Triclosan vs. Sodium Fluoride: Which is More Effective at Killing *Streptococcus mutans*?

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Abstract

Much research has been done to learn how to combat the negative effects of microorganisms on humans. In the present study, we are interested in understanding whether a combination of sodium fluoride and triclosan will be more effective at eradicating harmful oral bacteria than each of them separately. To test this question, the bacteria *Streptococcus mutans*, a bacteria that causes dental caries, was used. The chemical treatments were added to paper disks and it was observed whether a zone of inhibition occurred in bacteria growing in direct contact with the disks. It was found that there is no difference in effectiveness between the mixture and the individual triclosan treatment (one-way ANOVA, $F_{2,21}$= 41.307, P= 5.265E-8). Assuming the bacteria would not evolve to be resistant against triclosan, our results imply that toothpaste companies could cut their costs by using only one chemical in their products. Further studies must be done in order to assess the development of bacterial resistance in *Streptococcus mutans*. Cullinan et. al. (2014) have been able to support the fact that the bacteria are not likely to develop some a resistance to triclosan, so this may not have been an issue for this research.

Introduction

An important portion of scientific research deals with the study, attack, and prevention of the spread of disease-causing pathogens. The utilization of different chemicals that are not only safe but also effective against such harmful pathogens has become more widespread in recent years. Triclosan and sodium fluoride are two chemicals commonly used to combat the multitude of bacteria in the mouth (Aas et.al. 2005). These chemicals can be used in conjunction or separately to attack microorganisms. In the present study, we compared these two commonly-

used chemicals to assess their efficacy alone and in a combination at preventing pathogenic bacteria growth in the mouth.

Previous research has established that individually, both sodium fluoride and triclosan are effective against bacterial growth in the mouth; their combined impact is less known. Nole et. al. (2005) found that long-term use of triclosan is effective at reducing the overall amount of harmful *Streptococcus mutans* (a pathogenic bacteria found in the human mouth). As for sodium fluoride, other scientists have observed that this too has an appreciable effect on *Streptococcus mutans* (Somaraj et. al. 2017). There is some evidence suggesting that fluoride added to a non-fluoride substance will be most effective at killing bacteria (Randall et. al. 2015). To our knowledge, there have not been any previous studies that combine both sodium fluoride and triclosan to test their efficacy against bacterial growth (Binney et. al. 1995). In the current research, we assessed whether using triclosan and sodium fluoride individually or in a combination is more effective at inhibiting *Streptococcus mutans* growth.

Our objective was to test the efficacy of a solution of triclosan, sodium fluoride, and a combination of the two against *Streptococcus mutans* growth. Given previous research (Randall et al. 2015), our hypothesis was that the sodium fluoride-triclosan mixture will be the best at eradicating bacteria overall. We used microbiological experimental procedures to test this hypothesis. This research is important as it sheds light on chemical-bacterial interplay in the body and will allow consumers to make smarter, more informed decisions as to what products they utilize on a daily basis.

Methods

In order to test our hypothesis, we used the bacteria *Streptococcus mutans* as our study specimen. *S. mutans* is a common microorganism found in the human mouth and is a main contributor to dental caries, or tooth decay. It clings to the human tooth and produces lactic acid as a part of its metabolism, upsetting the pH balance of the mouth and creating an inhospitable environment for other species of oral microorganisms. When *S. mutans* finds itself in an acidic and warm environment (18 to 40 degrees Celsius), its population can increase exponentially, leading to a buildup of plaque on the tooth's surface. Triclosan and sodium fluoride are two chemicals found to inhibit the growth of *S. mutans* (Finkelstein et. al. 1990), and so these chemicals were the focus of our study.

The first step in testing chemical effectiveness against *S. mutans* growth involved the creation of the four chemical solutions. For the two chemical solutions of solely triclosan and sodium fluoride, 0.3 grams of each chemical was measured and mixed with 100 mL of water to create a 0.3% chemical solution, the same concentration of each respective chemical in toothpaste. The sodium fluoride-triclosan mixture was created using 0.15 grams of each respective chemical in 100 mL of water, and the control solution was 100 mL of carefully distilled water. In order to aid in the dissolving of triclosan, 2300 μL of sodium hydroxide (NaOH) was added to each solution.

