AN EVALUATION OF ANTIMICROBIAL SUSCEPTIBILITY TO RETAINER DISINFECTING AGENTS USING A DISK DIFFUSION METHOD

by

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Orthodontic retainers are essential appliances in the orthodontic profession. After completion of treatment, patients receive a retainer to aid in the retention of teeth. The insertion of the retainer into the oral environment can result in the exposure of the appliance to of an array of microorganisms. Regular use can lead to the accumulation of pathogenic organisms and give rise to the formation of bacterial biofilms. The resultant biofilm formation can lead to odor and adverse effects on the oral cavity and body.

A recurrent conundrum faced by orthodontists is recommending a cleansing protocol. Commonly suggested strategies include physical debridement with a toothbrush or the use of antimicrobial disinfecting agents. One of the most difficult issues facing consumers is selecting an antimicrobial disinfecting agent due to the number of products.

This study proposes to evaluate the inhibition of growth of bacteria (*Staphylcoccus aureus, Escherichia coli, Enterococcus faecalis, Streptococcus pyogenes, and Streptococcus mutans*) using various disinfecting agents; Chlorihexidine Gluconate, Retainer Brite, Smile Saver 1, and Smile Saver 2. Saline will be used as a control. The study will use a disk diffusion protocol to evaluate each disinfecting agents ability to inhibit growth of bacterial species. The study will also evaluate if there is a statistical significance between Smile Saver 1 and Smile Saver 2, which are two previously unresearched disinfectants. It is hypothesized that this study will find the antimicrobial agents to be similar to one another in their ability to reduce and eliminate microbial pathogens.

The results showed that Chlorihexidine Gluconate was able to inhibit the growth of all five bacterial species. Retainer Brite was able to inhibit the growth of S. aureus and E. coli. The smile saver variants only inhibited the growth of S. aureus and E. faecalis.

The following conclusions can be made about the disinfecting agents; (1) All four chemical cleaners displayed some level of bacterial inhibition, (2) Chlorihexidine Gluconate displayed the greatest variety of bacterial inhibition, limiting the growth of all tested bacterial species and (3) There is no statistical difference in antimicrobial inhibition between Smile Saver 1 and Smile Saver 2.

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1.0 INTRODUCTION

1.1 RETENTION AND RELAPSE

1.1.1 Theory

Following the completion of orthodontic treatment, patients are often given a retention appliance to hold the dentition in its ideal esthetic and functional position (Graber et al. 2011). Without retention, teeth have the tendency to return back to their original, pre-treatment positions. Factors such as age, severity of malocclusion, tissue health, muscular pressure, and distance of tooth movement can all play a factor into the likelihood of relapse following treatment (Graber et al. 2011).

According to Proffit (2013), there are 3 main reasons that contribute to orthodontic relapse: 1) gingival and periodontal tissue reorganization, 2) teeth in unstable positions due to interaction with soft tissues, and 3) changes due to growth of the patient. It is known periodontal ligament (PDL) fibers can take 3-4 months to reorganize (Proffit 2013). During that time period the teeth are susceptible to soft tissue and occlusal forces. As the PDL fibers reorganize over a period of months, the teeth become more stable and resistant to occlusal and soft tissue forces (Proffit 2013). In addition, collagen fibers within the gingival tissues can take 4-6 months to reorganize while elastic fibers can take as long as a year to reorganize. During that time period an unretained dentition will be subjected to forces from soft tissue fibers in the gingiva that can cause relapse of the teeth into their previous positions (Proffit 2013).

After a patient's gingival fibers and PDL have reorganized, soft tissue forces from the musculature of the face and mouth may continue to act on the dentition. In non-growing

patients, the teeth may have been orthodontically compensated into an unfavorable position, pitting soft structures such as the tongue, lips, and cheeks, against the dentition. Although reorganization of the bone, PDL, and gingival fibers has be completed, the teeth could still be subjected to imbalanced forces from the soft tissue components of the face. Without continuous retention it would be possible for the teeth to move back towards their original position until a balance between soft tissue forces is met. (Proffit 2013).

In many adolescent patients there is a tendency to experience a period of growth during orthodontic treatment. This growth is often used to the advantage of the orthodontist when treating the patient's malocclusion. Although most of the growth of the patient's upper and lower jaws has completed by the time the orthodontic appliances are removed, many patients will experience some growth after the completion of treatment. Behrents (1984) notes that vertical and anterior growth may continue throughout one's life, although at a much slower rate. This potential growth throughout life can once again pit the dentition against forces created by the soft tissues of the face, causing a shift the occlusion that can result in orthodontic relapse (Proffit 2013).

Studies have shown that many orthodontic patients will follow a specific retention protocol of wearing a removable retainer full time for 3-4 months, followed by a phase of night-time wear until 12 months is reached. Retention protocol may vary depending on the malocclusion, although there is not a clear set protocol in terms of time or style of retention that should be used (Andriekute et al. 2017; Melrose & Millett 1998; Proffit 2013).

