An Investigation of the Anti-bacterial Properties of Orbit and Trident brands of Chewing Gum

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Abstract

Chewing gum producing companies have claimed publicly that their product helps fight oral diseases. But, these companies have not publicly shown what effect their product has on the oral flora. Specifically they do not mention the effect on Streptococcus mutans, bacteria known to cause some oral diseases. This study was an in vitro study on the effects of the chewing gums Trident® and Orbit® on S. mutans, more specifically these gums’ ability to inhibit the growth of this oral pathogen. A solid and liquid media study was performed to help simulate the overall conditions in the oral cavity. Cultures were cultivated on nutrient agar and in nutrient broth while being exposed to the gum. Measurements were used during the solid media portion using Kirby/Baur method, while optical densities were taken to compare growth in the liquid phase of the testing. During both phases of this study, Orbit® showed an approximate inhibition of S. mutans of 30-50% compared to the inhibition exhibited by Colgate Total™ Toothpaste (known in the literature to inhibit this bacterial species) with a zone of inhibition mean of 1.74 cm. Trident® exhibited little or no observable inhibition during both phases. This experiment supported the hypothesis that Orbit® should be considered to supplement traditional oral healthcare procedures for a healthier mouth.

Introduction

Chewing gum has served many functions over the years of its popularity. It is recommended to passengers of airplanes to help equalize pressures across the tympanum to prevent barotraumas (damage to the tympanum resulting from rapid changes in pressure). Chewing gum helps prevent halitosis and even fights boredom (it was recommended to me by my driver’s education instructor to fight highway hypnosis). Recently, companies such as Wrigley’s and Trident have made claims that chewing gum helps fight tooth decay. However, little research has been published in the scientific literature about the specific effects of these gums on the bacterial flora of the mouth. More specifically, there is a research gap in the effects of these gums on the bacteria Streptococcus mutans, bacteria shown to increase the risk of developing certain oral diseases.

Lang et al. (1987) performed a study on the correlation between infection of S. mutans and development of dental caries in humans. In this study, the sample was 48 elementary age children that were known to have low populations of S. mutans in their mouths. Microbiological assays and radiographic images were taken every three months from each participant from multiple sites in each mouth. The assays were plated and analyzed along with the radiographs which were examined for evidence of S. mutans. Many of the lesions and decalcification sites (areas where enamel had been eaten away) that were sampled from each participant’s mouth showed evidence of S. mutans growth. The
discovery of $S.\ mutans$ growth in many of the cavity sites suggests that uncontrolled $S.\ mutans$ growth can lead to an increased risk of developing cavities. The research group noted this in their conclusions saying their study showed evidence that supported $S.\ mutans$ as an initiator in the process that develops dental caries (Lang et al., 1987).

Controlling populations of pathogenic bacteria in the oral cavity is done mainly by regularly brushing teeth with toothpaste using standard techniques that are usually taught to elementary age children, or younger, by their parents, their dentist, or a representative from the local health department. One of the leading brands of toothpaste is Colgate Total™. Williams and Ummins (2005) examined the chemistry of Colgate Total™ and stated that this toothpaste has a fairly simple, but effective chemistry. Total™ uses a copolymer of maleic acid and polyvinyl methyl ether coupled with the antibiotic material triclosan. This copolymer serves to delay the breakdown of triclosan so that it can be present longer in the oral cavity and prolong its antibacterial effect. Scientists at Colgate Industries have been able to detect therapeutic levels of triclosan up to twelve hours after its introduction via brushing with Colgate toothpaste. Brushing one’s teeth with Colgate toothpaste has been shown to decrease bacteria populations in the oral cavity and decrease many other factors that lead to tooth decay and bad breath (Williams, 2005).

Currently, research on gums related to oral health is centered on using the gum as a preventive measure against dental diseases and using the gum to decrease the symptoms of, or even to treat, some oral diseases. Dr. Brailsford et al. (2001) conducted research on residents of an assisted living home. They provided residents with gums, one to two times daily for one year, containing varying chemicals that were suspected to have some oral health benefit. This was beneficial research, because these patients have difficulty with manually providing themselves with proper dental care due to dexterity problems related to different pathologies. In this study, it was determined that chewing gum containing chlorhexidine acetate or xylitol can help reduce gingival disease and plaque buildups in elderly citizens of assisted living facilities. It was also suggested that providing patients with these gums should be used, in addition with other oral healthcare procedures, in the normal oral maintenance of people with limited manual dexterity (Brailsford et al., 2001). However, older subjects are not the only people that chew gum, and therefore, not the only group that could benefit from having a biologically active gum.