The second step of our study involved applying the chemicals and the bacteria to the growth medium before incubation. We used a bunsen burner, gloves, and ethanol to sterilize the experimental area before, during, and after the application had taken place to minimize the effects of contamination on our end results. 150 μL of *S. mutans,* in a liquid growth medium, was applied to each petri dish using a micropipette. The bacteria was spread evenly around the petri

dish, and then the solution was applied to the sample using small disks of filter paper lightly soaked in the respective chemical solution. Two disks were applied to each dish with a total of eight replicates per solution (four plates per chemical solution). The dishes were immediately sealed with parafilm, labeled with tape, and stored in the incubator at 37 degrees Celsius for about one to two days. For more information about the husbandry and care of *S. mutans*, refer to Appendix 1.

The final step of the experiment involved measuring the zones of inhibition for each petri dish. Once the incubation period was completed, the petri dishes were placed upside-down and the diameter of inhibited bacterial growth around the paper disk was measured using a ruler (See Appendix 2). The diameters for each chemical treatment were recorded and the area of the zone of inhibition was calculated using $\pi r^2_{zone} - \pi r^2_{disk}$. The results for each replicate were recorded in a table (See Appendix 3).

We used a one-way ANOVA test to assess whether the mean zone of inhibition varied across chemical treatments. Since our data was not normal, we used a square root transformation to meet the assumption of normality for our subsequent tests. We further used a Tukey test to do post-hoc comparisons between groups. We put our data for each replicate into the statistical software R (R core team 2008) for data analysis.

Results

We found that sodium fluoride on its own had little effect on inhibiting bacterial growth and that the antimicrobial effects of the triclosan solution and the mixture solution were the same. The one-way ANOVA test confirmed that the difference in chemical effectiveness against bacterial growth was significant (one-way ANOVA, $F_{2,21}$= 41.307, P= 5.265E-8). This allowed

us to reject our null hypothesis and proceed to the next test. The post-hoc test allowed us to compare treatment groups pairwise and revealed that the sodium fluoride solution performed significantly worse than the other two solutions (Tukey, $P < 0.05$; see Figure 1) and the mixture and triclosan solutions have basically the same effectiveness (Tukey, $P= 0.1663796$; see Figure 1).

Discussion

Our research assessed whether triclosan, sodium fluoride, or a triclosan-sodium fluoride combination is the best at eradicating *Streptococcus mutans* bacteria. We utilized the method of zones of inhibition to test the effectiveness of these chemicals. Our results indicated that the sodium fluoride solution performed significantly worse than the triclosan solution and the sodium fluoride-triclosan mixture. Interestingly, we also found that the triclosan and sodium fluoride-triclosan mixture both had the same level of efficacy in the inhibition of bacterial growth. These results suggest that since there is no difference in utilizing a combination of sodium fluoride and triclosan or just triclosan on its own, perhaps such a combination is not necessary at all in the production of hygienic products.

During experimentation, we ran into some unprecedented issues in the dipping of the filter paper, the medium of growth, and replications that may have caused some slight, though likely innocuous, variations (also, see Appendix 4 for notes on the bacterial form). Since there was no way for us to control how much solution was saturated in the filter paper disk when we dipped it into the solution, the final amount of chemical solution we applied to the petri dishes might have varied somewhat across all of the dishes. This could have led to a slight skew in our results meaning that the diameter values we obtained could have been somewhat exaggerated.

Also, initially, we tried to grow the bacteria in blood agar with no success. We switched over to using a standard tryptic soy agar that did not have blood in it further into our research. However, we are confident in our results as the same people were preparing the dishes using a uniform methodology as stated above, therefore making it unlikely for any procedural mistakes or biases to affect our data. Since all of the treatments were subject to these same conditions, these errors should have been controlled for across all of the treatment groups. Furthermore, the experimentation that we completed should be redone with more than merely 8 replicates to increase the reliability of the data, keeping in mind all of the other issues we ran into during our own research so that the results of another experiment are generated in a timely and resource-effective manner.

