no inflammation
1	Localized mild inflammation
2	Generalized mild inflammation
3	Moderate inflammation
4	Severe inflammation

Tongue coating was recorded according to Oho et al.2001, in which the total area and thickness of the tongue coating was determined by visual inspection. The area was recorded as a score of 0 to 3 (0, no tongue coating; 1, tongue coating covering less than 1/3 of tongue dorsum; 2, tongue coating covering 1/3-2/3 of tongue dorsum; and 3, tongue coating covering more than 2/3 of tongue dorsum). The thickness was recorded as a score of 0 to 2 (0, no tongue coating; 1, thin tongue papillae visible; and 2, thick tongue papillae invisible). The tongue coating score was derived by multiplying the area score by the thickness score (Table 2.3) (Oho et al. 2001).

Table 2.3 Tongue Coating Score (TCS) (Oho et al. 2001)

Tongue Coating (0-6) = Area score × thickness score			
Area		Thickness	
0	No tongue coating	0	No tongue coating
1	<1/3 tongue dorsum surface covered	1	Thin tongue coating (papillae visible)
2	1/3–2/3 tongue dorsum surface covered	2	Thick tongue coating (papillae invisible)
3	>2/3 tongue dorsum surface covered		

2.3.2 Retainer Assessment

The appliance (Figure 2.6) was placed in a Teflon bag for 1 minute. A gas sample from the inside the Teflon bag containing the appliance was then collected using a gas tight syringe from the bag valve through the injection septa for gas chromatography analysis (1ml), an image demonstrating this can be seen in Figure 2.7. The appliance was then taken from the bag for an organoleptic assessment and a score was taken and recorded according to Rosenberg and McCulloch, using a 0–5 scale; no detectable odor was given score 0, hardly detected odor given score 1, lightly odor given score 2, moderate odor given score 3, strong odor given score 4, and finally extremely strong odor given score 5 (Table 1.1) (Rosenberg and McCulloch 1992).

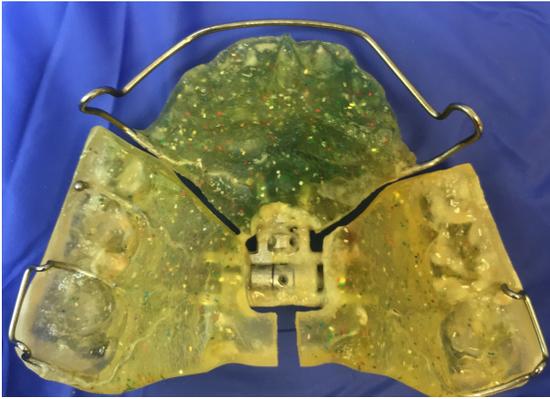


Figure 2.6 Removable Orthodontic Appliance

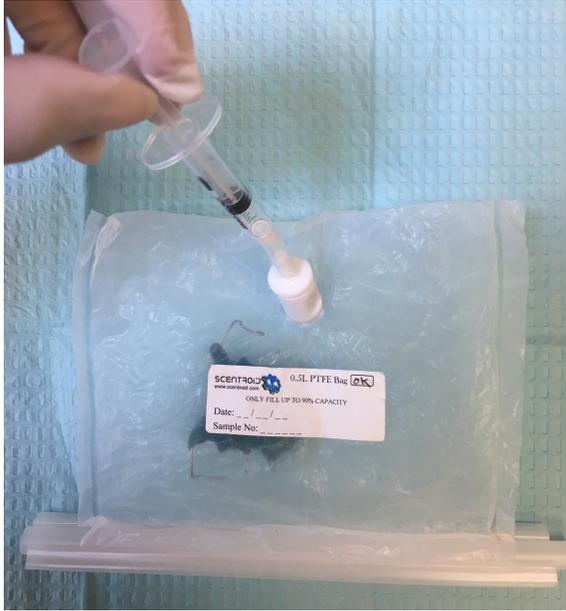


Figure 2.7 Gas Sample Taken from the Teflon Bag in which the Removable Orthodontic Appliance was Placed

2.3.3 Microbial Sample

The microbial sample was taken from the appliance using a sterile cotton swab and placed in a sterile tube and stored in a refrigerator overnight ($< 4\text{ }^{\circ}\text{C}$) so that microbes did not continue to grow. The next day the swab samples were soaked in 5 ml of BMI for 1 minute using a stopwatch then 1 ml was taken and serially diluted. 20 ml of each solution was plated on a BHI agar plate and incubated for one week at $37\text{ }^{\circ}\text{C}$ in an anaerobic bag for anaerobic testing while for the aerobic condition it was incubated for two days. Colonies were counted and results were expressed as colony-forming units per milliliter CFU/ml.

2.3.4 The Appliance Surface Porosities

The appliance surface porosity was tested and observed under scanning electron microscopy (SEM). In order to do this, a mold was made with putty. An impression was taken from both surfaces of the appliance (polished and unpolished) using a light body polyvinylsiloxane impression material; impressions were made on the right side of the retainer for consistency as shown in Figure 2.8. A positive replica of the impression was made using epoxy resin, after which the surface was prepared and an image was obtained with a scanning electron microscope (Hitachi S-3000N).



Figure 2.8 Process of Appliance Surface Impression

2.4 Randomization

After all clinical assessment and measurements were taken, the participants were provided with the mouth-rinse corresponding to their number given for the study; both the investigator and the participants were unaware of which solution the participant was being given. Written instructions (Appendix A.6) detailing how to use the mouth rinse were provided to the participants, which matched the instructions that were on the bottle

label. All the participants were instructed to brush the appliance then soak it in the solutions for 15 minutes daily for the next week and to continue their habitual oral hygiene procedures. They were instructed to soak the appliances in the rinse in a given denture cup with a marked line, which was standardized to make sure all the participants used the same amount of rinse.

A usage log (Appendix A.9) was given and the participants were instructed to return the bottles and the usage log at the re-evaluation visit.

2.5 Re-evaluation Visit

All participants were seen one week after the initial assessment with all the baseline clinical assessments, measurements, impressions of the retainer and microbiological samplings repeated. The usage log and the bottles were collected from the participants.

2.6 Data Analysis

The baseline data as well as the one-week re-evaluation visit data were collected at the above mentioned time points. The data analysis was conducted to assess the difference of the intervention within the same group (intra-group) and between the groups (inter-group).

The changes in the oral hygiene, tongue coating and gingival indices before and after the intervention were tested using Wilcoxon Signed-Rank test and Paired t-test with the significant level set at $P < 0.05$.

Before any analysis was performed the difference between the pre and post

treatment scores of each participant was taken regarding microbial changes, organoleptic scoring and the OralChroma™ gas reading

$$\Delta\text{Score} = \text{Post Score} - \text{Pre Score}$$

All subsequent analysis, with one exception, was then performed on this change in score. The exception is the transformation of microbial data to a log scale.

One-way Analysis of Variance (ANOVA) and Kruskal-Wallis were used to determine whether significant differences existed among groups with regard to: organoleptic score, VSCs and log of bacterial counts. The level of significance was $P < 0.05$ as well as Bonferroni Correction.

Chapter 3: Results

3.1 In-Vitro: Microbial killing Test

The BFMR showed an 84.61% reduction in bacterial count after soaking for 30 seconds. At, 1 minute and 3 minutes the BFMR had over a 99% bacterial count reduction, indicating a strong ability for the test solution to kill bacteria contained within a biofilm, and that a minimum of 1 minute of soaking would be required for the in-vivo test. (Table 3.1)

Table 3.1 Microbial killing Test of the BFMR

	Control (water)	BFMR (30 Sec)	BFMR (1 min)	BFMR (3 min)
Average bacterial	13×10^4	2×10^4	5.33×10^1	8.165×10^1
count	130000	20000	53.3	81.65
Applied/contx100		15.38%	0.041%	0.062%
Killing Effect %		84.61%	99.95%	99.93%

The microbial killing test of the BFMR after 30 seconds, 1 minute and 3 minutes compared to the water

3.2 In-Vivo: Pilot Clinical Trial

Data collection began in February 2016 and continued until December 2016. During this period, 67 potential participants were identified and 27 consented to participation. Reasons for non-participation were either no interest in participation or inability to attend two appointments. Of the participants, 19 were male and 8 were female, ranging from 8-16 years of age with a mean age of 11 ± 2 years old. The participant's distribution by sex and age is shown in Table 3.2

Table 3.2 Patients' Distribution by Sex and Mean Age at Initial Observation

	N	Gender Male/Female	Age (years) (Mean (SD))
Group 1 (BFMR)	9	5/4	11 (2.5)
Group 2 (CHX)	9	6/3	10 (1)
Group 3 (Water)	9	8/1	11 (1.3)

3.2.1 Controlled Variables

The results of data differences of the oral hygiene is reported in Table 3.3, showed no significant differences using paired t-test ($p > 0.05$). The tongue coating and the gingival indices showed no significant differences using Wilcoxon Signed-Rank test ($p > 0.05$) throughout the study, indicating that oral hygiene behaviors were not altered by the participants due to their involvement in this study.

