An investigation of the levels of antimicrobial efficacy in commercial
dentifrices on *Streptococcus mutans* and *Lactobacillus*

Chris W. Leyster, Saint Martin’s University, 5300 Pacific Avenue SE, Lacey, WA 98503. This work was supported by Saint Martin’s University.

**Abstract**

This study was undertaken to determine the various efficacies of the antimicrobial properties in common commercial dentifrices on the prominent oral disease causing bacteria *Streptococcus mutans* and *Lactobacillus*. Oral bacteria are the primary cause of oral decay and disease. Toothpaste is the most accessible means of preventative oral healthcare, and is therefore a primary defense against oral decay. Four commercial dentifrices (Crest, Colgate, Aquafresh and Arm n’ Hammer) containing the active ingredient fluoride, and one outlier toothpaste (Tom’s of Maine), which does not contain fluoride were tested to examine the relative effects of non-fluoride and fluoride containing toothpastes on oral bacteria. Simple diffusion experiments were performed to examine the average zones of inhibition for each toothpaste/bacteria sample. The samples contained either a *Lactobacillus* or *S. mutans* spread on nutrient agar in a Petri dish. A 3 mm diameter filter disc containing 15 µL of a 1g toothpaste per 2ml distilled water concentrated slurry was applied to the center of each plate. These samples were incubated at 37º C for 24 and 72 hours and the subsequent zones of inhibition were then measured. In each of the four separate tests performed Arm n’ Hammer obtained the greatest mean zone of inhibition (21.63 mm, 29.4 mm, 25.75 mm, 22.9 mm) suggesting that it provides the greatest antimicrobial effectiveness when exposed to *S. mutans* and *Lactobacillus*. Additionally, each toothpaste exhibited different mean zones of inhibition (P < 0.05) illustrating that the tested toothpastes vary in their antimicrobial effectiveness. The non-fluoride containing toothpaste, Tom’s of Maine, obtained average zones of inhibition which were higher than all toothpastes other than Arm n’ Hammer in each of the four tests. These results would suggest that fluoride as an active ingredient in oral dentifrices is not necessary to achieve antimicrobial effectiveness upon *S. mutans* and *Lactobacillus* in an in vitro study.

**Introduction**

Oral health has a strong correlation with the overall health of an individual, specifically in humans. The oral cavity (mouth) is the primary orifice of entry for dietary and respiratory elements. The principal anatomical structures of the human mouth include the tongue, salivary glands, a hard and soft palate, and multiple teeth. The lining of the interior is composed of a mucous membrane and extends in a posterior fashion to the laryngo-pharynx, ultimately forming the pharynx and subsequent biconcavity of the esophagus and trachea. The mouth is continually bombarded by the various rudiments of nature, and when coupled with a perpetual warm, moist climate serves as a hot bed for the residence and development of numerous microbial populations (Wistreich and Lechtman, 1988). To combat these invading and potentially harmful microbes, ingredients possessing antimicrobial activity are included in several, if not all, oral hygiene products.

A majority of the microbes present in the oral cavity reside on the posterior
portion of the tongue, as a result of the tongue’s large surface area (Vazquez and Pilch, 2003). As explained by Wistreich and Lechtman (1988), the first exposure to the numerous antimicrobial substances encountered during life occurs as a fetus is birthed. While descending through the birth canal the fetus is exposed to many microbial substances that are already present within the mother. Some of the colonizing microbes resulting via contact with the reproductive system include: Lactobacilli, Micrococci, Streptococci, and Coliforms; while Staphylococci and Pneumococci are acquired via the air. This is the beginning of the symbiotic relationship between microbes and a newborn’s oral cavity.