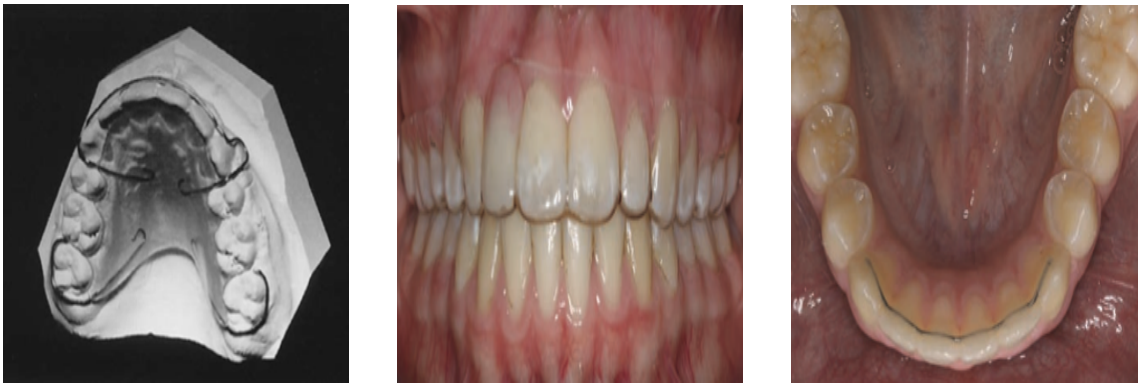
1.1.2 Retention Appliances

There is a wide variation on the types of retention appliances and protocols used around the world. There is no agreement among orthodontist in regards to the style, materials used, or length of retention needed (Andriekute et al. 2017; Littlewood et al. 2016). Andriekute et al. (2017) noted that surveys from various countries showed a trend of using a fixed retention appliance in the mandibular arch. The maxillary arch showed less agreement among orthodontists, with the Hawley retainer, vacuum-formed retainer, and fixed retainer being the most prevalent.

The Hawley retainer (Figure 1a) is a retention appliance made with an acrylic palatal base, a wire labial bow, and wire clasps. It is one of the most frequently used appliances and can come in a variety of designs depending on the type of retention required (Graber et al. 2011). Advantages of a Hawley retainer includes its adjustability, simplicity of fabrication, the ability to close space, allowance of posterior bite settling, and its potential use as an anterior bite plate in deep bite occlusions. Disadvantages of using a Hawley retainer are that they take longer to fabricate, are less esthetic than other retainer designs, and rely on patient compliance to be effective (Proffit 2013).

Vacuum-formed retainers (Figure 1b) are fabricated by heating a plastic material until it is pliable and then sucking it down tightly over a dental cast. They are among the most popular retention appliances used worldwide (Littlewood et al. 2016). The popularity of the vacuum-formed retainers stems from its esthetic appeal and ease of fabrication. Though popular, Vacuum-formed retainers do have a few disadvantages. They are difficult to adjust, can wear down, crack, and discolor over time, necessitating the need for replacement, and rely on patient compliance to be effective (Proffit 2013). Vacuum-formed retainers also do not allow for posterior settling of the occlusion.

Orthodontic fixed retainers (Figure 1c) consist of a thin or braided wire that is adapted and bonded to the lingual surface of the dentition in order to maintain its position. They can be used in the maxillary or mandibular arch, but are most frequently used in the mandibular arch. According to Proffit (2013) there are four main indications for using a fixed retainer: 1) maintenance of lower incisors position, 2) diastema maintenance, 3) maintenance of a pontic, and 4) keeping extraction spaces closed in adults. Fixed retainers are often used in combination with removable appliances and do not rely upon patient compliance (Andriekute et al. 2017). The disadvantages to fixed retainers include difficulty in placement, bond failure, and difficulty in performing oral hygiene procedures (Proffit 2013).



(a) Hawley (b) Vacuum-Formed (c) Fixed Retainer

Figure 1: Types of Retention Appliances (Graber et al. 2011)

1.2 MICROORGANISMS

1.2.1 Oral Pathogens

Orthodontic appliances, in addition to genetic, dietary, and environmental factors, have been shown to change the ecology of the microflora inhabiting the oral cavity (Pathak & Sharma 2013). The appliances can serve as a substrate for which bacteria and other organisms can attach to with the use of organic molecules (Kolenbrander & London 1993). Children who have orthodontic appliances have shown an increase in the presence of *S. mutans* and other oral microbes in comparison those without orthodontic appliances (Rosenbloom 1991; Batoni et al. 2001; Pathak & Sharma 2013). A study by Hagg (2004) found an increase in the variety of coliforms found in plaque after the insertion of fixed orthodontic appliances. Even after the removal of fixed appliances, the use of removable or fixed orthodontic retainers appears to have an effect on the overall number and types of microbes present in the oral cavity. A study by Türköz et al. (2012) indicated that thermoplastic orthodontic retainers could create an oral environment that is conducive to growth and proliferation of *S. mutans* and *Lactobacillus* species. This growth is enabled by preventing the normal flushing effect of saliva on teeth and the surrounding soft tissues. Although they are made from different materials, there was

no difference between Hawley and vacuum-formed retainers in the number and proportion of organisms isolated from them (Groosh et al. 2015). An increase in these organisms can lead to a higher caries incidince rate, halitosis, and gingivitis (Loesche 1986; Pathak & Sharma 2013).