Table 3.3 Comparison of the Oral Hygiene Index Before and After Treatment with BFMR, CHX and Water

	Groups	Baseline mean (SD)	Reevaluation mean (SD)	p-value
OHI	Group 1 BFMR	0.85 (0.33)	0.77 (0.32)	0.288 ⁺
	Group 2 CHX	0.66 (0.28)	0.69 (0.36)	0.692 ⁺
	Group 3 Water	0.75 (0.35)	0.76 (0.31)	0.889 ⁺

⁺ Analyzed using paired t-test, Significance at $p < 0.05$

3.2.2 Microbial Results

A significant difference was detected between the groups by both the ANOVA and Kruskal-Wallis ($p < 0.05$) in aerobic and anaerobic bacterial count before and after the treatment. A significant reduction in both the aerobic and the anaerobic bacterial count in

the BFMR group ($p < 0.05$) a moderate, but not significant reduction in the CHX group and a minimal reduction in the water group (Table 3.4, 3.5) with corresponding boxplots shown in Figure 3.1

Table 3.4 Comparison of the Aerobic Bacterial Count Before and After Treatment with BFMR, CHX and Water

Log(Aerobic Microbe +1)	ANOVA p-value	Corrected ANOVA p-value (factor of 9)	Kruskal Wallis p-value	Corrected Kruskal Wallis p-value (factor of 9)
Comparison of group means	0.01901*	0.171	0.00231*	0.021
Pre-Post group effect.	Estimated mean score	Standard error	p-value	Bonferroni adjusted p-value
Group 1 BFMR	-4.69E+00	1.50E+00	0.00452*	0.04068
Group 2 CHX	-2.21E+00	1.50E+00	0.15227	1
Group 3 Water	3.00E-01	1.50E+00	0.84279	1

Results for the log transformation of the aerobic bacterial count

* $P < 0.05$

Table 3.5 Comparison of the Anaerobic Bacterial Count Before and After Treatment with BFMR, CHX And Water

Log(Anaerobic Microbe+1)	ANOVA p-value	Corrected ANOVA p-value (factor of 9)	Kruskal Wallis p-value	Corrected Kruskal Wallis p-value (factor of 9)
Comparison of group means	0.02041*	0.184	0.01018*	0.092
Pre-Post group effect.	Estimated mean score	Standard error	p-value	Bonferroni adjusted p-value
Group 1 BFMR	-4.81E+00	1.54E+00	0.00471*	0.04239
Group 2 CHX	-2.23E+00	1.54E+00	0.16133	1
Group 3 Water	-1.73E-01	1.54E+00	0.91187	1

Result for the log transformation of the Anaerobic bacterial count

* $P < 0.05$

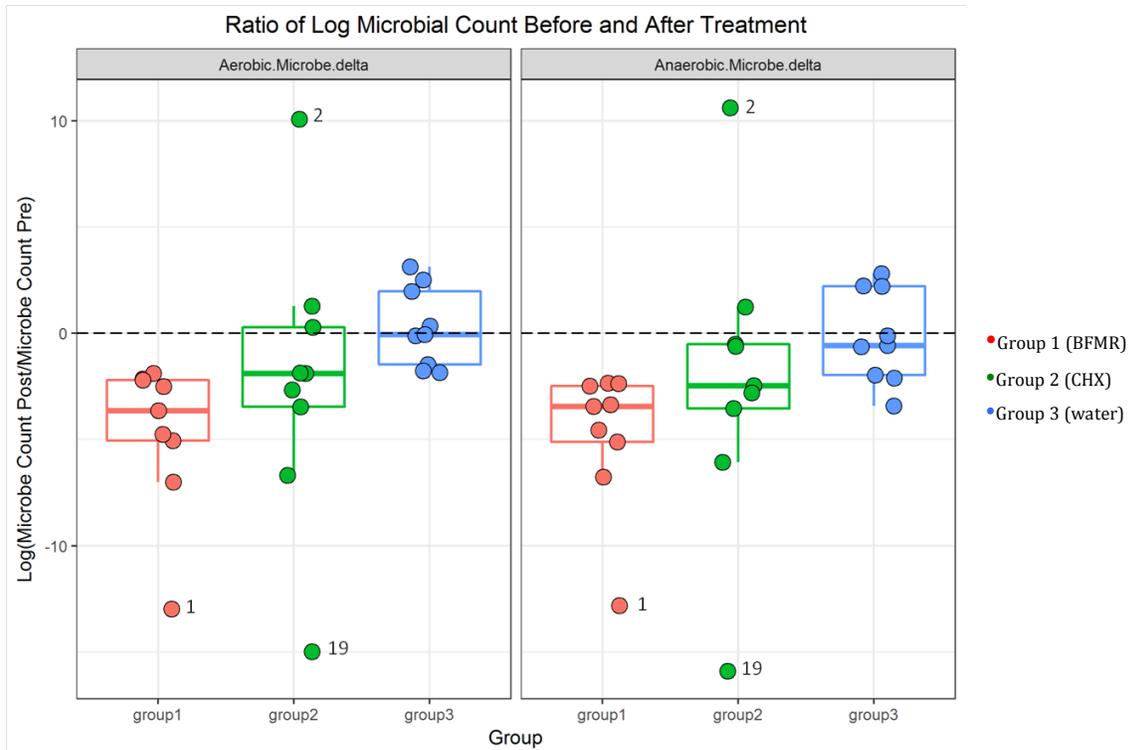


Figure 3.1 Boxplot of the Changes in Aerobic and Anaerobic Microbial Counts Before and After Treatment in the Three Test Groups. The red represent group 1 which was given BFMR, the green represent group 2 which was given CHX and the blue represent group 3 which was given water

The BFMR group showed a $94.4\% \pm 5.8$ aerobic and $96.1\% \pm 3.9$ anaerobic bacterial reduction, with all (100%) participants in the group showing a bacterial reduction. While the CHX group showed a $93.2\% \pm 6.9$ aerobic and $88.0\% \pm 20.9$ anaerobic bacterial reduction, with 6 of 9 (66%) participants in the group showing a bacterial reduction. The water group showed a $51.9\% \pm 40$ aerobic and $65.7\% \pm 35.8$ anaerobic bacterial reduction, with 5 of 9 (55%) subjects in the group showing a bacterial reduction. (Table 3.5)

Table 3.5 Microbial killing Effect in Each Group

Groups	Number of participants	Mean bacterial reduction \pm SD%	
		Aerobic	Anaerobic
Group 1 BFMR	9	94.4 \pm 5.8%	96.1 \pm 3.9%
Group 2 CHX	6	93.2 \pm 6.9%	88.0 \pm 20.9%
Group 3 Water	5	51.9 \pm 40%	65.7 \pm 35.8%

3.2.3 Organoleptic Results

A significant difference was detected between the groups by both the ANOVA and Kruskal Wallis ($p < 0.05$) in the organoleptic score of the odor before and after the treatment. Of the three individual groups, the BFMR group had a significant improvement ($p < 0.05$) from the pre to the post test in regards the organoleptic score, but neither the CHX nor the water group had a significant difference. (Table 3.6 figure 3.2)

Table 3.6 Comparison of the Organoleptic Score Before and After Treatment with BFMR, CHX and Water

Organoleptic score	ANOVA p-value	Corrected ANOVA p-value (factor of 9)	Kruskal Wallis p-value	Corrected Kruskal Wallis p-value (factor of 9)
Comparison of group means	0.018*	0.161	0.043*	0.387

Post-Pre group effect.	Estimated mean score	Standard error	p-value	Bonferroni adjusted p-value
Group 1 BFMR	-1.333	0.407	0.003*	0.02871
Group 2 CHX	-0.444	0.407	0.286	1
Group 3 Water	0.222	0.407	0.590	1