One type of symbiosis, mutualism, is the relationship oral flora have with their human host. Lee (2004) cites the relationship between oral microbes and the host as normal, and even indicates the importance of a balance among the different bacteria. Changes in the balance associated with a healthy oral cavity can be a precursor to an infection or disease. Lee (2004) discusses findings in previous research concerning the shifts in periodontopathic, cariogenic, or opportunistic flora and the subsequent prosperity of resistant strains that ultimately result from exposure to certain antibacterial ingredients found in normal toothpastes. It is suggested that infections and diseases of the oral cavity are significant in the health of an individual, and could quite possibly be exacerbated by some of the ingredients found in toothpaste.

A bio-film known as dental plaque, which provides ground for the formation and inhabitance of pathogenic bacteria, is formidable in its contribution to various factors that lead to tooth decay, gingivitis, and periodontitis (Steinberg et al., 2003). Steinberg et al. (2003) discusses the principles of adhesion that occur to form the bio-film, which is located on the outermost layer of the enamel of the tooth. The construction of dental plaque occurs in three stages: initial adhesion, proliferation, and steady state. They explain the interactions among the bacteria located in the oral cavity and the enamel of the tooth surface as being dependent upon several factors. These interactions can be, and ultimately are, influenced by the physical and chemical properties of the microbes interacting with the specific anatomy and physiology of the human mouth. It is noted that hydrophobic properties, contact angles, surface free energy, exposed chemical functional groups, and degree of roughness on the enamel are all critical in the formation of dental plaque.

The cooperative effects of brushing and using toothpaste containing certain chemical agents aid in the removal of this hazardous biofilm, as well as reducing the repopulation of bacteria on the enamel (Steinberg et al., 2003). The practice of tooth brushing twice daily (in addition to flossing) is the standard routine suggested by the American Dental Association (ADA) to effectively preserve good oral health. Approximately 80% of the European and North American population follow the professional advice (Moran and Addy, 1988). Tooth brushing is highly correlated with levels of bacteria, in that it maintains the desired balance of bacterial repopulation on the surface of the tooth which ensures a healthy balance of oral flora (Steinberg et al., 2003). The action of abrasion, coupled with the main ingredients found in different brands of toothpaste, provides an effective means of microbe population control.

The inclusion of certain chemical agents in toothpaste to combat oral pathologies has been widely accepted and is the standard practice in the professional care of teeth (Kornman, 1986; van der Ouderea, 1991; Addy and Renton-Harper, 1996; as cited by Moran and Newcombe, 2005). Some of the earliest and most studied
chemical groups found in toothpastes include the metal salts such as zinc and tin (Hanke, 1940; as cited by Moran and Newcombe, 2005). Zinc and tin salts earned a popular reputation among health care professionals. The compounds primarily benefited from receiving the majority of public exposure in regards to their positive antimicrobial effects and being safe for consumer use (Scheie, 1989; as cited by Moran and Newcombe, 2005). In commercial toothpastes available today, there are a few different main ingredients which have varying effects on the oral flora population. These ingredients include triclosan, sodium lauryl sulfate, chlorhexidine (Gaffar et al., 1990), sodium fluoride, sodium monofluorophosphate, zinc citrate trihydrate, and hexetidine (Moran and Addy, 1988).

It is common for toothpastes to contain different main ingredients, and this leads to an inquiry concerning the efficacy of these various ingredients and the toothpaste product as a whole. Lee’s study (2004) concerning the antimicrobial effects of varying brands of natural toothpastes indicates that more testing is required to empirically compare the efficacy of the dentifrices studied. Although the efficacies of the fourteen tested toothpastes in the study require further testing, it is frequently accepted that the exclusion of fluoride from toothpaste dramatically inhibits its caries-preventive benefits by reducing antimicrobial properties.

It is difficult to reach a definitive conclusion on the antimicrobial properties of the different toothpastes, because studies differ in their specific aims. For example, in Lee (2004), there were four different strains of oral bacteria being tested, including Streptococcus mutans, Streptococcus sanguis, Actinomyces viscosus, and Candida albicans. The outcomes of the study were minimized as a result of testing only natural herbal dentifrices and not common commercial brands. In addition, Lee and his coauthors are careful not to overlook the negative side effects of these dentifrices, and even suggest that the use of some of the ingredients found in the natural herbal toothpastes could be hazardous if used incorrectly. The majority of the population does not use herbal toothpastes, and these are not endorsed by health care professionals or by the Food and Drug Administration (FDA).