1.2.2 Opportunistic Pathogens

Wearing orthodontic appliances may not only change the prevalence of commonly found oral bacteria, but may increase the prevalence of opportunistic bacteria, especially in those who are immunocompromised (Kitada et al. 2009). Studies have shown that orthodontic retainers can harbor bacteria and microorganisms that do not regularly inhabit the oral cavity. A study by Nisayif (2009) found the presence of textitEnterobacter, *E. coli*, *Streptococcal*, and *Staphylcoccal* species on removable orthodontic appliances. Groosh et al. (2011) found an increased proportion in *Streptococcal*, *Staphylcoccal*, and *Candida* species in those wearing orthodontic retainers in comparison to non-retainer wearers. In addition, Groosh et al. (2011) isolated methicillin - resistant *Staphylcoccus aureus* from an orthodontic retention appliance. A study by Brook & Gober (1998) found that Group A β-Hemolytic Streptococci were isolated from the orthodontic retainers of children who were previously treated for pharyngotonsillitis. The presence of these pathogens in orthodontic retainers after treatment suggest a potential source for reinfection, especially in the immunocompromised. These studies demonstrate and highlight the need for cleaning and disinfection on a regular basis.

1.3 CLEANING METHODS

As noted in the previous section 1.2, there are multitude of pathogenic organisms, oral and non-oral alike, that can colonize and inhabit removable orthodontic retention appliances. The importance of cleaning these appliances becomes all the more apparent knowing that they can serve as a source of reinnoculation and infection to those who are immunocompromised. Various methods such as mechanical removal with a toothbrush, chemical disinfectants, and

vibration have been proposed as ways to help disinfect and clean orthodontic retention appliances (Shpack et al. 2014). A study by Farhadian et al. (2016) suggested fabricating retention appliances from materials that inhibit microbial growth as a solution.

Although there are many ways to disinfect retention appliances, a survey conducted by Eichenauer et al. (2011) showed there was no consistent recommendation as to how to disinfect the appliance. One of the more commonly suggested protocols is mechanically cleaning with retention appliance with a toothbrush in conjunction with a chemical disinfectant (Levrini et al. 2016). Numerous chemical disinfectants such as Chlorihexidine Gluconate, persulfate tablets, sodium hypochlorite, and even vinegar have been proposed as potential disinfecting agents. (Eichenauer et al. 2011).

1.4 ANTIMICROBIAL SUSCEPTIBILITY

1.4.1 Disk Diffusion Method

There are various methods to determine the susceptibility of microorganisms to certain drugs or chemicals. The disk diffusion method is one of the simplest methods used. (Balouiri et al. 2016). Developed in 1940, the disk diffusion method is now one of the oldest and most commonly used procedures to determine antimicrobial resistance to antibiotics and other agents (Matuschek et al. 2014).

The procedure is accomplished by inoculating a sterile agar plate with a known microorganism. Disks containing antibiotics or an antimicrobial agent in a known concentration are added to the surface of the agar. The agent is able to diffuse through the agar layer. The plate is then incubated for a period of time. During the incubation phase, the microorganism is able to proliferate until it reaches the agar that has been impregnated with the antimicrobial agent, inhibiting its growth. The area of growth inhibition around the disks can then be measured and compared to known standards (Balouiri et al. 2016). Figure 2

According to Balouiri et al. (2016), the disk diffusion method offers the advantages of "simplicity, low cost, the ability to test enormous numbers of microorganisms and antimi-

crobial agents, and the ease to interpret results provided." Despite these advantages, the disk diffusion method cannot differentiate if an antimicrobial agent is bactericidal or bacteriostatic, only if the agent has the ability to inhibit growth (Balouiri et al. 2016).