* $P < 0.05$

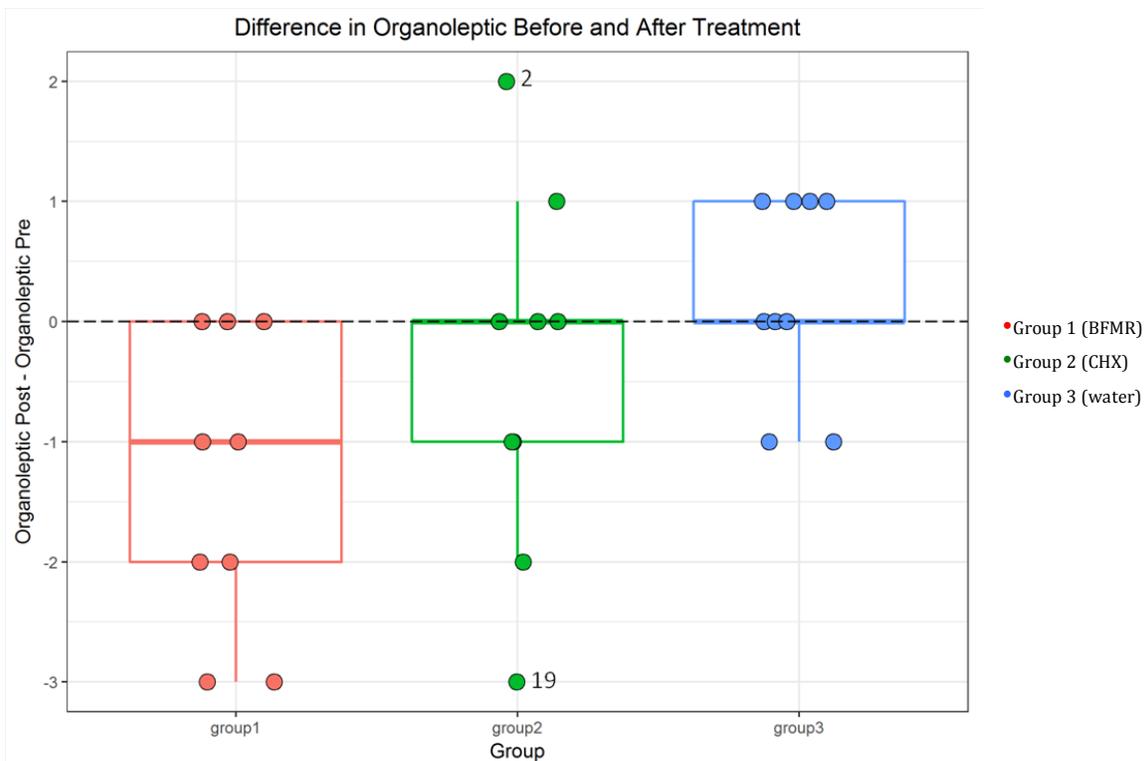


Figure 3.2 Boxplot of the Changes in Organoleptic Score Before and After Treatment in the Three Test Groups. The red represent group 1 which was given BFMR, the green represent group 2 which was given CHX and the blue represent group 3 which was given water

In the BFMR Group 6 of the 9 participants showed odor reduction by an average decrease of 1-2 score in the organoleptic score scale. However, in the CHX group only 4 of the 9 participants showed odor reduction by an average decrease of 1 score in the organoleptic score scale. In the water group 2 of the 9 participants showed odor reduction by an average decrease of 1 score in the organoleptic score scale.

3.2.4 OralChroma™ Results

There was no evidence of difference between any of the groups for the pre and post scores for either $(\text{CH}_3)_2\text{S}$ (Figure 3.3, table 3.7), CH_3SH (Figure 3.4, table 3.8) and H_2S (Figure 3.5, table 3.9) for both the appliance gas sample and the breath sample.

These results may trend in score similar to the organoleptic and microbial results, however the variance of the data is too large to resolve any effect that may exist in this particular pilot study.

Table 3.7 Comparison of the Level of Dimethyl Sulfide ((CH₃)₂S) of the Retainer and Breath Air Sample Before and After Treatment with BFMR, CHX and Water

(CH₃)₂S retainer	ANOVA p-value	Corrected ANOVA p-value (factor of 9)	Kruskal Wallis p-value	Corrected Kruskal Wallis p-value (factor of 9)
Comparison of group means	0.763	1.000	0.654	1.000
Post-Pre group effect.	Estimated mean score	Standard error	p-value	Bonferroni adjusted p-value
Group 1 BFMR	0.899	0.995	0.375	
Group 2 CHX	-0.057	0.995	0.955	
Group 3 Water	0.586	0.995	0.562	

(CH₃)₂S breath	ANOVA p-value	Corrected ANOVA p-value (factor of 9)	Kruskal Wallis p-value	Corrected Kruskal Wallis p-value (factor of 9)
Comparison of group means	0.424	1.000	0.093	0.835
Post-Pre group effect.	Estimated mean score	Standard error	p-value	Bonferroni adjusted p-value
Group 1 BFMR	-0.200	0.816	0.809	
Group 2 CHX	1.357	0.816	0.110	
Group 3 Water	-0.233	0.816	0.777	

Non significant p>0.05

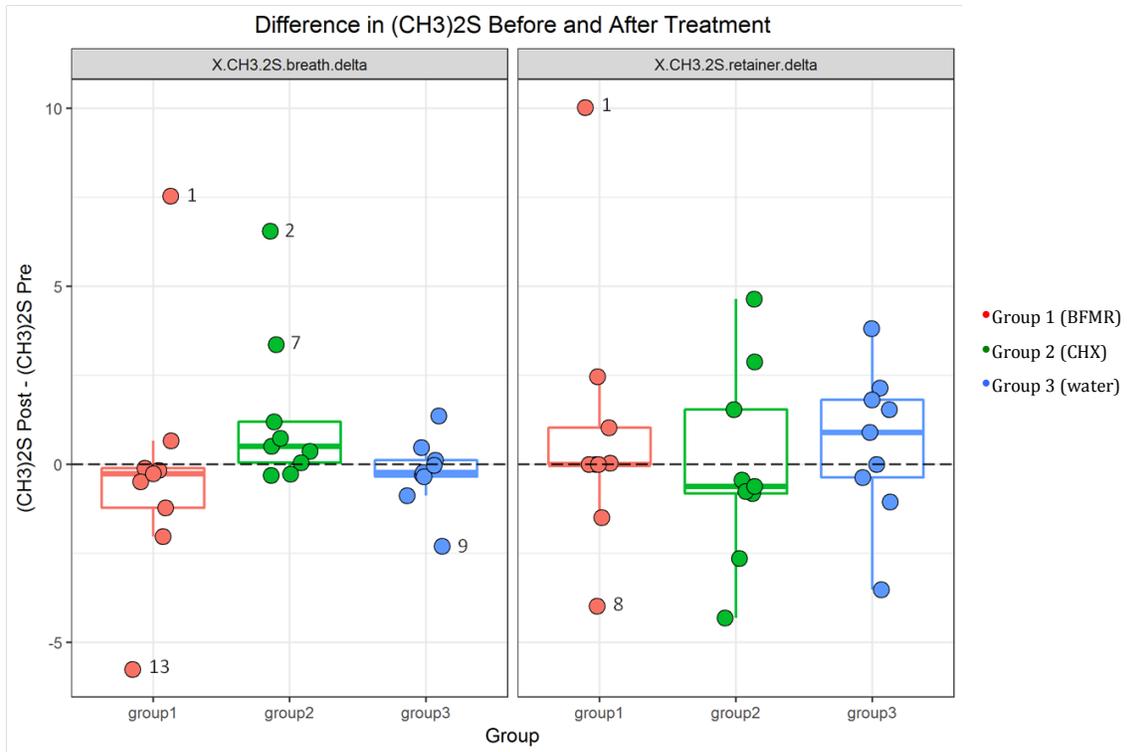


Figure 3.3 Boxplot of the Changes in the Level of Dimethyl Sulfide ((CH₃)₂S) Before and After Treatment in the Three Test Groups. The red represent group 1 which was given BFMR, the green represent group 2 which was given CHX and the blue represent group 3 which was given water

Table 3.8 Comparison of the Level of Methyl Mercaptan (CH₃SH) of the Retainer and Breath Air Sample Before and After Treatment with BFMR, CHX and Water

CH₃SH retainer	ANOVA p-value	Corrected ANOVA p-value (factor of 9)	Kruskal Wallis p-value	Corrected Kruskal Wallis p-value (factor of 9)
Comparison of group means	0.766	1.000	0.688	1.000
Post-Pre group effect.	Estimated mean score	Standard error	p-value	Bonferroni adjusted p-value
Group 1 BFMR	-0.040	0.234	0.866	
Group 2 CHX	0.228	0.234	0.340	
Group 3 Water	0.097	0.234	0.683	

CH₃SH breath	ANOVA p-value	Corrected ANOVA p-value (factor of 9)	Kruskal Wallis p-value	Corrected Kruskal Wallis p-value (factor of 9)
Comparison of group means	0.061	0.545	0.050	0.449
Post-Pre group effect.	Estimated mean score	Standard error	p-value	Bonferroni adjusted p-value
Group 1 BFMR	0.060	0.299	0.843	
Group 2 CHX	-0.237	0.299	0.436	
Group 3 Water	0.834	0.299	0.010	