In the study conducted by Moran and Newcombe (2005), the effects of the antimicrobial properties among different toothpastes were measured by levels of plaque inhibition. They compared plaque re-growth between two zinc citrate/triclosan formulations, one which contained bromochlorophene, and evaluated whether the addition of the phenol group had any effect. Furthermore, they also tested whether or not these two pastes had any significant differences in plaque accumulation versus a proprietary fluoride paste. They failed to find any significant difference in plaque formation among the three pastes. Although there was no data indicating which types of microbes were being tested, their analyses were based on levels of plaque formation, which are dependent upon the balance of oral bacteria in the biofilm (Steinberg et al., 2003).

An additional study by Moran and Addy (1988) studied bacteria such as Streptococcus mitior, Actinomyces odontolyctius, Bacteroides intermedius, Staphylococcus aureus, and Peptostreptococcus asaccharolyticus to determine antimicrobial activity amongst various toothpastes available in the United Kingdom. By determining minimum inhibitory concentrations (MIC) using an agar dilution method, the researchers were able to evaluate the antimicrobial activity of the various brands. The results, as indicated
by low MIC’s (zones of inhibition),
determined that there was significant
antimicrobial activity amongst the majority
of the tested UK toothpastes.

The study carried out by Vazquez
and Pilch (2003) consisted of testing for the
control of mouth-odor-causing bacteria,
such as *Prevotella intermedia* and *P.
melaninogenica*. Test subjects provided a
baseline salivary sample to be used as
reference and after seven days of using the
tested product, *Colgate Total Advanced Fresh*,
the researchers collected a final
salivary sample from the subjects. These
final samples were then diluted in a sterile
phosphate-buffered saline and duplicate-
plated onto a lead acetate agar. After 96
hours the numbers of hydrogen-sulfide
producing colonies were counted, and the
results confirmed the positive antimicrobial
activity of the dentifrice.

In this study I focused on testing
the antimicrobial effectiveness of several
toothpastes currently being sold in Lacey,
WA. I chose five different commercial
toothpastes (Crest, Colgate, Aquafresh, and
Arm n’ Hammer, and Tom’s of Maine), four
of which contain the active ingredient
fluoride. To test the claims made
concerning the importance of the inclusion
of fluoride as an effective antimicrobial
dentifrice, I included one outlier toothpaste,
Tom’s of Maine. I propose that there will be
a variance in antimicrobial effectiveness
among the four toothpastes, although they
all contain fluoride as the active ingredient.
I also suggest that the exclusion of fluoride
from a dentifrice will not drastically reduce
its antimicrobial activity. Two bacteria
strains commonly found in the bio-film,*
*Streptococcus mutans* and *Lactobacillus*,
were used in this study to test each
dentifrices’ antimicrobial activity.

I obtained results concerning the
effective antimicrobial activity of these
brands by comparing the antimicrobial
properties of the different brands of
toothpastes using subsequent zones of
inhibition and statistical analysis. I then
analyzed the data to determine the relative
antimicrobial effectiveness of the tested
dentifrices. As mentioned before, the act of
tooth brushing is the most common and
accessible means of preventive oral health
care available to the greater population. As
such, it is the most practical and efficient
means for providing microbe inhibition in
the general population.

