

EFFECT OF TOOTHPASTE FORMULATIONS ON THE NUMBER OF VIABLE BACTERIA LEFT ON TOOTHBRUSHES FOLLOWING ROUTINE BRUSHING

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Abstract

The antimicrobial triclosan is being added to toothpaste formulations to reduce the number of potentially pathogenic bacteria remaining on toothbrush heads after brushing. Because of recent concerns regarding the safety of triclosan, this study investigated the effect of different toothpaste formulations on the survival of bacteria remaining on toothbrush heads to assess whether the addition of triclosan in toothpaste is warranted. Fifteen subjects were provided three different toothpaste formulations (regular, baking soda, and triclosan) and asked to brush following their usual brushing routine. After 1 week of brushing the number of viable bacteria left on toothbrush heads were estimated using the standard plate count method and enriched and selective media. The number of colony forming units (CFU) per milliliter remaining on toothbrush heads ranged from 3.3×10^6 (triclosan toothpaste) to 2.3×10^7 (regular toothpaste). While the number of surviving bacteria were significantly lower for triclosan formulated toothpaste when samples were plated onto enriched media compared to the number of surviving bacteria enumerated for regular and baking soda formulations, there was no significant difference in the number of surviving bacteria between formulations when samples were plated onto media designed to select for oral bacteria implicated in the formation of dental caries. Results from this study suggest that triclosan toothpaste formulations are no better than regular formulations in reducing the number of potentially pathogenic oral bacteria remaining on toothbrush heads after brushing.

Introduction

With an estimated 700 or more different types of bacteria comprising complex microbial communities that inhabit the mouth, it is important to maintain good oral hygiene. The mouth provides an environment that permits the growth of diverse groups of microorganisms including lactic acid bacteria belonging to the genera *Lactobacilli* and *Streptococcus* and members of the genus *Actinomyces*. It has been well established that bacteria inhabiting the mouth can negatively affect the health of teeth and gums and if left unchecked can lead to periodontal disease (Bergquist, 2009; Mazumdar et al., 2009). More recently, studies are suggesting that in addition to dental caries and periodontal disease, oral microbiota can have negative impacts on other aspects of our health (Li et al., 2000; Berquist, 2009). Joshipura et al. (1996) examined the potential link between poor oral health and coronary heart disease (CHD). After controlling for standard CHD factors (for example, level of physical activity, diet, smoking, family history), significantly higher incidence of CHD was observed among men with 10 or fewer teeth compared to males with no previously documented periodontal disease. This study proposed that oral bacteria enter the blood stream via inflamed gums and after traveling to the heart, trigger the formation of atherosclerotic plaques increasing one's risk of heart attack (Joshipura et al., 1996). Other

studies suggest that bacteria need not gain entry into the circulatory system. Instead, bacteria leach toxins resembling proteins associated with arterial walls into the blood stream. The body responds to these toxins by launching an innate immune response that leads to inflammation and the eventual damage of vessel walls (Thoden van Velzen et al., 1984; Li, 2000). Similar links have been investigated between chronic inflammation caused by oral microbiota with insulin resistance and Type 2 diabetes (Kim & Amor, 2006).

The Centers for Disease Control and Prevention (CDC) suggest that everyday use of a toothbrush is essential to maintaining optimum oral health, but studies suggest that brushing may also represent one of the primary ways in which potentially harmful bacteria can be introduced into the body. Tools such as tooth- and inter-dental brushes can collect, harbor, and spread bacteria throughout the oral cavity and transfer bacteria between individuals who share these devices. Edman et al. (1975) showed that dental floss can transfer strains of streptomycin resistant *Streptococcus mutans* into the posterior proximal sites of the mouth. After the bacteria have established themselves, they can migrate from initial sites to contiguous adjacent tooth surfaces as well as sites on the opposite side of the mouth. Similarly, Loesche et al. (1979) demonstrated that a streptomycin resistant strain of *S. mutans* was transferred by a dental explorer within 7-9 days from an implanted artificial fissure to a sterile artificial fissure that was placed in a crown on the opposite side of the mouth.