Non significant p>0.05

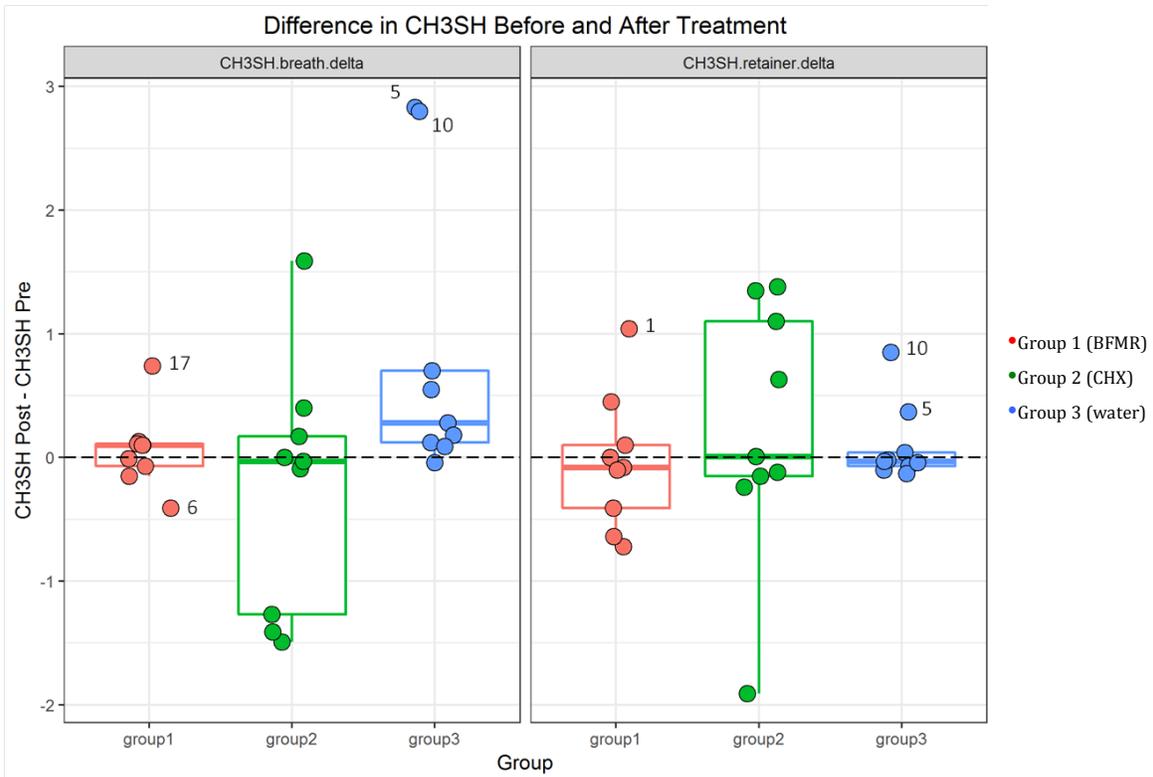


Figure 3.4 Boxplot of the Changes in the Level of Methyl Mercaptan (CH_3SH) Before and After Treatment in the Three Test Groups. The red represent group 1 which was given BFMR, the green represent group 2 which was given CHX and the blue represent group 3 which was given water

Table 3.9 Comparison of the Level of Hydrogen Sulfide (H₂S) of the Retainer and Breath Air Sample Before and After Treatment with BFMR, CHX and Water

H ₂ S retainer	ANOVA p-value	Corrected ANOVA p-value (factor of 9)	Kruskal Wallis p-value	Corrected Kruskal Wallis p-value (factor of 9)
Comparison of group means	0.348	1.000	0.839	1.000
Post-Pre group effect.	Estimated mean score	Standard error	p-value	Bonferroni adjusted p-value
Group 1 BFMR	-0.940	0.837	0.272	
Group 2 CHX	-0.104	0.837	0.902	
Group 3 Water	-1.236	0.837	0.153	

H ₂ S breath	ANOVA p-value	Corrected ANOVA p-value (factor of 9)	Kruskal Wallis p-value	Corrected Kruskal Wallis p-value (factor of 9)
Comparison of group means	0.714	1.000	0.377	1.000
Post-Pre group effect.	Estimated mean score	Standard error	p-value	Bonferroni adjusted p-value
Group 1 BFMR	-0.770	0.971	0.435	
Group 2 CHX	-0.702	0.971	0.476	
Group 3 Water	-0.457	0.971	0.642	

Non significant p>0.05

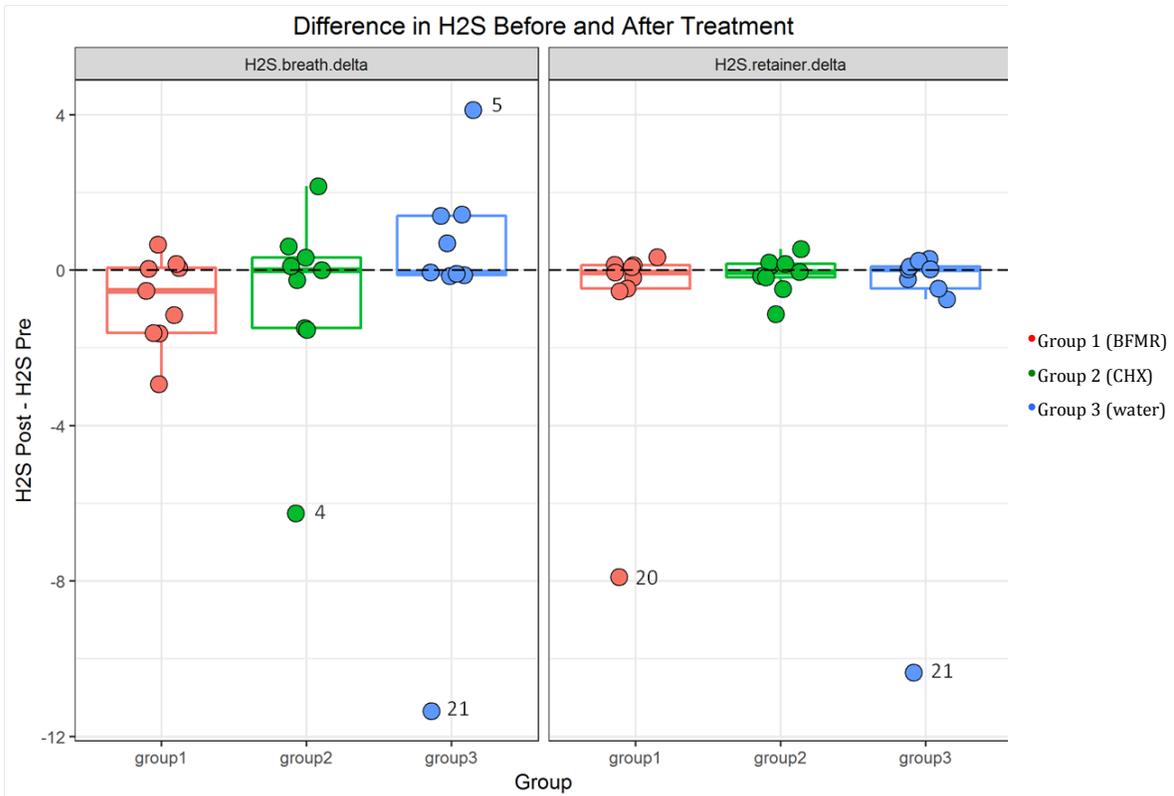


Figure 3.5 Boxplot of the Changes in the Level of Hydrogen Sulfide (H₂S) Before and After Treatment in the Three Test Groups. The red represent group 1 which was given BFMR, the green represent group 2 which was given CHX and the blue represent group 3 which was given water

3.2.5 Scanning Electron Microscope: Appliance Surface

Impressions were initially taken to assess the surface porosities and to compare any changes from the baseline to the follow-up. However, several challenges occurred such as bubbles and artifacts in the impression material and the replica, which was hard to exclude. However, the porosities were clearly visible on the surface of the appliance scanned under the SEM, as shown in Figure 3.6.

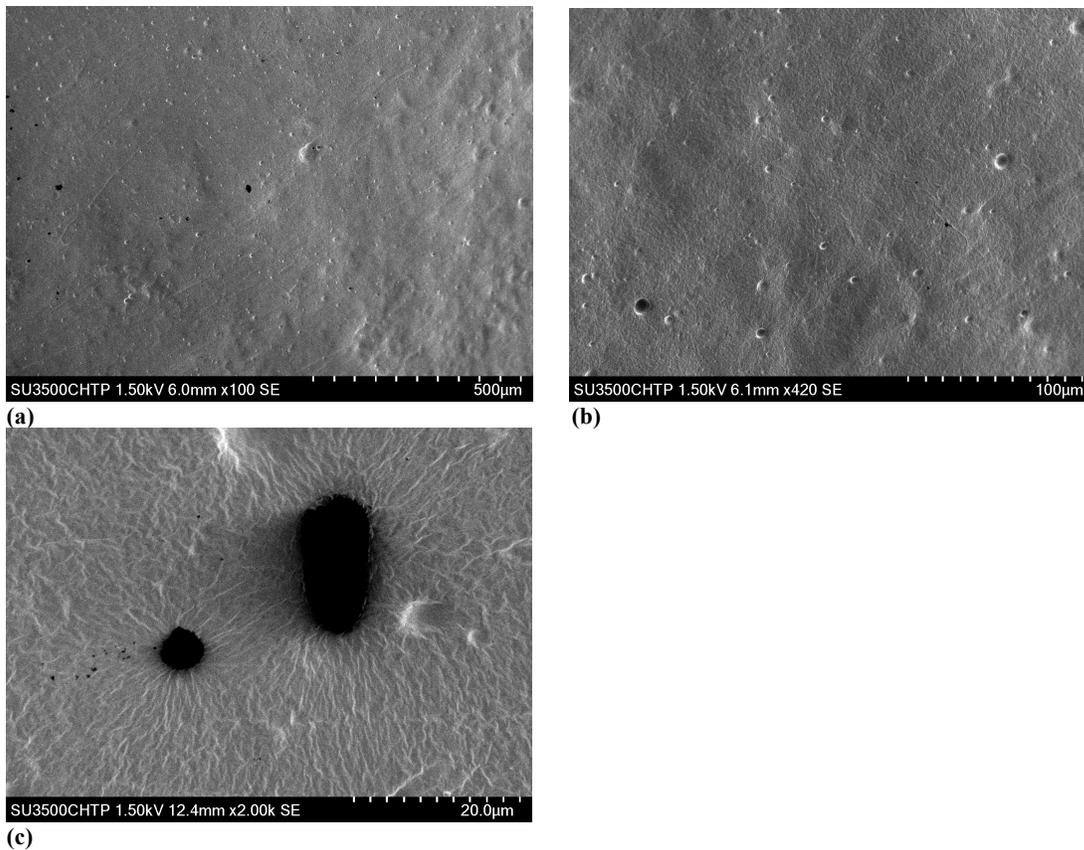


Figure 3.6 Orthodontic Removable Appliance Surface Porosities: viewed at (a) 100X magnification, (b) 420X magnification, and (c) 2000X magnification using Scanning electron microscopy (SEM)

3.2.6 Compliance Assessment Results

A usage log was used by all participants to monitor the compliance of the participants, all the participants completed the usage log and soaked the appliances for 7 days except one participant in the BFMR group who soaked the appliance just for two days. The rinse bottles all returned empty except for the same participants that soaked the appliance for two days.