**Methods**

The antimicrobial properties of the
individual toothpastes were measured by
testing the zones of inhibition on the
bacteria strains *S. mutans* and *Lactobacillus*,
which are known to cause decay within the
oral cavity by demineralizing and
weakening the enamel of the tooth
(Steinberg *et al.*, 2003). In regards to the
testing of zones of inhibition, many methods
have been devised and employed with
concerns for cost and time. One of the most
popular methods used today, as explained by
Cormican *et al.*(1991), is to measure zones
of inhibition by a disc diffusion
susceptibility method (also known as the
Kirby-Bauer method). This method is quick,
easy, and inexpensive, making it ideal for
research done on a low budget with time
constraints. It involves applying a thin
d paper disc containing the antibacterial agent
on a culture of bacteria grown on the agar
media. The simple diffusion of the agent
through the paper and onto the agar plate
containing the bacteria provides an effective
means to evaluate the differences among the
toothpaste’s antibacterial properties. This is
the method I used to test and measure the
zones of inhibition. I added 30 µL of either
*S. mutans* or *Lactobacillus* grown in nutrient
broth with a bent glass rod in a spreading
fashion. The liquid solution of the
individual bacteria was spread uniformly over the agar. After the bacteria were applied, the sterile disc was placed on the plate. After placement of the 3 mm diameter disc on the center of each plate, I applied 15 µL of the antimicrobial slurry, consisting of toothpaste and water in a concentration of 1g toothpaste/ 2 ml water, to the sterile disc. The agent was then allowed to diffuse through the disc and onto the plate, ultimately resulting in inhibition of growth of the bacteria. The resulting diameter in which the bacteria were inhibited was indicative of the toothpaste’s antibacterial potential on the specific bacteria.

The zones of inhibition on the growth of the test strains were defined by the location where visible growth had been inhibited. The measurements of the diameter were made in whole mm, and were measured with a ruler.

The tests for zones of inhibition involved 8 replicates for each toothpaste, and time frame, on each bacteria culture. The zones of inhibition were then measured after either 24 or 72 hours for each test sample (Moran and Addy, 1988). This variance in incubation time allowed for observation involving time as a factor in the resulting zones of inhibition. The averages for each toothpaste solution on each bacteria strain were calculated, and these are the resulting representative zones of inhibition.

Preparation of test culture and media

A standard nutrient agar gel solution was used as the environment for the growth of the S. mutans and Lactobacilli test strains (Moran and Addy, 1988). The preparation of the solid media was prepared by adding Difco standard nutrient agar (5.0 g peptone, 3.0g beef extract, 15.0 g agar powder) to one liter of distilled water. This liquid media was then autoclaved at 121 ° C for 20 minutes using a Barnstead 14-48823.

Preparation of the S. mutans culture

I mixed 69 grams of standard nutrient agar powder with three liters of distilled water. The solution was then transferred to a hot plate, stirred, and allowed to heat for approximately thirty minutes. This allowed sufficient time for the solution to become a homogenous and uniform liquid. I transferred 12 ml of the agar solution into individual screw-top test tubes using a pre-measured test tube. After this was done, I sterilized the solution in the test tubes by autoclaving them at 121° C for a total of 15 minutes. I distributed the media, pouring the contents of each test tube into corresponding Petri dishes. This was repeated for each test tube. I added 30 µL of the S. mutans bacteria strain, grown at 37º C for one week, with a bent glass rod in a spreading fashion ensuring that I used enough to cover the dish (Steinberg et al., 1988). After this was done I applied sterilized, autoclaved discs prepared by hole punching individual filter paper discs. One disc was applied to the center of each plate containing the agar growth medium.

The addition of the toothpastes was in the form of slurry. I prepared the slurry to a concentration of 5g toothpaste/10ml distilled water and mixed with a sterile stirrer until a uniform solution had been prepared (Moran and Addy, 1988). Fifteen micro liters of toothpaste in the slurry form was then added to each paper disc located on the Petri dishes. For each toothpaste/bacteria combination, I allowed eight plates to incubate for 24 hours at 37º C, and eight to incubate at the same conditions for 72 hours. The resulting zones of inhibition, as described above, were measured in mm. A total of 32 test trials for each toothpaste/bacteria combination were conducted, and the average zones of inhibition were then calculated.
Preparation of the *Lactobacilli* culture