Since its first use by the Egyptians around 5000 B.C., toothpaste has undergone many transformations to meet the needs and desires of consumers worldwide (Fischman, 2000). Today, most toothpaste brands contain a number of common ingredients that include fluoride, flavorings, colorings, sweeteners, and compounds that allow the paste to remain smooth, moist, and palatable. To satisfy the growing consumer interest in eliminating oral bacteria, manufacturers have begun adding antibacterial chemicals to toothpastes. One common antimicrobial additive is triclosan. Triclosan (2,4,4'-trichloro-2'-hydroxyphenyl ether) was first introduced as a pesticide in 1969. Today, triclosan is used as an antimicrobial compound and is incorporated into a number of consumer products for the purpose of slowing or preventing the growth of microorganisms. Products that contain triclosan include hand soaps, plastics, and toothpaste, but despite its wide use, very little data is available describing the potential effects that triclosan may have on human health. In 2008, the US Environmental Protection Agency (USEPA) conducted a human health risk assessment for triclosan with emphasis on endocrine effects, developmental and reproductive toxicity, chronic toxicity, and carcinogenicity. The Agency determined that use of triclosan as a pesticide meets national safety standards, but concluded that further studies would be needed to determine thyroid and endocrine effects that may be related to triclosan exposure (USEPA 2008). While more studies are needed to assess the potential effects of triclosan on human health, studies have found that sub-lethal levels of triclosan can induce antibiotic resistance in medically important bacteria (McBain et al., 2003; Christensen et al., 2011). Christensen et al. (2011) grew 8 strains of *Listeria monocytogenes*, a food-borne pathogen, in media containing two sublethal concentrations (1 and 4 µg/ml) of triclosan. After a 24h incubation period, susceptibility to gentamicin, kanamycin, and penicillin was assessed using the microdilution method and Brain Heart Infusion (BHI) broth. All 8 strains were found to have become resistant to a 16-fold increase in gentamicin after exposure to sublethal levels of triclosan. Triclosan exposed strains were also found to become resistant to other closely related aminoglycosides such as streptomycin. This finding is important because gentamicin and related antibiotics are the only antibiotics currently used to treat this pathogen. In addition, because of the widespread use of triclosan in personal care products including toothpaste and other household items, this biocide has been detected in wastewater effluent and subsequently in river

EFFECT OF TOOTHPASTE FORMULATIONS ON THE NUMBER OF VIABLE BACTERIA LEFT ON TOOTHBRUSHES FOLLOWING ROUTINE BRUSHING

ecosystems. There is now a grave concern that the presence of triclosan in rivers could contribute to the spread of antibiotic resistance among environmental microorganisms (Pycke et al., 2010).

As data indicating a link between oral bacteria and poor health has accumulated, triclosan-containing toothpastes have become readily available to consumers. Currently the benefits of triclosan in toothpaste are thought to outweigh any risks that might be associated with its use. The objective of this current study was to compare the effect of toothpaste formulations on the number of bacteria surviving on toothbrushes after brushing. Three toothpaste formulations were assessed; (1) regular, (2) baking soda, and (3) triclosan. The number of bacteria remaining on each toothbrush head after brushing was determined using the Standard Plate Count Method and Tryptic Soy (TS) and Snyder Test agars. TS agar, an enriched media, was chosen for its ability to encourage the growth of a variety of bacteria and was used to enumerate the total number of surviving bacteria. Snyder Test agar was chosen for its ability to select for fermenting bacteria that can withstand acidic environments and which are implicated in the destruction of enamel and facilitate periodontal disease.

Materials and Methods

Subjects and Toothbrushing Regimens

This study was submitted to and approved by the Rivier University Institutional Review Board. Fifteen subjects volunteered for participation in this study. Subjects were randomly placed into one of three groups and assigned a test kit as outlined in Table 1. Group assignments for each trial are outlined in Table 2. Subjects were asked to brush twice daily following their usual routine for seven days using their assigned kits. Kits were collected after seven days, completing Trial 1. The procedure was repeated for Trials 2 and 3.

Table 1. Contents of kits 1, 2, and 3, containing a new toothbrush and one of three toothpaste formulations.

Kit #	Toothpaste Formulation
1	Regular
2	Baking Soda
3	Triclosan

Table 2. Kit distributions among subjects during trials.