Chapter 4: Discussion

4.1 Principal Findings

In this study, after controlling the variables that might affect the malodor changes which include the oral hygiene, tongue coating and gingival condition, we found that the BFMR was more effective than CHX and water in reducing microbial counts and malodor related to removable orthodontic appliances among participants who had complained of malodor from their appliances. Our results showed that CHX was a somewhat effective antimicrobial, and minimally effective in reducing malodor from the removable orthodontic appliances. As expected water, which was used as a negative control, had little to no effect on microbial counts and malodor.

As we know the tongue is the most populated area with bacterial biofilm, which produce VSC's (Kazor et al. 2003, Roldan et al. 2003, Scully and Greenman 2008), and has been shown to be strongly associated with halitosis among patients attending a breath clinic (Quirynen et al. 2009). Moreover, periodontal diseases are also associated with oral malodor, and several studies have shown a relation to both gingivitis and periodontitis (Miyazaki et al. 1995, Delanghe et al. 1997, Bornstein et al. 2009, Bornstein et al. 2009, Quirynen et al. 2009). In this study we measured the reduction in microbial colony forming unit and reduction in malodor related to the removable orthodontic appliance and not a change in oral hygiene habits that could have affected the results. Therefore, having no significant changes in oral hygiene, tongue coating and gingival condition throughout the study in all groups, it indicated that these factors that can contribute to the malodor changes were controlled and did not appear to contribute to the reductions observed.

To our knowledge there are no previous studies that have tested the antimicrobial or the anti-malodor effect of bioflavonoids on removable orthodontic appliances. Even though, bioflavonoids are well known antimicrobial agents tested in-vitro (Kumar and Pandey 2013), this study involved testing the BFMR in-vivo on an oral biofilm. Unlike planktonic microbes, microbes in a biofilm are more resistant to the toxic effects of some chemicals (Kouidhi et al. 2015). To ensure that the test BFMR was able to have an antimicrobial effect, we first assessed in-vitro its ability as an antimicrobial with a human oral biofilm. The results of that test showed that in addition to being able to kill particular microbes as indicated by lab test performed by the manufacturer, the BFMR was able to do the same with an oral biofilm that contained both aerobic and anaerobic species of oral bacteria. These results further support the antimicrobial properties of the BFMR when used as a solution to soak removable oral appliances. Furthermore, our results have shown that the BFMR has the ability to reduce malodor from oral appliances as assessed by organoleptic measures.

The microbial swab of the removable orthodontic appliance was done in addition to the malodor assessments to determine whether the effect of the mouth rinses on malodor were due to a masking effect such as the effect of chewing gum, rinsing products, cloves and mint tablets (Sterer and Rubinstein 2006), or a reduction of microorganisms that cause malodor. Due to the high kill counts of the microbes we can conclude that the effect of the BFMR on malodor was most likely a result of its antimicrobial activity rather than from a masking effect that is commonly seen in other commercial mouth rinses.

The appliances in the CHX group did not show any significant reduction in the malodor assessed by organoleptic and OralChroma™ measurement, which was not in agreement with previous studies that have shown that CHX has good anti-malodor effect (De Boever and Loesche 1995, Bosy kulkarni et al.1994). The malodor assessment using the organoleptic score showed significant reduction in the BFMR group only. However, the malodor assessment using the OralChroma™ did not change significantly which may indicate that the sensitivity of the machine to pick the gases changes from the Teflon bag was not high; therefore, we just used the organoleptic score since it is still considered the gold standard in oral malodor assessment.

Our results are interesting in that CHX, which is widely used as an antimicrobial agent for cleaning removable orthodontic appliances, did not perform as well as the BFMR (Brex et al. 1993). Several studies have shown the effectiveness of CHX in reducing microbial counts. Lessa et al, found that acrylic baseplates of removable orthodontic appliances sprayed with 0.12% CHX showed significant efficacy in reducing *Streptococcus mutans* colonies/biofilms on acrylic surfaces, and performed better than a CPC based rinse as well as sterile tap water (Lessa et al. 2007). Peixoto et al, also found that baseplates sprayed with 0.12% CHX once or twice a week reduced the *Streptococcus mutans* contamination on acrylic surfaces (Peixoto et al. 2011). The difference between this study and the previous studies may be explained by the sample size or participant cooperation; however, the cooperation in this study was assessed by a usage log and the amount of rinse returned in the bottles. Another possible explanation is that CHX is less effective on biofilms compared to microorganisms in their free form as others have reported (Bonez et al, 2013).

Impressions were initially taken of the appliances surface to assess the effect of the BFMR on the surface porosities and to compare any changes from the baseline to the follow-up. However, after conducting initial scans of the surface under the SEM several difficulties were present such as bubbles and artifacts in the impression material and the replica, which was hard to exclude so the plan for identifying and comparing the pre- and post- treatment surface porosities of the appliances changes could not be achieved. However, given that we had the impressions and the SEM scans the only thing that we can conclude is the surface porosities of the appliances, which support what was mentioned previously about the microorganism retentive site on the appliances (Lessa et al. 2007). These biofilms are difficult to remove with routine hygiene measures, therefore, having an antimicrobial as an adjunct to the mechanical cleaning produced superior antimicrobial and anti-malodor effect. Future studies that assess changes in the surface porosities or roughness after soaking the appliances in the BFMR would be useful. Furthermore, similar to the work by Collares, an assessment of the effect of the BFMR on acrylic resin hardness and color would provide important information to the potential for adverse effects to the orthodontic appliances.

4.2 Strengths of the Current Research

This pilot randomized clinical trial has evaluated the malodor with a standard organoleptic method that is the gold standard for oral malodor assessment. Furthermore, the microbial test confirmed the real antimicrobial effect of the mouth rinses used in each group to exclude the masking effect.

Assessment of the oral hygiene, gingival condition and tongue coating add

strength to this study by excluding any factor that can contribute to the malodor and the bacterial count changes. Furthermore, having no changes in the baseline and follow-up measurements showed that the participants did not change their behavior because they were included in the study.

A usage log allowed for the monitoring of compliance of the patient as well as the amount of solution that was used. All participants completed the log and recorded usage for the seven days. All of the rinse bottles were returned empty except one from a participant in the BFMR group who indicated that she just soaked the removable appliance for two days. Interestingly, even two days of soaking reduced the microbial count significantly.

4.3 Limitations of the Current Research

The most significant limitation of this study is the small sample size. However, in this pilot study the sample size was enough to reach statistical significance, but further investigation with a larger sample should be conducted to confirm our preliminary results. Another limitation of the present study is the low recruitment rate. Of the sixty-seven potential participants that were contacted, only twenty-seven agreed to participate. Future studies among this population will need to ensure that multiple recruitment sites are involved in order to reach a number that could provide a large enough sample size to represent the variety in the population.

Furthermore, the variance of the data was too large to resolve any effect that may exist in the gas sample that was collected from the appliances placed in a sealed Teflon bag and analyzed by the GC. The use of a chamber instead of a Teflon bag might allow

for the detection of the gases and reduce adsorption of the gases to the bag surface. However, the organoleptic score was obtained for each retainer and is a recognized appropriate assessment for oral malodor.

Another limitation, was that all participants were asked to soak the appliances for 15 minutes and brush the appliance as they would normally, however there was no clear instruction for how long to brush the appliance and since the mechanical cleaning has an effect on reducing the microbial count this could have further impacted the results.

Finally, the presence of outliers reduces the effectiveness of the tests. However outliers should only be removed if there is a justifiable reason such as a measurement error or deviation from protocol. The removal of participants 1 and 19, while very tempting due to the resulting improved significance of the mean effect of the CHX-group, is not good idea practice. It would be of interest to know whether these two participants differed from other participants in their management of the appliance cleansing or oral hygiene practices.