Other studies that are related to the one we carried out loosely outline the conclusion we were able to draw regarding triclosan and sodium fluoride separately having an effect on bacterial growth, though their research included additional variables that we did not test for. In an experiment done by Yu-Ting Xu et. al. (2015), the experimenters used sodium fluoride and triclosan but also tested these two against BAG, a bioactive glass that can, under some circumstances, eradicate bacterial microorganisms. They did not combine triclosan and sodium fluoride in their work. Their results showed that more bacteria on the bioactive glass were eradicated in comparison to the sodium fluoride and triclosan plates, but they did find that sodium fluoride and triclosan had some sort of antibacterial effect, though not to the extent of the bioactive glass.

The question still remains as to whether or not the bacteria will develop some sort of resistance to the chemicals used. Sodium fluoride is able to hinder bacterial growth through inhibition of enolase, an enzyme used by the bacteria in glycolysis, which decreases ability to

intake and metabolize sugars (Subramaniam and Nandan 2011). Likewise, triclosan also inhibits glycolysis by irreversibly inactivating critical enzymes needed for this portion of bacterial respiration. Bacteria are widely able to generate an immunity to chemical usage, so this must be taken into consideration. According to a study carried out by Cullinan et. al. (2014), they found that *Streptococcus mutans* did not develop resistance to triclosan. However, this is merely one study, so it may not be enough to establish the lack of sensitivity to triclosan. In a piece of scholarly literature written by Ying Liao et. al. (2017), the scientists wrote about how the evolution of sodium fluoride resistance has been observed in *S. mutans*. Such a bacterial strain that is unaffected by sodium fluoride due to chromosomal alterations has been developed in a laboratory successfully by many scientists; therefore, it has been established that fluoride resistance may occur in this bacteria. Such a relationship between the bacteria and sodium fluoride - triclosan mixture has yet to be discovered. Thus, further research needs to be done on the evolution of chemical resistance in *S. mutans* before any further recommendations can be made specific to our research.

Our study could potentially aid companies creating hygienic products in what chemicals they should incorporate into their merchandise. As our research shows that there is no difference in efficacy between using a triclosan-sodium fluoride mixture and just using triclosan on its own, commercial companies can possibly just use one in their products and conserve resources and money that way. This may also prompt companies to find a better combination of chemicals that is more lethal to harmful bacteria.

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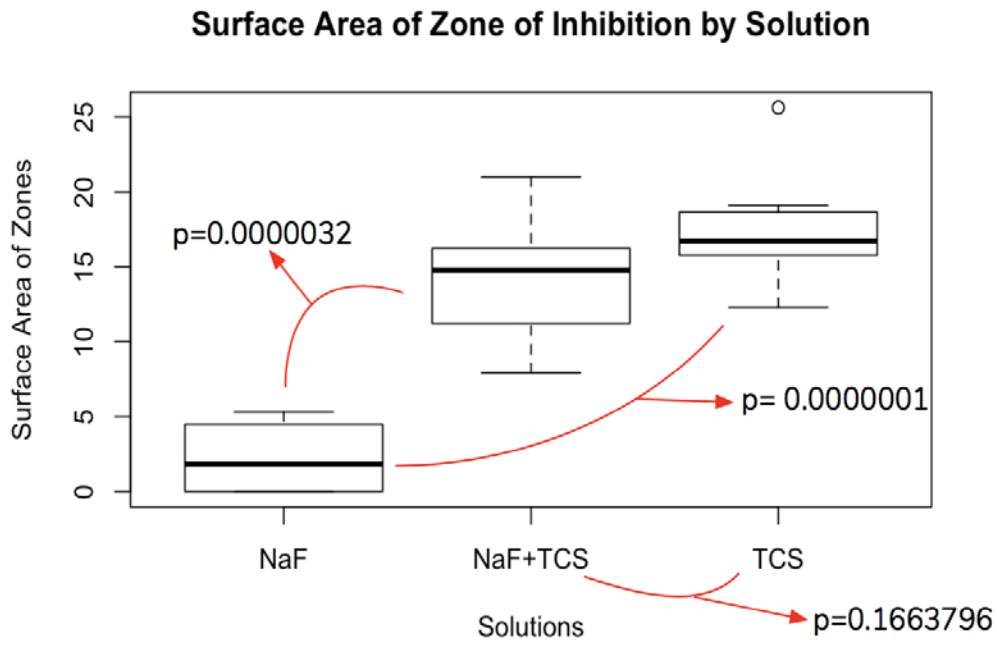
Figure legends