A study by Chestnutt (2003) examined the transmission of $S.\ mutans$ from mothers to their newly born children. In this study, pregnant mothers who were known to have high salivary levels of $S.\ mutans$ were given gums containing xylitol to be chewed during pregnancy and for three years after their children were born. Salivary samples of $S.\ mutans$ were taken from the mother and the children at regular intervals in order to determine the level of transmission from mother to child. It was shown that children of the mothers who chewed the xylitol containing gum showed a significantly slower colonization of their mouth by $S.\ mutans$ than mothers who did not. However, there were no long term decreases in the populations of $S.\ mutans$ in the mothers’ mouths themselves (Chestnutt, 2003). This indicates it is likely that the gum did lower the populations of the bacteria short term, but it did not eliminate the colonies completely, leaving them to repopulate the mouths of the subjects after the gum was no longer present.

According to the Wrigley Company (Wrigley’s Co., 2005) chewing Wrigley’s
gum improves concentration, eases tension, freshens breath, provides a low-calorie snack, and helps to fight tooth decay. It is with this last claim on which my research was centered. However, there is no mention of the gum’s effect on \textit{S. mutans}. Does chewing Wrigley’s Co. gums help control the populations of \textit{S. mutans} therefore helping to maintain long-term oral health?

My research examined this question. I conducted an \textit{in vitro} study of the effects of Wrigley’s Co. (and other brands) gum on \textit{S. mutans} bacteria. To examine the effects of gum on \textit{S. mutans} populations, I subjected colonies in the exponential growth phase of the bacterial life cycle to different brands of gum in varying environments in order to simulate the behavior of both gum and bacteria in the oral environment. All of the incubations for the population studies were done at physiological ranges of temperature and pH. This was an attempt to simulate the oral environment in order to obtain medically relevant results. Due to the effects of gum in the previous studies, my study was designed to test if common brands of chewing gum are useful as cavity fighting agents by testing their ability to prevent normal growth patterns of one of the bacterial species that produces tooth decay, \textit{S. mutans}.

The implications of this research could be far reaching. Millions of Americans chew tons of gum each day. Wrigley reported a net sales of $3,648,592 in 2004 (Wrigley Co., 2005). At about $1 per pack, that is a large amount of gum. I have been treated for about 5-10 cavities throughout my life, and have no reason to believe that my experience is abnormal for the average American. If it could be shown that by chewing gum one could help to prevent some tooth decay, this could mean millions of dollars in saved money for the American people, as well as better overall oral health. If commercially sold gum can be shown to have some inhibitory properties of \textit{S. mutans}, this gum could be prescribed by dentists to those who have high populations of \textit{S. mutans} in their oral cavity to supplement already existing oral healthcare practices. Gum could also be distributed to people that can not afford, or are not aware of, other oral care practices.

Before gum can be advertised or distributed to people as an oral healthcare supplement its effectiveness must be examined. My study was a first step into doing this \textit{in vitro}. I predicted that commercially distributed gums, such as those distributed by Wrigley Co., will show anti-microbial properties when incubated with \textit{S. mutans} in a simulated physiological environment.

**Methods**

This research tested the ability of common chewing gums to negatively influence the growth of the bacterial species \textit{S. mutans}. It was separated into two main focus areas. One focus area dealt with the commercially obtained dental gums “Orbit®” and “Trident®” and their effect on the growth rates of \textit{S. mutans} on solid nutrient agar media when incubated at 37º C. The second component dealt with the same brands of gum and the same species of bacteria, but examined their interactions while immersed in liquid media rather than on solid media. The oral environment was a mixture of both types of environments with \textit{S. mutans} colonies found on both solid surfaces, such as the tongue and teeth, and colonies suspended in saliva. To get an accurate picture of \textit{S. mutans} interaction with the gums in the oral environment both media needed to be studied and in order to maintain control of variables, they were done independently. Azrak \textit{et al.}(2004) conducted a study involving many different species of bacteria, including \textit{S. mutans}, and
their preferred media was nutrient agar media which showed no growth inhibition of *S. mutans*. Nutrient broth should also demonstrate no inhibition because the recipes for the two are nearly identical with only agar added to the solid media recipe.