4.4 Significance of the Current Research

Compliance of wearing a removal orthodontic appliance is greatly impacted by the patient's perception of appliance odor. Since these appliances are difficult to clean by typical mechanical measures, the addition of a chemical cleanser is often employed. Our results have shown that a natural, over the counter solution has a greater antimicrobial and anti-malodor effect on removable orthodontic appliances and may offer a superior option to a prescription oral rinse that has side effects. Furthermore, if such a solution improves compliance of the appliance wearer, treatment could ultimately be improved.

4.5 Options for Future Experiments

There are several different ways this study can be used to improve upon further experiments.

4.5.1 Reducing Variability

The data from this study are quite variable. Some participants were maintaining their own oral hygiene while others had the appliance maintained by their parents. These and other differences in the effective treatment probably contributed to the variability of the observed data. If the expected variability could be reduced, for example through tighter inclusion/exclusion rules or more careful monitoring/instructions, reproducibility of future studies would be more certain.

4.5.2 Improving Power

While one way to improve the effectiveness (ie power) of the test is by increasing the number of observed patients, another way is to increase the expected difference between the treatments. There are a number of possible improvements in cleaning procedure such as longer soaking time or the use of ultrasonic cleaning bath.

4.5.3 Experimental Design

The design used for this study was appropriate as a pilot study. The simplicity made the study relatively easy to run and the statistics easy to perform and interpret. However, a more complicated study design could do a better job of accounting for the

variability within the population of appliance users. One example of a different design is the crossover study. A crossover design provides all the treatments to each participant, permitting control for each participant's individual eccentricities. This could be done by assigning the treatment to each patient in a random order and then measuring the participant's pre- and post- scores for each treatment. To be statistically useful this design requires an assumption that the patient will "reset" to the starting condition after each treatment. For this reason, the study would require a considerably greater time commitment for each participant.

4.5.4 Improved Instrumental Analysis

Improvement of the method that was used to assess the odor of the retainer using OralChroma™, the usage of a chamber instead of a Teflon bag might allow the detection of the gases changes. Another improvement that might be considered is the process of impression taking of the retainer, the direct scanning of the retainers might give better results.

Chapter 5: Conclusion

Malodor of removable orthodontic appliances is most often caused by the incomplete mechanical removal of microbial biofilm that produce VSCs. This odor might decrease compliance of the patient wearing the appliance and could potentially affect the efficiency of orthodontic treatment. The addition of an antimicrobial solution such as CHX to aid in cleaning such appliances has been somewhat successful, but has drawbacks. This pilot study has shown that a non-prescription, natural, commercially available BFMR with no known side effects has superior antimicrobial and anti-malodor effect compared to CHX. The results demonstrated a significant reduction in the microbial count in the BFMR group with a $94.4\% \pm 5.8$ aerobic and $96.1\% \pm 3.9$ anaerobic bacterial reduction. Malodor assessed using the organoleptic method was significantly reduced in the BFMR group relative to the CHX and water groups. While the gas chromatograph measurements of appliance odor did not show significant reductions, there was considerable variety, and the changes that were detected were in the same direction as the organoleptic scores. Further studies with a larger sample size that utilize a cross-over design are needed to definitively determine how effective BFMR is at reducing malodor compared to CHX.

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Appendix

Appendix A – Consent forms and Data Collection Forms

Copies of the consent forms, assent forms and Data collection forms are included below for reference.

A.1 Invitation e-mail

UBC DENTISTRY



Invitation Letter

Dear,

My name is Hajer Alsabban. I am a graduate student in the orthodontic department at the University of British Columbia. I am conducting a randomized clinical trial to study the efficiency of a natural mouth rinse on oral malodor. If there is any patient that is complaining of malodor related to an orthodontic appliance or you noticed an appliance with malodor, please let me know so I can arrange to meet with the patient and explain the study.

Title of the Study: Assessing the efficacy of a bioflavonoid-based mouth rinse in reducing oral malodor on removable orthodontic appliances in comparison to Chlorhexidine

Principal Investigator: Dr. Leeann Donnelly

Co-Investigator: Dr. Hajer Alsabban

Purpose: To assess the anti-microbial and the anti-malodor efficacy of the BFMR.

Patients that meet the following criteria may be eligible:

- Males and females with age ranging between 8-20 years old
- A removable orthodontic appliance that has an obvious odor, will be assessed by an examiner
- Exclusion criteria will include an allergy to citrus or CHX, active caries, or antibiotic use in the previous month

Information of the visits:

The procedures and visits will include the following:

- A visual dental exam to check gums, plaque and tongue
- Oral malodor assessment
- Microbial swab from the appliance

That visit will take 15 minutes. Then the participants will be blindly randomized, into three groups with different mouthwash to soak the appliance. A re-evaluation visit will be done after one week with all the above procedures repeated and will take 15 minutes.

We will be happy to answer any questions you have about the study. You may contact me using the contact information provided below.

Thank you for your time and consideration.

With kind regards,
Dr.Hajer Alsabban

A.2 Invitation Letter

PARTICIPANTS NEEDED FOR RESEARCH IN TREATING ORTHODONTIC RETAINER ODOR

We are looking for volunteers to take part in a study on different treatments for orthodontic retainer odor.

As a participant in this study, you would be asked to: come to the Frontier Clinical Research Center at the University of British Columbia; have a dental exam; provide a breath sample and use one of 3 rinses to soak your retainer.

Your participation would involve 2 sessions,
each of which is approximately 20-30 minutes.

In appreciation for your time, you will receive re-imburement for parking at UBC, all examinations and rinses related to the study free of charge

For more information about this study, or to volunteer for this study,
please contact:

Dr. Hajer Alsabban

**This study has been reviewed by, and received ethical approval
through a University of British Columbia, Clinical Research Ethics Board.**

A.3 Participant Information and Consent Form

UBC DENTISTRY



DEPARTMENT OF ORAL BIOLOGICAL AND MEDICAL SCIENCES

PARTICIPANT INFORMATION AND CONSENT FORM

Assessing the efficacy of a natural mouth rinse in reducing oral odor on removable dental appliances

WHO IS CONDUCTING THE STUDY

Principal Investigator: Dr. Leeann Donnelly

Co-Investigator: Dr. Hajer Alsabban

If you are a parent or legal guardian of a child who may take part in this study, permission from you and the assent (agreement) of your child may be required. When we say “you” or “your” in this consent form, we mean you and/or your child; “we” means the doctors and other staff.

1. THE INVITATION

You and your child are being invited to take part in this research study conducted by the University of British Columbia because you or your child is complaining of oral malodour associated with their removable orthodontic appliance.

2. YOUR PARTICIPATION IS VOLUNTARY

Participation is entirely voluntary. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts. If you decide to participate, you or the child may still choose to withdraw from the study at any time without any negative consequences to the dental care, or other services to which you are entitled or are presently receiving from the University of British Columbia.

If you wish to participate, please sign on Page 5. You do not have to provide any reason for your decision not to participate. You are still free to withdraw yourself or your child at any time without giving any reasons for your decision by contacting the Principal Investigator

Please take time to read the following information carefully and to discuss it with your child, family, friends, and doctor/dentist before you decide.

3. BACKGROUND

Oral malodor is a complaint of many individuals, especially with those who wear a removable orthodontic appliance. There are several treatments however, chlorhexidine mouthwash is the most commonly used. This study is testing the effectiveness of a natural mouthwash (Citrarinse Natural) in treating oral malodor. If this natural, non-prescription solution is as effective at reducing odor related to your removable orthodontic appliances it may offer a more realistic and safer option than a prescription oral mouthwash as chlorhexidine that has side effects such as staining of the teeth and might cause burning sensation.

4. WHAT IS THE PURPOSE OF THE STUDY?

The purpose of this study is to assess the anti-microbial and the anti-malodor efficacy of the natural mouthwash

5. WHO CAN PARTICIPATE IN THE STUDY?

Children and adolescents between the age 8-20 years attending the UBC Dental Clinic or a private practice Orthodontist and complaining of oral malodor related to removable orthodontic appliance.

Exclusion from the study will include Allergy to citrus or Chlorhexidine, active caries and antibiotic use in the previous month

6. WHAT DOES THE STUDY INVOLVE?

This study will take place at UBC Dentistry. A total of approximately 30 participants will be enrolled for the entire study. Access to the health records will be done to check the allergy and medications

The procedures and visits will include the following:

- A visual dental exam to check gums, plaque and tongue
- Oral malodor assessment – sniff test that will be done by two judges to sniff air coming from the mouth and give a score of the odor. Breath sample that will be taken by inserting a plastic needled syringe into the oral cavity then inserted and analyzed by a machine
- Microbial swab from the appliance

That visit will take 15-30 minutes. You will then be blindly randomized by computer to one of the three groups, the chance will determine which group you will be in; one group will be given the natural mouthwash the other will be given chlorhexidine and one group will be given water. You will be instructed to brush the appliance then soak it in the solutions for 15 min daily for a week and to continue with habitual oral hygiene procedures. A re-evaluation visit will be done after one week with all the above procedures repeated and will take 15 minutes.

The sample that will be taken will be destroyed immediately after the records as the breath sample will be injected in the machine and disappear and the microbial swab will be destroyed after taking the measurements.

The collected information will remain confidential and will be used for research and service planning purposes only.