The preparation of the *Lactobacilli* culture growth media followed the same procedures as described for the production of the *S. mutans* growth media. I used the same agar medium for both bacteria strains. The volume of agar prepared above was enough to fill plates for both strains. I followed the same procedure for dispensing and autoclaving the test tubes. The same procedures were also followed for the application of the *Lactobacilli* test strain. The discs were autoclaved as described above, and placed one to a plate in the center. As with the *S. mutans*, I used eight plates consisting of one disc for each bacteria/toothpaste/time combination. The same toothpaste slurry as described above was applied. Eight plates for each bacteria/toothpaste combination were incubated at 37° C for 24 hours, and eight plates for each combination was incubated at the same temperature for 72 hours. The subsequent zones of inhibition were measured for each plate. My control group consisted of distilled water applied to the bacteria in the same fashion as the toothpaste slurry. Table 1, shown below, illustrates the numerical components of the study.

Table 1. Data concerning the number of replicates per time frame and bacteria for each toothpaste and control. Listed are the subtotals for each toothpaste, as well as the total number of replicates for the entire study.

<table>
<thead>
<tr>
<th>Toothpaste</th>
<th>Streptococcus</th>
<th>Lactobacillus</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 Hours</td>
<td>72 Hours</td>
<td>24 Hours</td>
</tr>
<tr>
<td>Aquafresh</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Arm n’ Hammer</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Crest</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Colgate</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Toms</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Control (H2O)</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>44</td>
<td>44</td>
</tr>
</tbody>
</table>

In regards to the statistical analysis concerning the antimicrobial activity of the tested toothpastes upon two different strains of bacteria, an ANOVA F-Test along with a Tukey comparison test was performed with the Minitab computer program (Minitab Inc., 2005). The variables in the experiment were the five toothpastes. The zones of inhibition for each toothpaste and time variable on the two strains of bacteria were analyzed separately.

Results

Results were obtained from the evaluation of common toothpastes on prominent cavity causing bacteria, *Streptococcus mutans* and *Lactobacillus*. Primary reference is directed towards Figures 2-5 depicting zones of inhibition resulting from various factors, including time of exposure and the bacteria used in each test. The control group for each test was distilled water, and the average zone of inhibition for the control was zero for each test (this data was omitted for practical purposes from each figure). Figure 1 illustrates the average zones of inhibition measured 24 hours after the application of each toothpaste on the test bacteria *Lactobacillus*.

Arm n’ Hammer toothpaste had an average zone of inhibition of 25.75 mm, while Colgate had an average zone of
Figure 1. The average zones of inhibition, measured in mm, for each toothpaste listed. The zones of inhibition were measured 24 hours after the application of each toothpaste on the test bacteria *Lactobacillus*. Each toothpaste consisted of 8 replicates which comprised the mean zone of inhibition. The error bars represent one standard deviation for each toothpaste/*Lactobacillus* group of samples.

inhibition of 19.75 mm (Figure 1). The outlier toothpaste, Tom’s of Maine had a mean zone of inhibition of 22.45 mm when exposed to the *Lactobacillus* culture for 24 hours. From the results of the ANOVA F-Test there was a statistical significant difference among the five toothpaste (F = 1.12, df = 4, P= 0.001). The results from the Tukey comparison test (ICL >99.8 %) indicates that Colgate and Aquafresh were not significantly different from each other. Crest, Arm n’ Hammer and Tom’s of Maine had the largest zone of inhibition and were statistically different from Colgate and Aquafresh, but not significantly different from one another.

Figure 2. The average zones of inhibition, measured in mm, for each toothpaste listed. The zones of inhibition were measured 72 hours after the application of each toothpaste on the test bacteria *Lactobacillus*. Each toothpaste consisted of 8 replicates which comprised the mean zone of inhibition. The error bars represent one standard deviation for each toothpaste/*Lactobacillus* group of samples.
Figure 2 illustrates the average zones of inhibition 72 hours after the application of each toothpaste on the test strain *Lactobacillus*.