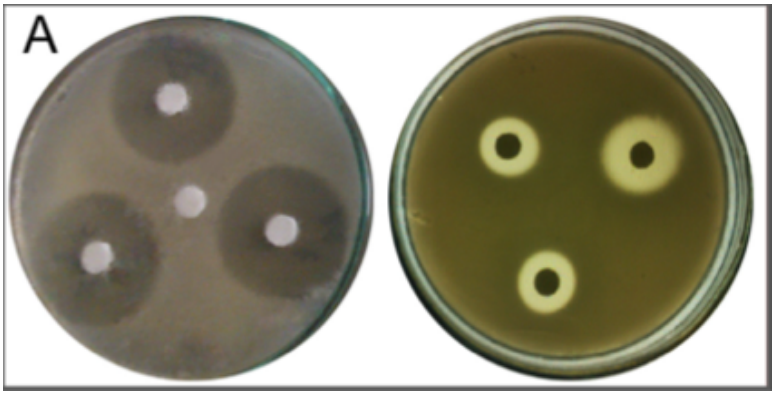


Figure 2: Disk diffusion method (Balouiri et al. 2016)

2.0 PURPOSE

The purpose of this study is to; (1) evaluate the antimicrobial susceptibility of various bacterial species to an assortment of chemical disinfectants using a disk diffusion method , (2) determine which chemical disinfectant displays the greatest inhibition, and (3) assess if there is a statistical difference between previously untested chemical formulas of Smile Saver 1 and Smile Saver 2. It is hypothesized that all chemical cleaners (Chlorihexidine Gluconate, Retainer Brite, Smile Saver 1, and Smile Saver 2) will inhibit growth of the bacterial species in comparison to the control (saline), but will display no statistical difference from one another.

3.0 METHODS AND MATERIALS

3.1 DISK DIFFUSION METHOD

The methodology performed in this study follows the European Committee On Antimicrobial Susceptibility Testing (EUCAST) disk diffusion method for antimicrobial susceptibility testing (Matuschek et al. 2014). The method was repeated three times, measuring each zone of inhibition to the greatest millimeter with a caliper. The zones of inhibition were then statistically analyzed.

3.1.1 Inoculum Creation

Pure bacterial cultures of *S. aureus*, *E. coli*, *E. faecalis*, *S. pyogenes*, and *S. mutans* were obtained as freeze dried pellets from the American Type Culture Collection (ATCC). The pellets were re-hydrated using a Brain-Heart Infusion (BHI) broth and individually plated on Muller-Hinton (MH) agar plates, a non-selective medium, using a quadrant streak technique. The quadrant streak technique was used to enable individual bacterial colonies to be isolated. The plates were incubated overnight at 35° Celsius. The following day, an inoculum suspension was created for each species. A suspension was created using a sterile inoculation loop to select independent, morphologically similar colonies from an overnight plate and added to a saline solution. The solution was vigorously mixed to reach an even turbidity. A spectrophotometer was used to ensure each solution was of equal turbidity. Every sample was compared to 0.5 McFarland standard solution to confirm the density of the suspension. Bacterial colonies or saline could added to the inoculum suspension in order to reach the proper turbidity. A 0.5 McFarland standard has a spectrophotometer absorbence reading of

0.08-0.1 at 625nm (Jorgensen et al. 2015).

3.1.2 Plate Inoculation and Disk Application

After the inoculum suspension had been calibrated to the correct turbidity, a sterile cotton swab was dipped into the suspension and spread on a growth medium. *S. aureus*, *E. Coli*, and *E. faecalis* were plated on Muller-Hinton agar plates without defibrinated horse blood (MH). *S. pyogenes* and *S. mutans* were inoculated on Muller-Hinton agar plates with defibrinated horse blood (MH-F). Each inoculum solution was swabbed evenly over an entire plate, within 15 minutes of creating the inoculum solution. Following inoculation, five sterile paper disks, individually soaked in a disinfecting solution (Chlorihexidine Gluconate, Retainer Brite, Smile Saver 1, Smile Saver 2, and Saline), were evenly applied across the surface of the inoculated growth plate. Saline was selected as a control agent. The cleaning solution soaked paper disks were added to the plate within 15 minutes of inoculation and the plate was then inverted (Matuschek et al. 2014).

3.1.3 Incubation

The inverted agar plates were placed in an incubator at 35° Celsius and allowed to grow for approximately 16-24 hours. The *S. pyogenes* and *S. mutans* samples were incubated in air with 4-6% carbon dioxide. An independent incubator with the ability to reach 4-6% carbon dioxide was unavailable for use at the chosen laboratory. A Candle-Jar method was used to increase the atmospheric carbon dioxide level in the incubator. All inoculated plates were placed in the incubator within 15 minutes of applying the chemical soaked paper disks (Matuschek et al. 2014).

3.1.4 Plate Reading

The growth plates were evaluated after 16-24 hours of incubation. The zones of inhibition on MH plates (*S. aureus*, *E. coli*, and *E. faecalis*) were read from the back of the agar dish, against a dark background using reflected light. The MH-F plates (*S. pyogenes* and

S. mutans) were read from the front with the lid removed using reflected light. A pair of calipers were used to make the measurements while holding the plates approximately 30 cm from the eye (Matuschek et al. 2014).