7. WHAT ARE THE POSSIBLE HARMS AND DISCOMFORTS?

There are no anticipated possible harms or discomforts related to this study other than those typically associated with a dental examination and by using Chlorhexidine as medicated mouthwash in the study that may cause an unpleasant taste, can give rise to a burning sensation, and can cause staining of the teeth but these risk are low due to soaking the appliances not direct application of Chlorhexidine

8. WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?

No one knows whether or not you will benefit from this study. There may or may not be direct benefits to you from taking part in this study. However, the group that will be given medicated mouthwash would see an improvement in oral malodor

We hope that the information learned from this study can be used in the future to benefit other people in a similar situation.

9. WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?

You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, you have the right to request the withdrawal of your information collected during the study. This request will be respected to the extent possible. Please note however that there may be exceptions where the data will not be able to be withdrawn for example where the data is no longer identifiable (meaning it cannot be linked in any way back to your identity) or where the data has been merged with other data. If you would like to request the withdrawal of your data, please let your study doctor know.

10. HOW WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator or his or her designate and by representatives of UBC Clinical Research Ethics Board for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that

disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a participant in this study. This number will not include any personal information that could identify you (e.g., it will not include your Personal Health Number, SIN, or your initials, etc.). Only this number will be used on any research-related information collected about you during the course of this study, so that your identity will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to insure that your privacy is respected. You also have the legal right of access to the information about you that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

11. WHAT HAPPENS IF SOMETHING GOES WRONG?

By signing this form, you do not give up any of your legal rights and you do not release the study doctor, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided at no additional cost to you. The costs of your medical treatment will be paid by your provincial medical plan.

12. WHAT WILL THE STUDY COST ME?

There are no costs for participating in this study and you will not be paid for your participation.

13. WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?

If you have any questions or would like further information about this study before or during participation, you can contact, the Principal Investigator, Dr. Leeann Donnelly

14. WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT DURING THE STUDY?

If you have any concerns or complaints about your rights as a research participant and/or your experiences while participating in this study, contact the Research Participant

Complaint Line in the University of British Columbia Office of Research Ethics by e-mail or by phone

STUDY TITLE: Assessing the efficacy of a natural mouth rinse in reducing oral odor on removable dental appliances

PARTICIPANT CONSENT

- I have read and understood the information in this consent form.
- I have had enough time to think about the information provided.
- I have been able to ask for advice if needed
- I have been able to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific purposes.
- I understand that my participation in this study is voluntary.
- I understand that I am completely free at any time to refuse to participate or to withdraw from this study at any time, and that this will not change the quality of care or services that I receive.
- I authorize access to my health records as described in this consent form.
- I authorize access to my samples as described in this consent form
- I understand that I am not waiving any of my or my child’s legal rights as a result of consenting to have my child participate.
- I understand that there is no guarantee that this study will provide any benefits to me or my child.

The parent(s)/guardian(s)/substitute decision-maker (legally authorized representative) and the investigator are satisfied that the information contained in this consent form was explained to the child/participant to the extent that he/she is able to understand it, that all questions have been answered, and that the child/participant assents to participating in the research.

SIGNATURES

[Redacted Signature Line]			
Participant’s Signature	Participant’s Printed Name	Date	
[Redacted Signature Line]			
Parent/Guardian Signature	Parent/Guardian Printed Name	Date	
[Redacted Signature Line]			
Signature of Person Obtaining Consent	Printed name	Study Role	Date

A.4 Adolescent Information and Assent Form

UBC DENTISTRY



DEPARTMENT OF ORAL BIOLOGICAL AND MEDICAL SCIENCES

Adolescent Information and Assent Form

Title of Study

Assessing the efficacy of a natural mouth rinse in reducing oral odor on removable dental appliances

WHO IS IN CHARGE OF THE STUDY?

The doctor in charge of the study is Leeann Donnelly. She is being helped by Dr. Hajer Alsabban. They will answer any questions I have about the study. If I am having an emergency and cannot talk to my parents or legal guardians, or if I am having any problems, I can call them.

INVITATION

I am being invited to take part in this research study because I am complaining of odor related to my removable dental appliances such as retainers. The following pages explain the study so that I can decide if I want to take part or not. It is up to me if I want to be in this study. No one will make me be part of the study and no one will get mad at me if I don't want to be a part of this study.

DO I HAVE TO BE IN THIS STUDY?

I do not have to participate in this study if I don't want to. If I choose to participate, I can stop being in it at any time. The doctors and my Orthodontist will take care of me as they have in the past, regardless of whether I am in the study or not.

If I want to participate in this study, I will be asked to sign this form. My parent/guardian will need to provide consent before I am enrolled in the study; but I do not have to participate even if they sign the consent form. The researchers will not enrol me into the study unless I agree to do so.

I should take time to read the following information carefully and to talk it over with my family, and if I wish, my doctor, before I decide. I understand that I should feel free to talk to the study doctors if anything below is not clear. I can choose to be in the study, not be in the study, or take more time to decide. Even if I agree now to be part of the study, I

can change my mind later. I can ask the study doctor or study coordinator any questions I may have at any time during my study participation.

WHY ARE WE DOING THIS STUDY?

This study is trying to find out if a natural mouthwash can reduce oral odor related to removable orthodontic appliances.

WHY ARE YOU INVITING ME TO BE IN THIS STUDY?

I am being invited to be in the study because I am complaining of oral odor related to removable orthodontic appliances.

WHAT WILL HAPPEN TO ME IN THIS STUDY?

If I choose to be in the study, I will be scheduled for an appointment to have a dental exam done and a breath sample taken as well as microbial swab from the appliance. Then I will be randomly assigned by computer to one of the three groups, the chance will determine which group I will be in and will be given either the natural mouthwash, medicated mouthwash or water to be used for one week to soak the appliance. After that another appointment will be scheduled to redo the dental exam, breath sample and the microbial swab.

CAN ANYTHING BAD HAPPEN?

There is nothing in the study itself that should cause anything bad to happen to me.

WHAT SHOULD I DO IF I AM NOT FEELING WELL?

If I feel sick or if I notice any strange or bad feelings during the study, especially if they are unexpected or severe, I will let the Doctors know right away.

CAN I GET BETTER BY BEING IN THE STUDY?

No one knows whether or not I will benefit from this study. The study doctors hope that the natural mouthwash will reduce oral odor as well as the medicated mouthwash.

WHO WILL KNOW I AM IN THIS STUDY?

My privacy will be respected. Unless I allow them to, the study team will not tell anybody else know I am or have been a part of this study. They will not release any information to anybody else that could be used to identify me, unless they are required to do so by law. For example, researchers are required to report if a participant is believed to be at risk for harming him/herself or others.

In order to protect my privacy, the study team will remove any information that may be used to identify me from any study documents, and instead of my name appearing on them, I will be identified by a specific study code number that applies only to me. Only this code number will be used on any research-related information collected about me for this study, so that my identity as part of the study will be kept completely private. Only Dr. Leeann Donnelly and Dr. Hajer Alsabban will have the ability to link this code number with my personal information, and the linking information will be kept in a locked cabinet in Room of the Faculty of Dentistry at UBC under the supervision and control of Dr. Leeann Donnelly.

WHAT WILL THE STUDY COST ME?

There are no costs for participating in this study and I will not be paid for participating.

WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?

If I have any questions or desire further information about this study before or during participation, or if I experience any side effects that were not outlined in this assent form, I can contact Dr. Leeann Donnelly.

WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A PARTICIPANT?

If I have any concerns or complaints about my rights as a research participant and/or my experiences while participating in this study, I should contact the Research Participant Complaint Line in the University of British Columbia Office of Research Ethics by e-mail or by phone.

FUTURE STUDIES

There is a chance that during or after this study the study team will find other questions needing answers that require future studies. If I am willing to hear about these future studies I will mark the “yes” box. This does not mean that I will have to take part in a new study, just that the study team will let me know about it.

Are you willing to be contacted by the researchers for future studies?
YES

Study Title: Assessing the efficacy of a natural mouth rinse in reducing oral odor on removable dental appliances

ASSENT TO PARTICIPATE

SIGNATURE

Participant Assent

My signature on this assent form means:

- I have read and understood this adolescent information and assent form.
- I have had enough time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had acceptable answers to my questions.
- I understand that all of the information collected will be kept confidential and that the results will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing the quality of care that I receive.
- I understand that I can continue to ask questions, at any time, regarding my participation in the study.
- I understand that if I put my name at the end of this form, it means that I agree to be in this study.

I will receive a signed copy of this assent form for my own records.

I agree to participate in this study.

Participant's Signature
Date

Printed name

A.5 Participant Assent Form

UBC DENTISTRY



DEPARTMENT OF ORAL BIOLOGICAL AND MEDICAL SCIENCES

PARTICIPANT ASSENT FORM

Title of Study

Assessing the efficacy of a natural mouth rinse in reducing oral odor on removable dental appliances

Invitation

I am being invited to be part of a research study. A research study tries to study the usefulness of a natural mouthwash for the treatment of mouth odor. It is up to me if I want to be in this study. No one will make me be part of the study. Even if I agree now to be part of the study, I can change my mind later. No one will be mad at me if I choose not to be part of this study.