Arm n’ Hammer had an average zone of inhibition of 29.4 mm, while Colgate had an average zone of inhibition of 20.75 mm. The non-fluoride containing toothpaste Tom’s of Maine had a mean zone of inhibition of 28.4 mm. The results of the ANOVA F-test regarding Figure 2 finds significant difference amongst the five toothpastes when exposed to *Lactobacillus* for 72 hours ($F =1.12$, df = 4, P-value = 0.001). The results of the Tukey comparison test indicates that Crest and Colgate were not significantly different from each other, but were significantly different from all others (ICL >99.8%). Aquafresh was significantly different from all other toothpastes, while Tom’s of Maine and Arm n’ Hammer had the largest zones of inhibition and were not significantly different from each other.

Figure 2. The average zones of inhibition, measured in mm, for each toothpaste listed. The error bars represent one standard deviation for each toothpaste/S. mutans group of samples.

Figure 3. The graph illustrates the average zones of inhibition, measured in mm, for each toothpaste listed. The zones of inhibition were measured 24 hours after the application of each toothpaste on the test bacteria *S. mutans*. Each toothpaste consisted of 8 replicates which comprised the mean zone of inhibition. The error bars represent one standard deviation for each toothpaste/S. mutans group of samples.

Arm n’ Hammer had an average zone of inhibition of 21.63 mm, while Aquafresh and Crest both possessed average zones of inhibition of 17.35 mm. The ANOVA F-test regarding Figure 3 found a statistical significant difference among the five toothpastes ($F=1.12$, d.f = 4, P=0.001). The Tukey comparison test regarding Figure 3 found that Colgate was not statistically different from Arm n’ Hammer and Tom’s of Maine. All other relations among the toothpastes displayed some degree of statistically significant difference with reference to Figure 3 (ICL >99.8%).

Figure 4 illustrates the average zones of inhibition 72 hours after the application of each toothpaste to the test strain *S. mutans*. 
Arm n’ Hammer had an average zone of inhibition of 22.9 mm, while Aquafresh had an average zone of inhibition of 17.35 mm. The ANOVA F-test found statistically significant differences among the five toothpastes when exposed to S. mutans for 72 hours (F=1.12, d.f = 4, P=0.001). The Tukey comparison test (ICL>99.8%) indicated that Arm n’ Hammer was significantly different from the other toothpastes concerning zones of inhibition on S. mutans over a period of 72 hours. In addition, the same Tukey comparison test indicated that Aquafresh was significantly different from each toothpaste in regards to zones of inhibition on S. mutans over a period of 72 hours.

Discussion

The results obtained in this study suggest differences among the tested dentifrices regarding antimicrobial properties. Each test comparing zones of inhibition amongst the oral bacteria, Streptococcus mutans and Lactobacillus, was accompanied by the dentifrices exhibiting a range of effectiveness (Figures 2-5). The smallest zone of inhibition achieved by any toothpaste, on either bacterium, was 16 mm by Crest on S. mutans. The largest zone of inhibition achieved by any toothpaste, on either bacterium, was 30 mm by Arm n’ Hammer on Lactobacillus. This discrepancy between zones of inhibition strongly suggests that the tested toothpastes exhibit a wide range of antimicrobial effectiveness. In each portion of this study, Arm n’ Hammer displayed the greatest average zone of inhibition. This would suggest that it provided the greatest antimicrobial activity on the two oral bacteria, S. mutans and Lactobacillus, tested in this study.

There was no single dentifrice that exhibited the smallest average zone of inhibition over each of the four tests. In fact, one of the dentifrices that exhibited the smallest average zone of inhibition on S. mutans, Colgate, was among those demonstrating one of the larger zones of inhibition on Lactobacillus (Figures 1-4).
The reason for this could be attributed to the differences in interactions between the two bacteria and Colgate. Further tests specifically testing the effectiveness of Colgate on the two bacteria would be necessary to validate these results. The differences among relative zones of inhibition created by Colgate upon \textit{S. mutans} and \textit{Lactobacillus} might be explored in future tests by increasing the sample size to allow a more robust statistical analysis, or by using a more concentrated toothpaste slurry. Crest and Aquafresh both obtained some degree of antimicrobial effectiveness as suggested by their moderate zones of inhibition compared to Arm n’ Hammer (Figures 1-4).