The effect of these gums on the overall oral flora needed to be studied *in vitro* in order to limit the amount of uncontrolled variables. Similar studies done *in vivo* have shown that other chemicals contained in certain gums demonstrated positive effects on oral health. However, as in many *in vivo* experiments other variables could have influenced the outcomes of those experiments. This *in vitro* study will be a small first step in validating these commercially obtained products as a legitimate contributor to oral health practices.

In the solid agar media portion of the study, *S. mutans* cultures were subjected to portions of a piece of gum on solid nutrient agar and incubated for a period of 120 hours at 37°C (ambient body temperature), which was sufficient to get an accurate gauge on the effectiveness of the gum. To produce the media needed for this portion of the experiment a recipe from *Understanding Microbes* for Nutrient Agar (0.005% peptone, .003% beef extract, .015% agar, 99.977 % distilled water) was used (Claus, 1988). The media came from a pre-mixed batch produced by BioPro. Once the ingredients were dissolved; the mixture was autoclaved for 20 minutes at 121°C at 15 psi. After cooling to approximately 55°C, 15 mL portions were poured into sterile plates for a total of 49 plates.

The required organism for the test was *S. mutans* ordered from Fisher Scientific (fishersci.com, 2005). Stock culture was received in BHI broth, and maintained on nutrient agar using the recipe previously stated. Two plates were inoculated and placed into the incubator at 37°C for 48 hours to ensure the inoculated colonies were in the exponential growth phase of the bacterial life cycle.

Two test tubes were obtained and 8 mL distilled water was added to each. Each tube was inoculated from one of the stock plates to create an emulsion of the desired test organism. These tubes were then incubated at 37°C for 48 hours to create a dense emulsion of test organism. Using a sterile micropipette I added 0.5 mL from the test tubes to one of the pre-prepared nutrient agar plates. To ensure even growth over the plate “spreading technique” was used (emulsion was added to the center of the plate, and using a sterile 90° bent glass rod, the emulsion was spread over the plate). A 0.5 gram piece of Orbit® was then added (exactly as it came out of the wrapper) to the center of one plate. This process was repeated for 20 plates for both brands of gum. The plates were incubated for 120 hours at 37°C.

Three more plates were inoculated in the same fashion as the test plates; these were used for positive controls. In these plates a portion of filter paper soaked in a 33% by weight Colgate Total™ Toothpaste solution (known to have inhibitory effects on *S. mutans* (Lang, 1987, Williams and Ummis, 2005)) was placed in the middle of the plate and incubated at 37°C. This process was repeated on three more plates for a negative control. In the center of these plates a one gram piece of Bubblicious® watermelon flavored bubble gum was placed for negative controls. These plates were also incubated at 37°C for 120 hours. Bubblicious® was used because it is also a gum, but not known or expected to have any anti-microbial action, chewing bubblegum may actually cause dental diseases (Dr. Robert Sears D.D.S., personal communication). The final two control groups were an inoculated plate with no other material and a plate that is not
inoculated with anything. Each control group consisted of five plates. Both groups of plates were incubated with the rest of the plates to ensure that they received the exact same treatment as the experimental plates. This was important to maintain the integrity of the controls. After incubation measurements were taken of the ring around each piece of gum where no bacteria grew. This ring is called the zone of inhibition.

In the liquid media portion of the experiment *S. mutans* samples were set into suspension in a liquid nutrient broth (.005% peptone, .003% beef extract, 99.992% distilled water (Marcus, 2000)). The BioPro pre-mix was also used for this media preparation. Each tube was inoculated from sample plates containing the original organism ordered from Fisher Scientific, and 85 experimental tubes were produced. I added a 0.5 gram piece of gum to each tube using sterile technique. The distribution for the liquid media tubes is shown in Table 1.

Bubblicious was used as a negative control, Colgate toothpaste as a positive control, and a blank tube for blank control. I incubated all tubes for 72 hours at 37 ºC. The controls were incubated with the experimental tubes to ensure they were exposed to the same environment for the same amount of time.