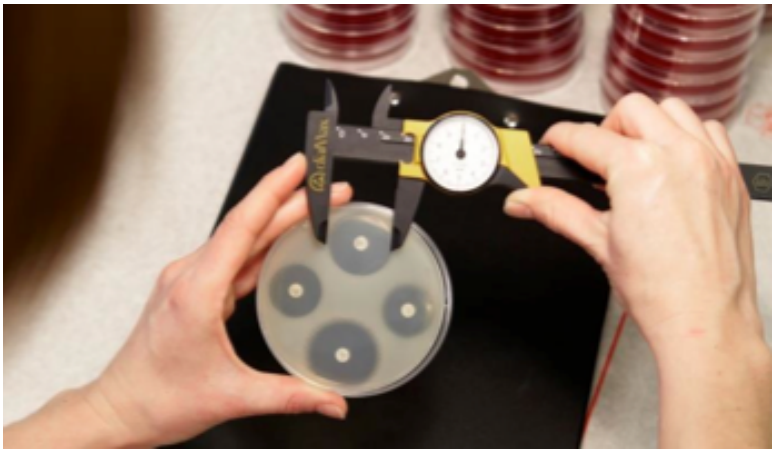


Figure 3: Plate Reading (Matuschek 2017)

4.0 STATISTICAL ANALYSIS

The Disk Diffusion Method was repeated three times for each species of bacteria. All data was entered into an excel spreadsheet and uploaded into STATA (StataCorp, College Station, TX). The zones of inhibition for each bacteria were averaged together. A one-way analysis of variance (ANOVA) was used to assess any statistical differences between the chemical cleaners' ability to inhibit bacterial growth. A p-value of less than 0.05 was considered significant

5.0 RESULTS

5.1 *S. AUREUS* SUSCEPTIBILITY

All of the retainer disinfectants significantly inhibited the growth of *S. aureus* compared to saline. Retainer Brite had the largest zone of inhibition at 23.33mm followed by Chlorihexidine (18.67mm), Smile Saver 2 (10mm) and Smile Saver 1 (8mm). Retainer Brite had a significantly larger zone of inhibition compared to Chlorihexidine, although both cleaners performed well overall. Retainer Brite performed significantly better than Smile Saver 1 and Smile Saver 2. Both Smile Saver 1 and Smile Saver 2 were significantly more effective than saline, although there was no significant difference between the two. (Figure 4)

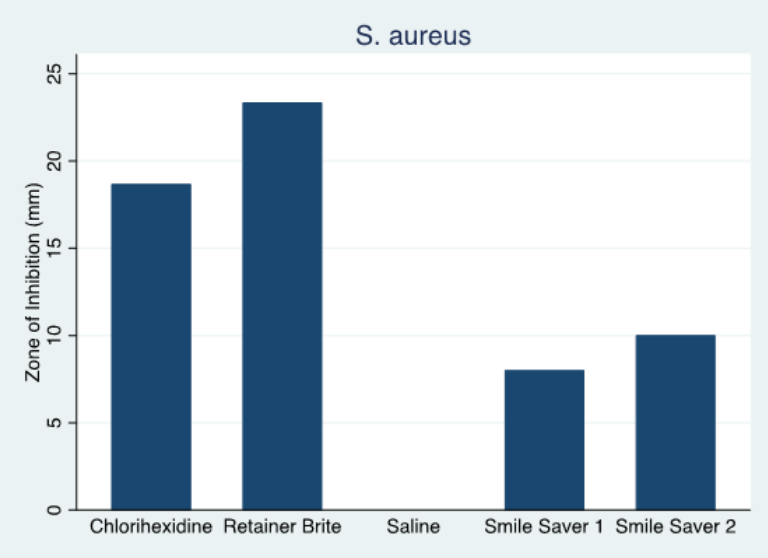


Figure 4: *S. Aureus* Zones of Inhibition

Table 1: *S. Aureus* Zone of Inhibition Comparison

Comparison	**Difference (mm)**	**p-value**	**Significant**
Saline v. Retainer Brite	23.33	0.000	YES
Saline v. Chlorihexidine	18.67	0.000	YES
Saline v. Smile Saver 1	08.00	0.000	YES
Saline v. Smile Saver 2	10.00	0.000	YES
Chlorihexidine v. Retainer Brite	04.67	0.010	YES
Chlorihexidine v. Smile Saver 1	10.67	0.000	YES
Chlorihexidine v. Smile Saver 2	08.67	0.000	YES
Retainer Brite v Smile Saver 1	15.33	0.000	YES
Retainer Brite v. Smile Saver 2	13.33	0.000	YES
Smile Saver 1 v. Smile Saver 2	02.00	0.761	NO

5.2 *E. COLI* SUSCEPTIBILITY

E.coli showed susceptibility to both Chlorihexidine and Retainer Brite. Although Chlorihexidine had a slightly larger zone of inhibition (17mm compared to 14.67 mm), no statistical significance was found. Both Smile Saver 1 and Smile Saver 2 showed no effect on the growth of *E. coli*. There was no statistical significance found Smile Saver 1 Smile Saver 2, and saline.