Four out of the five toothpastes tested in the study contained the active ingredient sodium fluoride, which has proven antimicrobial effects as suggested by Moran and Addy (1988). Tom’s of Maine was the only toothpaste tested in the study that did not include some type of active fluoride ingredient. The results obtained by this study failed to support the hypothesis that fluoride is an essential ingredient in an antimicrobial dentifrice as explained by Lee (2004). The zones of inhibition displayed by Tom’s of Maine were consistently among the top two for both the \textit{S. mutans} and \textit{Lactobacillus} tests.

In addition to the differences regarding the absence of fluoride in Tom’s of Maine, there were differences in average zones of inhibition among the four toothpastes containing fluoride. Although all four of these dentifrices contained fluoride in their active ingredient, the results of this study suggest that the effectiveness of each dentifrice’s antimicrobial properties relating to \textit{S. mutans} and \textit{Lactobacillus} differ.

The results obtained by this study reflect the individual dentifrices effectiveness when merely exposed to the bacteria. The control of oral bacteria, which forms a bio-film on the outside layer of the enamel, is achieved by the combination of both chemical and physical means (Steinberg \textit{et al.}, 2003). The chemicals used to aid in the denaturation of the bio-film (Moran and Addy, 1988) were present in each dentifrice tested as evidenced by their measurable zones of inhibition. The physical means used in tandem with these chemicals includes the act of brushing. The bristles, comprising the head of the toothbrush, and the mechanics of abrasive brushing both aid considerably in the removal of the organic bio-film, and are therefore essential to the effective control of oral bacteria (Steinberg \textit{et al.}, 2003).

The control of oral bacteria is compounded as well by the ingested diet. Diets consisting of higher concentrations of sugar are more likely to perpetuate the formation of a thicker bio-film. Furthermore, individual microbes will likely consist in greater concentration in the bio-film, thereby increasing the odds of caries formation (Steinberg \textit{et al.}, 2003).

The results of this study suggest that the mere presence of the tested dentifrices will aid in the inhibition of \textit{S. mutans} and \textit{Lactobacillus}, and therefore decrease the potential for extreme formation of a caries inducing bio-film. The inclusion of a larger sample size, i.e. 1000 replicates for each toothpaste/bacteria trial, as well as an accurate representation of the conditions found in the oral cavity would be of greatest benefit to establishing a sound study. Larger sample sizes would aid in the reduction of any human error made while recording measurements and an accurate replication of the oral cavity provides the only true means of creating a beneficial oral healthcare product. This study included only two of the many bacteria present in various concentrations in the oral cavity. Additional oral bacteria that would be of significance to test include \textit{Staphylococcus}}
aureus and Actinomyces odontolyitus, among others, as these are also included in the oral flora. Future studies testing the effectiveness of these dentifrices would benefit by the inclusion of more oral bacteria, with the possibility of directly testing the oral cavities of test participants for specific microbial population control.

Additionally, claims such as those made by Moran and Addy (1988) supporting the presence of fluoride compounds in toothpaste would benefit from additional studies including Tom’s of Maine and other non-fluoride containing toothpastes. Results from this study show non-fluoride containing toothpastes to exhibit competitive antimicrobial activity in relation to fluoride containing toothpastes, but again, these results could also be expanded upon by performing laboratory tests in an environment that replicates the human oral cavity. Although these antimicrobial ingredients did exhibit positive inhibition upon S. mutans and Lactobacillus in this research, the environment of the oral cavity, the composition of the diet, and the practice of sound oral care all ultimately play an important role in the factors contributing to oral disease.

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Literature Cited