After 72 hours of incubation (less time is needed than for the solid media portion, because the solid media portion required solid lawn growth for accurate measurements) all tubes were removed from the incubator and analyzed using a spectrophotometer. To obtain a measurement of population density, the spectrophotometer measured the amount light of a specific wavelength (680 nm) is absorbed when shone through the sample. The %Transmittance was converted to optical density using the equation O.D. = \( \log (100/\%T) \).

These two procedures were done in tandem represent how these gums behave in the oral cavity. Once all data was collected, the Analysis of Variance test was done to test if there was a statistical difference between the groups. Multiple comparisons were made using Tukey’s test to determine specific differences between groups. These tests were done using the MiniTab14 program (MiniTab Inc., 2005).

### Results

After a 120 hour incubation (plates were checked every 24 hours until sufficient growth was achieved) the solid nutrient agar plates were removed from the incubator. The inoculated plates with no inhibitors present showed uninterrupted lawn growth over the entirety of the agar surface on all five plates, the plate that was not inoculated with bacteria grew nothing, the remaining experimental plates were grouped according to the inhibitor they contained and the zones of inhibition were measured (all zones were circular in shape and the zone was measured as a diameter). The inhibition zone is shown in Figure 1.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Trident®</th>
<th>Orbit®</th>
<th>None</th>
<th>Colgate Total™</th>
<th>Bubblicious®</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of tubes</td>
<td>35</td>
<td>35</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
Figure 1. The dark circular region represents agar surface covered with bacterial growth. The white area is the zone of inhibition. The square in the middle represents the piece of inhibitor. The line through the middle shows the way in which the zone was measured.

Table 2. There were 20 replicates in the experimental groups, and 5 in the control groups.

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Orbit</th>
<th>Trident</th>
<th>Colgate Toothpaste</th>
<th>Bubblicious</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhibition range</td>
<td>1.4 cm – 2.2 cm</td>
<td>0</td>
<td>3.4 cm – 3.6 cm</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Average inhibition zone</td>
<td>1.76 cm</td>
<td>0</td>
<td>3.55 cm</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The measurements were taken and averaged for an overall picture of each inhibitor’s performance. Table 2 is a summary table of the results.

Orbit seemed to work about 50% as well as toothpaste as an inhibitor for *S. mutans*. Trident did not exhibit a zone of inhibition when examined (uninoculated control, bubblegum control, and uninhibited control also did not show a zone of inhibition). Trident® did inhibit bacterial growth on the piece of gum itself and underneath where it was sitting, but did not show any outward radiating zone of inhibition. According to the Analysis of Variance test (Minitab, 2005) there is a significant difference in the zones of inhibition for the different inhibitors (F = 32.65 ; df = 4 ; p < 0.005). However, there was no significant difference between the uninhibited control the Trident® brand gum and the Bubblicious control. These three groups did not exhibit any inhibition on the bacterial growth. The Colgate Toothpaste performed the best, with Orbit® the second best.

In the liquid media portion of the experiment a percent transmittance was recorded from the spectrophotometer and converted to optical density using the equation given in the methods.

An ANOVA indicated there was a significant difference in the experimental groups (F = 54.29; df = 4; p < 0.0005, reject H₀). Tukey 95% Simultaneous Confidence Interval test revealed that there was not a significant difference between Trident® and the uninhibited control but that all other groups had a significant statistical difference with a confidence level of 99.34%. Again, Colgate Toothpaste exhibited the highest amount of inhibition, and Orbit® was second highest.
The experimental tubes are depicted in Figure 3.
Again, in this portion of the test, it does not seem that Trident inhibited the growth of the bacteria at all, it actually seemed to increase the rate of growth in the liquid media portion of the experiment as its optical density value was slightly higher (though not statistically significant, $P > 0.05$) than the uninhibited control. Orbit did show some inhibition over the uninhibited control, but did not maintain its 50% effectiveness when compared to Colgate Total™ Toothpaste.

![Figure 2](image1.png)

Figure 2. The No Inhibitor column was used as a control. It appears that Trident® brand gum and Bubblicious actually increase the rate of growth of *S. mutans*. The error bars represent one standard deviation of each group. Orbit® and Trident® had 35 replicates in their groups, there were 5 in each of the other groups.