Group	Trial 1 – Kits	Trial 2 – Kits	Trial 3 - Kits
5A	1	2	3
5B	2	3	1
5C	3	1	2

Determination of CFU/ml

After each trial, kits were brought back to the lab. Toothbrush heads were removed and aseptically placed into appropriately labeled sterile test tubes containing 10.0 ml of sterile physiological saline (NaCl 0.85 % w/v). Test tubes containing toothpaste heads were vortexed for 2.0 minutes. Serial dilutions (10^{-1} - 10^{-5}) were prepared for each toothbrush head. Aliquots (100 μ l) for each dilution were aseptically transferred and dispensed into labeled sterile Petri plates. Approximately 20.0 ml each of

molten Tryptic Soy (TS) or Snyder Test agar were added to the appropriately labeled Petri plate. Triplicate samples were plated per dilution per test subject. Inoculated agar plates were incubated at 37°C for 18-24 hours under high CO₂ conditions using a BD GasPack CO₂ containment system. Following incubation, plate counts were determined by counting the number of colony forming units (CFU) for each dilution. Dilutions that resulted in plates with 30-300 CFU were used to estimate the number of bacteria remaining on the toothbrush head after one week of brushing.

Statistical Analysis

Viable cell count data were log₁₀ transformed and tested for normality using the Shapiro-Wilk Normality Test (p=0.05). The statistical *t*-test (α=0.05) was used to determine if there was a significant difference in the number of bacteria surviving on toothbrush heads when samples were plated on TS agar compared to samples plated on Snyder Test agar. Analysis of Variance (ANOVA) with repeated measures (α=0.05) was conducted to determine if there was an effect of toothpaste formulation on the total number of viable bacteria (TS agar) and fermentative oral bacteria (Snyder Test agar) left on toothbrushes after one week of brushing.

Results

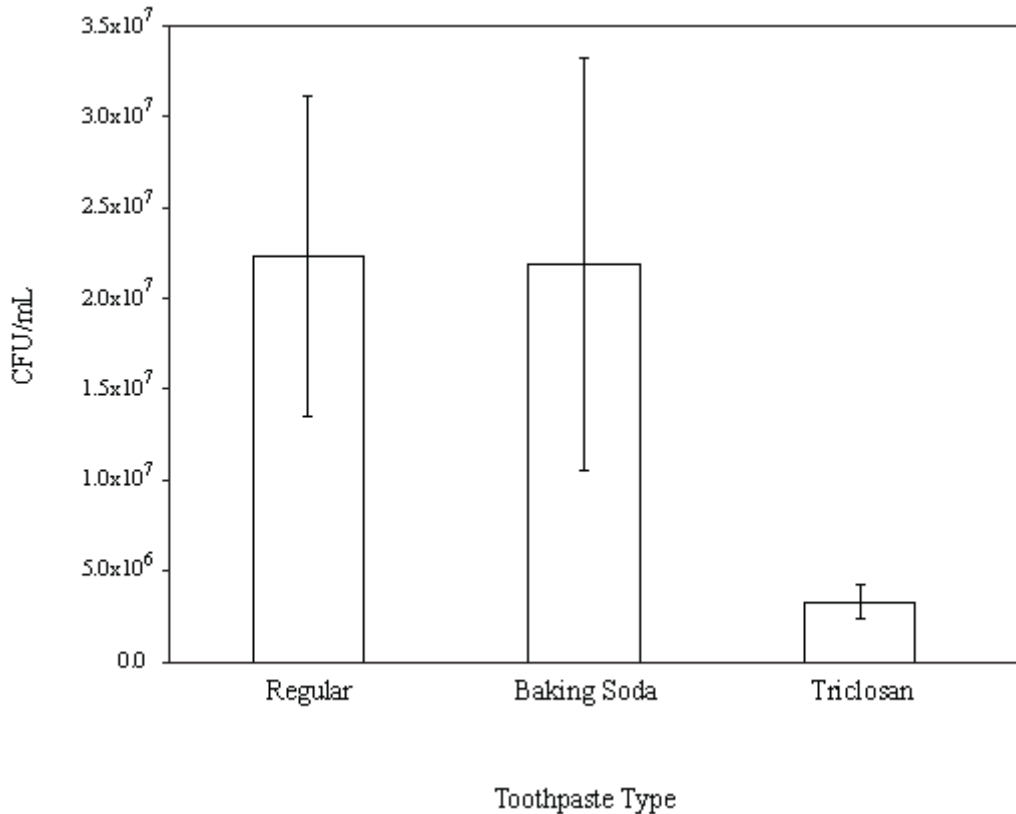


Figure 1. Number of Colony Forming Units (CFU) per milliliter grown on Tryptic Soy agar for trials 1, 2, and 3. Error bars represent standard deviations for n=15.