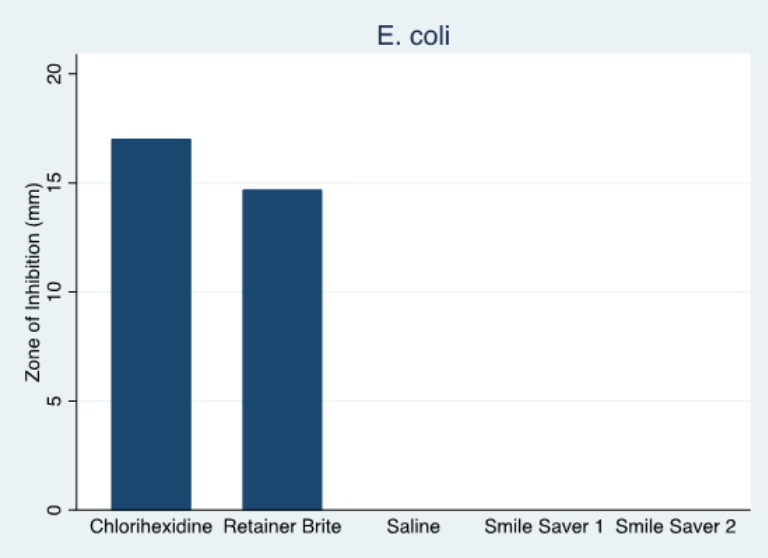


Figure 5: *E. Coli* Zones of Inhibition

Table 2: *E. Coli* Zone of Inhibition Comparison

Comparison	**Difference (mm)**	**p-value**	**Significant**
Saline v. Retainer Brite	14.67	0.000	YES
Saline v. Chlorihexidine	17.00	0.000	YES
Saline v. Smile Saver 1	00.00	1.000	NO
Saline v. Smile Saver 2	00.00	1.000	NO
Chlorihexidine v. Retainer Brite	02.33	0.294	NO
Chlorihexidine v. Smile Saver 1	17.00	0.000	YES
Chlorihexidine v. Smile Saver 2	17.00	0.000	YES
Retainer Brite v Smile Saver 1	14.67	0.000	YES
Retainer Brite v. Smile Saver 2	14.67	0.000	YES
Smile Saver 1 v. Smile Saver 2	00.00	1.000	NO

5.3 *E. FAECALIS* SUSCEPTIBILITY

E. faecalis demonstrated susceptibility to Chlorihexidine, Smile Saver 1, and Smile Saver 2. Chlorihexidine showed the greatest zone of inhibition (13.67mm) followed by Smile Saver 2 (9mm) and Smile Saver 1 (2.67mm). Although Smile Saver 1 was found to be able to inhibit bacterial growth, it was found to be statistically insignificant in comparison to saline. Both Retainer Brite and saline were unable to inhibit growth of *E. faecalis*.

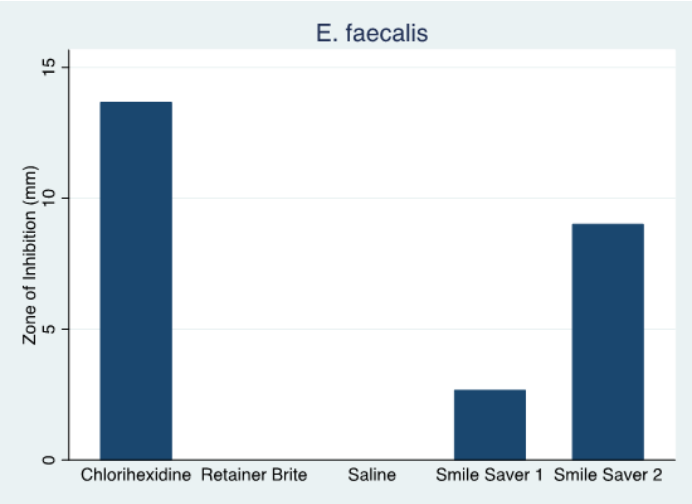


Figure 6: *E. Faecalis* Zones of Inhibition

Table 3: *E. Faecalis* Zone of Inhibition Comparison

Comparison	**Difference (mm)**	**p-value**	**Significant**
Saline v. Retainer Brite	00.00	1.000	NO
Saline v. Chlorihexidine	13.67	0.000	YES
Saline v. Smile Saver 1	02.67	1.000	NO
Saline v. Smile Saver 2	09.00	0.009	YES
Chlorihexidine v. Retainer Brite	13.67	0.000	YES
Chlorihexidine v. Smile Saver 1	11.00	0.002	YES
Chlorihexidine v. Smile Saver 2	4.67	0.355	NO
Retainer Brite v Smile Saver 1	02.67	1.000	NO
Retainer Brite v. Smile Saver 2	09.00	0.009	YES
Smile Saver 1 v. Smile Saver 2	06.33	0.080	NO

5.4 *S. PYOGENES* SUSCEPTIBILITY

Chlorihexidine was the only chemical disinfectant to demonstrate the ability to inhibit growth of *S. pyogenes*. Retainer Brite, Smile Saver 1, and Smile Saver 2 did not exhibit any ability to inhibit growth of *S. pyogenes* and were statistically no different than saline.