![Figure 3](image2.png)

Figure 3. Experimental tubes of bacteria and gum. The darker regions represent “clumps” that were suspended. These clumps were likely to be made out of suspended bacteria with some of the gum or coating from the gum. The tubes were swirled before data collection to disperse these clumps so that the solution was uniform in appearance.
**Discussion**

The inhibition of *S. mutans* in this experiment does follow a rather peculiar trend. It seems that Orbit® gum, produced and sold by Wrigley Co. does in fact have a significant inhibition on *S. mutans*. However, Trident® showed no such inhibition. In the liquid portion of the experiment the Trident gum showed no significant statistical difference between its inhibition and the uninhibited control tube (P>0.05). However, the Orbit® brand gum showed a statistically significantly higher zone of inhibition than the other controls (P<0.05). This trend was repeated in the solid media portion of the experiment. Again, Orbit® had a statistically significant higher inhibition of the bacteria when exposed on nutrient agar (P<0.05). It was approximately 50% as effective as Colgate Total™ toothpaste, known in the literature to effectively inhibit *S. mutans* (Lang *et al.*, 1987, Williams and Ummins, 2005). Trident, on the other hand, showed no zone of inhibition on the nutrient agar plate, the same result as the uninhibited control and the Bubblicious® control.

These results do not prove that Trident® has no inhibitory effect on *S. mutans*. Although there was no observable zone of inhibition exhibited by Trident® while on the agar media, there were no bacterial colonies observed on the piece of gum itself. These results were not mirrored by the bubblegum control; in fact the bubblegum control had changed colors because the bacteria had so thoroughly infested it. Likewise, underneath the Trident® the agar was uninhabited by bacteria. There was a perfect outline of the piece of gum on the agar surface. Again, these results were not observed in the Bubblicious® control.

During the liquid media portion of the experiment, Trident® seemed to slightly enhance growth of *S. mutans* in nutrient broth. The enhancement in growth does not follow the pattern of limited inhibition displayed in the solid agar portion of the test. This can be partly explained by examining the surface of the gum itself. Trident® is covered with a thin layer of white powder to prevent it from sticking to the wrapper while in the package. This could explain some of the clumping found in suspension during the test. One of the clumps was removed, examined and incubated on agar for 24 hours. This test showed that there were bacteria present on the clumps, but the sparse growth indicates that not all of the suspension was comprised of bacterial cells. This phenomenon was also observed in the Orbit® tubes, but since there is much less coating on the outside of Orbit®, the suspensions observed were also less dramatic. With this taken into account, it is reasonable to suggest that Trident® probably did exhibit some inhibition on the bacterial growth, since its optical density was the same as the uninhibited control even though some non-bacterial suspension was present in the mixture.

The ingredient lists from both gums include xylitol. This chemical has been noted in the past to be beneficial to oral health (Brailsford *et al.*, 2001). In the case of Orbit® gum, my experiment supports the claims in Brailsford, *et al.*, (2001) that xylitol containing gums should be considered for supplemental oral healthcare for those people who are not able to adequately clean their teeth using conventional methods. In order to better test these results, as well as the Brailsford *et al.*, 2001 results, a shorter experimental test should be performed, perhaps 30 minutes, an average time that people keep gum in their mouth at one time. This would be to establish an amount of time needed for
inhibition of bacteria to occur. In order to establish the usefulness of this kind of oral healthcare, doses of the chemical xylitol must also be examined. An effective dose for different people would be a necessary factor to establish. Also, it would be necessary to examine the behavior of the chemical xylitol when exposed to the enzymes that are present in the oral environment. In order to be used, xylitol must maintain its integrity while in the harsh environment of the human mouth. Overall, my data supports the use of these types of xylitol containing gum as a viable support mechanism in oral healthcare. More testing needs to be done before it should be marketed as such, but this in vitro test was a preliminary study of this concept.

My study supports the hypothesis that xylitol containing gums can inhibit the growth of *S. mutans*, and could therefore be considered for supplemental oral healthcare procedures. However, since xylitol is contained in many other products (including but not limited to non-fluoride toothpaste) these products should also be further studied for their effectiveness at inhibiting this bacteria. Lozenges, pastes, and other forms of delivery of this chemical could all be viable alternatives (and additives) to complete the process to a healthy mouth.

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