EFFECT OF TOOTHPASTE FORMULATIONS ON THE NUMBER OF VIABLE BACTERIA LEFT ON TOOTHBRUSHES FOLLOWING ROUTINE BRUSHING

Figure 1 shows the average CFU/ml were estimated at 2.3×10^7 for regular toothpaste, 1.9×10^7 CFU/ml for baking soda toothpaste, and 3.3×10^6 CFU/ml for triclosan toothpaste when samples were plated onto TS agar. For each toothpaste type, these counts were found to be significantly higher ($p < 0.05$) than those estimated when samples were plated onto Snyder Test agar. The average CFU/ml for regular toothpaste was 2.5×10^6 , 2.9×10^6 for baking soda toothpaste, and 1.2×10^6 for triclosan toothpaste when samples were plated on Snyder Test agar (Figure 2). Although the #CFU/ml estimated for samples plated onto TS agar were higher than those reported for Snyder Test agar, data indicate that a large proportion of bacteria enumerated in this study are typical oral bacteria represented by individuals in the Lactobacilli and Streptococcal genera.

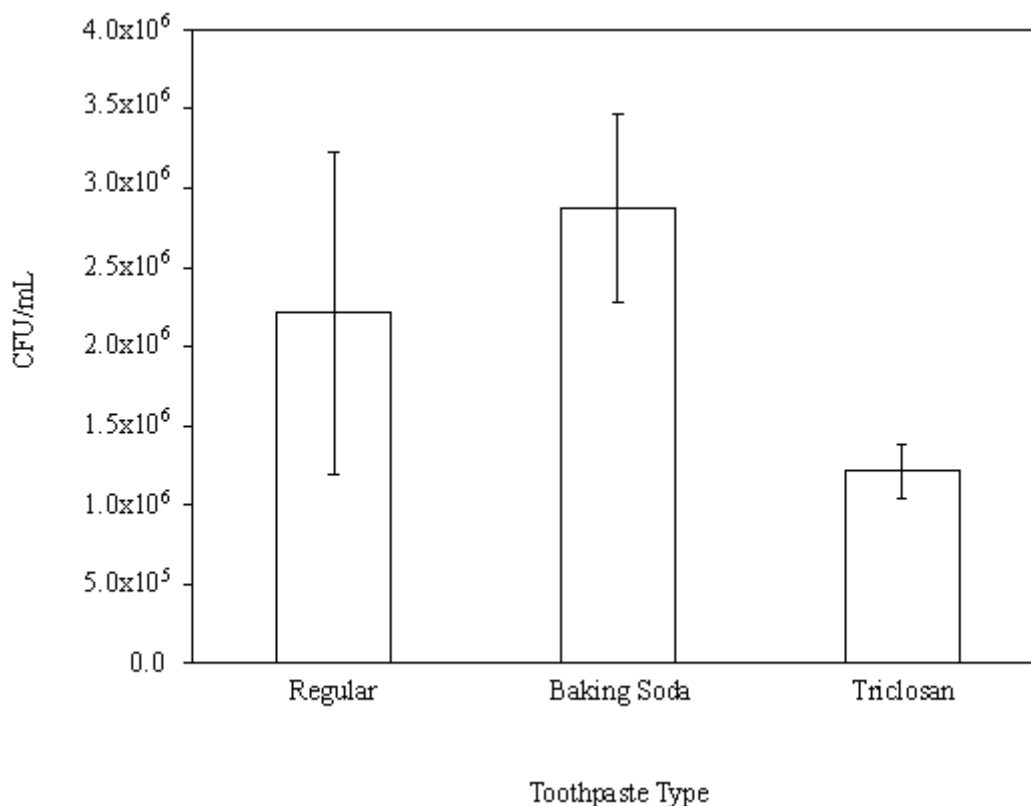


Figure 2. Number of Colony Forming Units (CFU) per milliliter grown on Snyder Test agar for trials 1, 2, and 3. Error bars represent standard deviations for $n=15$.