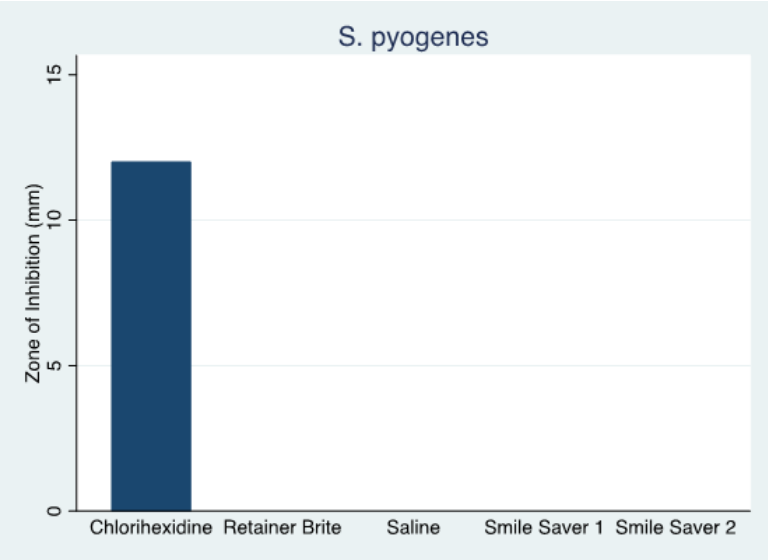


Figure 7: *S. Pyogenes* Zones of Inhibition

Table 4: *S. Pyogenes* Zone of Inhibition Comparison

Comparison	**Difference (mm)**	**p-value**	**Significant**
Saline v. Retainer Brite	00.00	1.000	NO
Saline v. Chlorihexidine	12.00	0.000	YES
Saline v. Smile Saver 1	00.00	1.000	NO
Saline v. Smile Saver 2	00.00	1.000	NO
Chlorihexidine v. Retainer Brite	12.00	0.000	YES
Chlorihexidine v. Smile Saver 1	12.00	0.000	YES
Chlorihexidine v. Smile Saver 2	12.00	0.000	YES
Retainer Brite v Smile Saver 1	00.00	1.000	NO
Retainer Brite v. Smile Saver 2	00.00	1.000	NO
Smile Saver 1 v. Smile Saver 2	00.00	1.000	NO

5.5 *S. MUTANS* SUSCEPTIBILITY

Similar to *S. pyogenes*, Chlorihexidine was the only chemical cleaner to demonstrate to ability inhibit growth of *S. mutans*. Retainer Brite, Smile Saver 1, and Smile Saver 2 did not exhibit any ability to inhibit the growth of *S. mutans* and were statistically no different than saline.

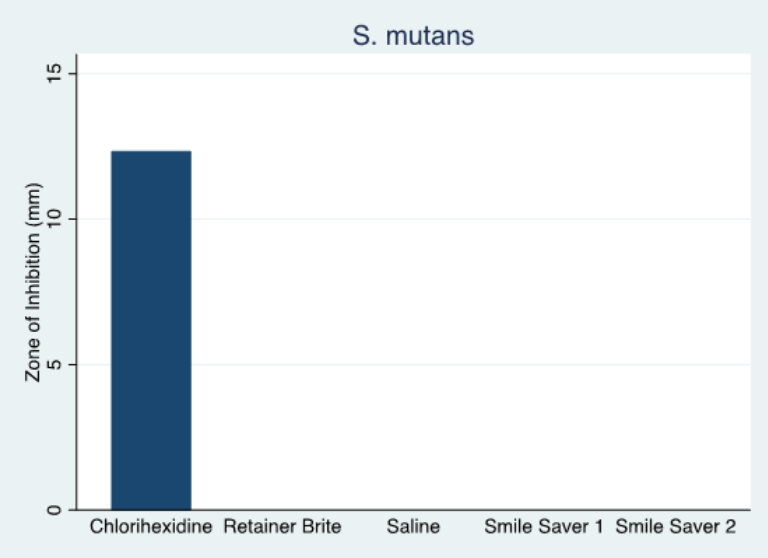


Figure 8: *S. Mutans* Zones of Inhibition

Table 5: *S. Mutans* Zone of Inhibition Comparison

Comparison	**Difference (mm)**	**p-value**	**Significant**
Saline v. Retainer Brite	00.00	1.000	NO
Saline v. Chlorihexidine	12.33	0.000	YES
Saline v. Smile Saver 1	00.00	1.000	NO
Saline v. Smile Saver 2	00.00	1.000	NO
Chlorihexidine v. Retainer Brite	12.33	0.000	YES
Chlorihexidine v. Smile Saver 1	12.33	0.000	YES
Chlorihexidine v. Smile Saver 2	12.33	0.000	YES
Retainer Brite v Smile Saver 1	00.00	1.000	NO
Retainer Brite v. Smile Saver 2	00.00	1.000	NO
Smile Saver 1 v. Smile Saver 2	00.00	1.000	NO