Figure 1. This is a box plot displaying the results of the ANOVA test. The red lines and the p-values the lines point to at the p-values generated by the Tukey test. The two treatment groups that the line bridges between are the two groups that were compared, and the subsequent p-value is the p-value for the pairwise comparison for the two groups.

Figures

Figure 1.



Appendix 1: Husbandry of Organisms

Our bacteria, Streptococcus mutans, are rod-shaped bacteria that are abundant in the mouth. They are facultative anaerobes so we do not have to worry about the environment in which we work with and store our bacteria in. We ordered and received our bacteria from a company by the name of Ward's Science and they sent the organism to us in the aerobic form which minimizes any problems that will occur with said organism since we work in oxygenated environments. Additionally, Ward's Science sent us this bacteria in the lyophilized form (frozen in a medium) which means that the tube of bacteria suspended in the medium must be stored at around 4 to 5° C which is the temperature of a regular refrigerator. After the bacteria is plated and growing, it must be kept at an incubation temperature of around 37° C which is the temperature of one of the incubators already present in the laboratory. We will not have to provide the bacteria with any form of sustenance as the medium the lyophilized bacteria is in and the bacteria growing on the tryptic soy blood agar will derive all their nourishment from the mediums they are currently residing in/on. *Streptococcus mutans* are also non-motile, indicating their inability to move, so this should not cause us any issues either.

Appendix 2: Diameters of Zones of Inhibition

This table show the diameters of the filter paper disks measured for each treatment group (measured in mm). Plates 1 and 2 were not used in the final analysis which is why they are absent in Table 2.

Plate	Diameters of Disks with Treatments with 2300 µL NaOH Added			
	Triclosan	NaF	NaF+Triclosan	Distilled H_2O
1	15	20	19	8

2	**N/A**	**N/A**	**20**	**8**
3	**20 and 19**	**10 and 8**	**18 and 16**	**8 and 8**
4	**16 and 23**	**8 and 9**	**20 and 14**	**8 and 8**
5	**30 and 20**	**8 and 8**	**19 and 12**	**8 and 8**
6	**21 and 22**	**10 and 9**	**25 and 20**	**8 and 8**

Appendix 3: Calculated Surface Areas of the Zones of Inhibition

This table shows the calculated surface areas using the diameter values from Table 1.

	Surface Area of Zone of Inhibition in mm^2 (minus 50.2655 mm^2 for filter paper disk)							
Plate	**Triclosan**		**NaF**		**NaF+Triclosan**		**Distilled H_2O**	
1	**263.9838**	**233.2633**	**28.2743**	**0**	**204.2035**	**150.7964**	**0**	**0**
2	**150.7964**	**365.2101**	**0**	**13.3518**	**263.8938**	**103.6726**	**0**	**0**
3	**656.5929**	**263.8938**	**0**	**0**	**233.2633**	**62.8319**	**0**	**0**
4	**296.0951**	**329.8672**	**28.2743**	**13.3518**	**440.6084**	**263.8938**	**0**	**0**

Appendix 4: Notes on Bacterial Form

When ordering our bacteria, the company we ordered it from (see Husbandry in Appendix 1 for more information on where we ordered from) sent it to us in the lyophilized form, which essentially just meant that the bacteria was already frozen in a medium. This made it very difficult for us to extract the bacteria to plate it. We had to refine our methods and basically cut up pieces of the frozen medium and homogenize them in another vial of liquid tryptic soy agar medium to the best of our ability and use this solution to spread on the plates. In further

experiments, it may be useful to order this bacteria from the company already in a liquid state to prevent these kinds of issues.