There was no significant difference between the average CFU/ml estimated for regular and baking soda formulations when samples were plated onto TS agar ($p=0.209$), but these formulations resulted in significantly higher numbers of bacteria compared to triclosan toothpaste (regular and triclosan, $p=0.001$; baking soda and triclosan, $p=0.019$) indicating that triclosan has a negative effect on the survival of certain populations of bacteria. In contrast, there was no significant difference in the CFU/ml between toothpaste formulations when samples were inoculated onto Snyder Test agar (regular and baking soda, $p=0.417$; regular and triclosan, $p=1.000$; baking soda and triclosan, $p=0.081$). This suggests that the regular and baking soda formulations reduce numbers of lactic acid bacteria to the same extent as the triclosan toothpaste.

Discussion and Conclusions

The number of viable bacteria remaining on toothbrush heads after brushing reported in this study are similar to bacterial survival rates on toothbrushes reported in other studies. Quirynen et al. (2001) found that toothbrushes used by individuals with periodontal disease with no toothpaste retained 10^7 CFU aerobic species and 10^8 CFU anaerobic species even after toothbrush heads were rinsed with water and stored dried for up to 24 h. When the same subjects added toothpaste containing detergents to their brushing regimen, the CFU declined by 2 log (10^5 CFU for aerobic species and 10^6 CFU for anaerobic species) indicating that this formulation decreased the survival rate of pathogenic species (Quirynen et al., 2001).

Because of the relationship between oral bacteria and overall health, triclosan is being introduced into traditional toothpaste formulations as a means to control the growth of potentially pathogenic bacteria. In the present study, the triclosan toothpaste formulation only affected the survival of bacteria when samples were plated onto TS agar. TS agar is considered an enriched media designed to encourage the growth of many kinds of bacteria including environmental bacteria that end up in the mouth or on the toothbrush head as well as oral bacteria implicated in periodontal disease. The significant decrease in CFU/ml observed for triclosan toothpaste samples compared to regular and baking soda formulations plated onto TS agar may be due to transient bacteria that are more sensitive to the effects of toothpaste in general. Snyder Test agar is a media that encourages the growth of oral bacteria, specifically *Lactobacilli* and *Streptococcal* spp., identified as important contributors in the formation of dental caries. These bacteria generate high levels of organic acids through fermentation. The acids accumulate on dental surfaces causing the decalcification and softening of enamel. Perforations resulting from soft enamel provide entry routes for bacteria into the body via the circulatory system. Results from this study suggest that triclosan toothpaste does not further reduce the number of important oral bacterial species compared to regular and baking soda toothpaste formulations. This result is supported by Warren et al. (2001) who looked at the survival of three test organisms (*Prevotella* sp., *Porphyromonas gingivalis*, and *Actinobacillus actinomycetemcomitans*) on toothbrush heads used by individuals with periodontal disease. Subjects brushed their teeth without toothpaste and with regular toothpaste or triclosan-containing toothpaste and found no significant difference in the isolation frequencies of the test organisms between regular toothpaste and Triclosan-containing toothpaste.

Modern oral healthcare products contain ingredients believed to improve oral health and lower one's risk of illness related to oral microbiota, but the potential health risks and mechanisms that result in the induction of resistance associated with the use of triclosan are not yet clear. In response to the growing concerns related to the toxic effects of triclosan, the US EPA plans to initiate an extensive review investigating whether the incorporation of this microbicide in personal care products is necessary. Results obtained in this study suggest that triclosan does not significantly reduce the survival of oral bacteria on toothbrushes compared to formulations that do not contain this microbicide.

Many bacterial inhabitants of the mouth are harmless, but research indicates that oral bacteria can enter the bloodstream and pose serious health risks. Maintaining proper oral health is one of the most effective ways to prevent disease associated with oral microbiota. Triclosan has emerged as a possible means to effectively control the survival and growth of bacteria. The number of consumer products containing triclosan has increased within the past decade, but further research is required to better understand the effects of triclosan on the survival of bacteria that commonly inhabit the mouth. The field of dentistry could benefit greatly from a better understanding of the use of triclosan in toothpaste formulations. This study and future research might allow dental professionals to develop and

EFFECT OF TOOTHPASTE FORMULATIONS ON THE NUMBER OF VIABLE BACTERIA LEFT ON TOOTHBRUSHES FOLLOWING ROUTINE BRUSHING

recommend more effective and safer oral health regimens to improve patient care while considering the impacts that widespread use of antimicrobials have on human health and the environment.

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