6.0 DISCUSSION

This study sought to evaluate the effectiveness of multiple chemical disinfecting agents' ability to inhibit growth of a variety of bacterial species. Multiple studies that have been previously conducted to answer similar questions. A study by Lessa et al. (2007) found that retainers disinfected with Periogard (Chlorihexidine Gluconate) had a significantly reduced presence of *Streptococcus mutans* in comparison to water. The present study's findings were in agreement with that conclusion, as well as studies conducted by Peixoto et al. (2011), Silva et al. (2008), and Shpack et al. (2014). Others have found that Chlorihexidine is not only effective against S. mutans, but other pathogenic species such as *E. coli*, *S. sanguinis*, *C. albicans*, and methicillin-resistant *S. aureus* (Chang et al. 2014; Emilson 1977). Those findings were supported by the finding of this study, showing effectiveness against *S. aureus*, *E. faecalis*, *E. coli*, *S. mutans*, and *S. pyogenes*.

In the realm of dentistry, Chlorihexidine is a very well known chemical disinfectant that can be used to decrease the overall microbial presence in the oral cavity. The use Chlorihexidine is not limited to the oral cavity though. Chlorihexidine has been suggested as a tool to reduce the possibility of complications after total joint arthroplasty (George et al. 2017). It has also been suggested for use in preventing surgical site infection during spinal surgeries (Anderson et al. 2017), prevention of Ventilator Associated Pneumonia (VAP) (Zuckerman 2016), disinfection of human musculoskeletal allografts (Mohr 2016),and reduction of infection during Caesarean section (Hadiati et al. 2014).

A previous study conducted by Albanna et al. (2017) evaluated the ability of Retainer Brite to inhibit the growth of *S. aureus*, *S. epidermis*, and *S. mutans* (among others) using a disk diffusion method. The study found Retainer Brite does not inhibit the growth of S. mutans, which would be in agreement with the current study's findings. However, Albanna

et al. found that Retainer Brite does not inhibit the growth of S. aureus, which is disputed with the current findings of this study. Overall it appears that Retainer Brite is effective against *S. aureus* and *E. coli*, but is ineffective against *E. faecalis*, *S. pyogenes*, and *S. mutans*.

Currently, there is no literature on the retainer disinfectant, Smile Saver. Two chemical variant formulas were tested in the present study. The findings showed that Smile Saver 1 and Smile Saver 2 were both effective in inhibiting the growth of *S. aureus* and *E. faecalis*, but not nearly as effective as Chlorihexidine Gluconate. Neither chemical variant was successful in inhibiting the growth of *E. coli*, *S. mutans*, or *S. pyogenes*.

Silva et al. (2008) examined six disinfectants on removing five microbial species, including *S. mutans*, *S. aureus*, and *E.coli*. They found that 1% sodium hypochlorite was the most effective disinfectant, significantly reducing the number of tested microorganisms. Sodium hypochlorite is successful as a disinfectant, but can be caustic and corrosive to orthodontic retainers with metal clasps. It would appear that Chlorihexidine Gluconate is the disinfectant of choice to inhibit growth of bacteria on orthodontic retainers. Retainer Brite appears to be next disinfectant of choice, followed by the Smile Saver variants.

There are many aspects of this study that could be improved. First, the disk diffusion method is a test that evaluates growth inhibition. It does not evaluate whether the chemical disinfectant prevents microbial growth or whether the microbes are killed by the disinfectant. Secondly, the study tested susceptibility of individual species as opposed to biofilms. Biofilms in the oral cavity are typically made up of multiple species and organic compounds. The microbiota makeup can vary based on the environment present in the oral cavity. In addition, the way a chemical effects bacteria can differ based on the components of the biofilm. Lastly, the current study was based on an in-vitro model as opposed to a clinical in-vivo model. The oral cavity is an ever-changing environment with many biological variants such as saliva, pH changes, immune factors, biofilms, and food particulate. A disk diffusion model is unable to replicate those variables.

7.0 CONCLUSIONS

Within the parameters of this study, the following conclusions can be made about the chemical disinfecting agents:

1. All four chemical disinfectants displayed some level of bacterial inhibition.
2. Chlorihexidine Gluconate displayed the greatest variety of bacterial inhibition, limiting growth of all tested bacterial species.
3. There is no statistical difference in antimicrobial inhibition between Smile Saver 1 and Smile Saver 2.

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