Why Are We Doing This Study?

This study is trying to find the effect of a natural mouthwash in treating odor related to removable dental appliances such as retainers.

What Will Happen in This Study?

If I choose to be in the study, I will be scheduled for an appointment to have a dental exam done and a breath sample taken as well as microbial swab from the appliance. Then I will be randomly assigned by computer to one of the three groups, the chance will determine which group I will be in and will be given either the natural mouthwash, medicated mouthwash or water to be used for one week to soak the appliance. After that another appointment will be scheduled to redo the dental exam, breath sample and the microbial swab.

Who Is Doing This Study?

Dr. Leeann Donnelly and other doctors from the University of British Columbia will be doing this study. They will answer any questions I have about the study. I can also call, if I am having any problems or if there is an emergency and I cannot talk to my parents.

Can Anything Bad Happen to Me?

We don't expect anything bad to happen to you while you are in this study

What Should I Do If I Am Not Feeling Well?

If I feel sick or if I notice any strange or bad feelings during the study, especially if they are unexpected or severe, I will let the Doctor know right away.

Could I Get Better By Being in the Study?

No one knows whether or not I will benefit from this study. The study doctors hope that the natural mouthwash will be good at reducing the oral odor as well as the medicated mouthwash.

Who Will Know I Am in the Study?

Only my doctors and people who are involved in the study will know I am in it. When the study is finished, the doctors will write a report about what was learned. This report will not say my name or that I was in the study. My parents and I do not have to tell anyone I am in the study if we don't want to.

When Do I Have To Decide?

I have as much time as I want to decide to be part of the study. I have also been asked to discuss my decision with my parents.

Signatures:

If I put my name at the end of this form, it means that I agree to be in the study.

Participant's Signature
Date

Printed name

A.6 Participants Instructions



Participants Instructions

Appointments for this protocol: Estimated length

Visit 1, Date _____ At ____AM/PM _____min

Instruction before the visit:

- Avoidance of spicy foods, garlic and onions the 2 days prior to examination
- Refrain from oral activities such as brushing, flossing, rinsing, tongue cleaning, eating and drinking for at least 3 hours before the measurements

Instruction after the visit:

- Brush the appliance then soak it in the solutions for 15 minute daily for the last week before the next appointment and to continue with habitual oral hygiene procedures.
- Use the denture cup fill it with the mouth-rinse till the line and soak the appliance.

Visit 2, after 1 week Date _____ At ____AM/PM _____min

Instruction before the visit:

- Avoidance of spicy foods, garlic and onions the 2 days prior to examination
- Refrain from oral activities such as brushing, flossing, rinsing, tongue cleaning, eating and drinking for at least 3 hours before the measurements
- Soak the appliance the day before and continue using it until the visit.
- Bring the bottle of the mouth rinse with you to the visit

If you have any question please contact
Dr.Hajer Alsabban

A.7 Examination Checklist

UBC DENTISTRY



Check List For the First Visit

<ul style="list-style-type: none"> • Medical history including Allergies, Antibiotics 	
<ul style="list-style-type: none"> • Inclusion Criteria and exclusion criteria: <p>Inclusion criteria: Removable appliance, Confirmation of a VSC level of >1.5 mg/10ml for hydrogen sulfide (H₂S) or 0.5mg/10ml for methyl mercaptan (CH₃SH) using the Oral Chroma, Full-mouth organoleptic odor score 3 or more</p> <p>Exclusion criteria will include an allergy to citrus or CHX, active caries, or antibiotic use in the previous month</p>	
<ul style="list-style-type: none"> • Instructions followed prior to their clinical assessment <p>Avoidance of spicy foods, garlic and onions the 2 days prior to examination and to refrain from oral activities such as brushing, flossing, rinsing, tongue cleaning, eating and drinking for at least 3 hours before the measurements</p>	
<ul style="list-style-type: none"> • Consent Forms 	
<ul style="list-style-type: none"> • Gas sample from the retainer (oral Chroma) <p>Remove the retainer put it in a sealed bag and take odor sample using Oral Chroma after ..Min</p>	
<ul style="list-style-type: none"> • Organoleptic scoring of the retainer for the bag 	
<ul style="list-style-type: none"> • Oral breath sample of the participant mouth <p>The patient should be upright, and a syringe inserted into the oral cavity until the flange reaches the lip. The patient will be instructed to softly bite to stabilize it, and children might need an assistant help. They will be instructed to breathe through the nose while keeping the lips sealed for 30 seconds, avoid touching the tip of the syringe with the tongue, after that the piston of the syringe is pulled and filled with oral breath, then push the piston and return the gas to the oral cavity. Pull the piston again to fill the syringe completely, at this time remove the syringe. Clean the saliva from the tip with tissue paper, slowly push the plunger to the 1 cc (ml) position and within one minute the gas should be injected at a right angle to the inlet of the Oral Chroma and push down strongly on the plunger in one stroke, then remove the syringe from the inlet</p>	
<ul style="list-style-type: none"> • Clinical Exam <p>Plaque index, gingival index and tongue coating</p>	
<ul style="list-style-type: none"> • Microbial swab 	
<ul style="list-style-type: none"> • Impression of the retainer 	

<ul style="list-style-type: none"> • Randomization and mouth rinse given • Instruction given • Usage Log 	
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Check List For the Re-evaluation

<ul style="list-style-type: none"> • Instruction Followed <p>Avoidance of spicy foods, garlic and onions the 2 days prior to examination and to refrain from oral activities such as brushing, flossing, rinsing, tongue cleaning, eating and drinking for at least 3 hours before the measurements</p>	
<ul style="list-style-type: none"> • Return of the Mouth rinse • Return of the Usage Log 	
<ul style="list-style-type: none"> • Gas sample from the retainer(oral Chroma) <p>Remove the retainer put it in a sealed bag and take odor sample using Oral Chroma after .. Min</p>	
<ul style="list-style-type: none"> • Organoleptic scoring of the retainer for the bag 	
<ul style="list-style-type: none"> • Oral breath sample of the participant mouth <p>The patient should be upright, and a syringe inserted into the oral cavity until the flange reaches the lip. The patient will be instructed to softly bite to stabilize it, and children might need an assistant help. They will be instructed to breathe through the nose while keeping the lips sealed for 30 seconds, avoid touching the tip of the syringe with the tongue, after that the piston of the syringe is pulled and filled with oral breath, then push the piston and return the gas to the oral cavity. Pull the piston again to fill the syringe completely, at this time remove the syringe. Clean the saliva from the tip with tissue paper, slowly push the plunger to the 1 cc (ml) position and within one minute the gas should be injected at a right angle to the inlet of the Oral Chroma and push down strongly on the plunger in one stroke, then remove the syringe from the inlet</p>	
<ul style="list-style-type: none"> • Clinical Exam <p>Plaque and calculus index, gingival index and tongue coating</p>	
<ul style="list-style-type: none"> • Microbial swab 	
<ul style="list-style-type: none"> • Impression of the retainer 	

A.8 Examination Form

UBC DENTISTRY



Participant Code:

Date:

First visit

Re-evaluation visit

Examination:

Oral exam:

Tooth no.	Debris	Calculus	Gingivitis
16 B			
11 B			
26 B			
36 L			
31 B			
46 L			
Total			
OHI-S			

Tongue Coating:

Area score	
Thickness	
Total	

Participant Code:

Date:

Organoleptic score:

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Oral Chroma:

Oral breath sample	
Retainer sample	

Microbial swab:

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Impression of the retainer surface:

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Notes

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Index:

Debris			Calculus
0	No soft debris or stain	0	No calculus
1	Less than 1/3 of surface covered	1	Less than 1/3 of surface covered
2	1/3 to 2/3 of surface covered	2	1/3 to 2/3 of surface covered
3	More than 2/3 of surface covered	3	More than 2/3 of surface covered

OHI-S = Total debris score divided by the number of teeth scored	
0	Excellent
0.1-0.6	Good
0.7-1.8	Fair
1.9-3.0	Poor

Gingival index		
Code	Description	Definition
0	No inflammation	No inflammation or edema
1	Localized mild inflammation	Slight change in color, little change in texture of any portion of the marginal or papillary gingival unit
2	Generalized mild inflammation	criteria as above but involving the entire marginal or papillary gingival unit
3	Moderate inflammation	glazing. Redness, edema, and/or hypertrophy of the marginal or papillary gingival unit
4	Severe inflammation	Marked redness and edema, or ulceration

Tongue Coating = Area score × thickness score			
Area		Thickness	
0	No tongue coating	0	No tongue coating
1	<1/3 tongue dorsum surface covered	1	Thin tongue coating (papillae visible)
2	1/3–2/3 tongue dorsum surface covered	2	Thick tongue coating (papillae invisible)
3	2/3 tongue dorsum surface covered Thickness		

Organoleptical scoring scale	
0	No detectable odor
1	Hardly detectable odor
2	Light odor
3	Moderate odor
4	Strong odor
5	Extremely strong odor

A.9 Usage Log



Usage log

Participant Code:

Date:

Please highlight the days that you soaked the appliance in the mouthrinse.

Weeks/Days	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Week 1							