Effectiveness of Plaque Removal of Two Different Toothpastes

BY

BENJAMIN Z BELAVSKY
B.S., University of Illinois at Urbana-Champaign, 2009
D.D.S., University of Illinois at Chicago, 2013

THESIS

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Defense Committee:

Carlotta Evans, Chair and Advisor
Christine D. Wu, Pediatric Dentistry
Grace Viana, Orthodontics
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LIST OF ABBREVIATIONS

ADA  American Dental Association
AHA  American Heart Association
CDC  Centers for Disease Control
CRC  Clinical Research Center
CRP  C-Reactive Protein
DPIA Digital Plaque Imaging Analysis
ENR  Enoyl-acyl Carrier Protein Reductase
FDA  Food and Drug Administration
FISH Fluorescence In Situ hybridization
HSRIC Health Services Research Information Central
IRB  Institutional Review Board
OPRS Office of the Protection of Research Subjects
OTC  Over the Counter
PCR  Polymerase Chain Reaction
QLF  Quantitative Light-induced Fluorescence
SD  Standard Deviation
UV  Ultraviolet
UIC  University of Illinois at Chicago
WHO World Health Organization
SUMMARY

Poor oral hygiene has been shown to cause physical and cosmetic damage to hard and soft tissues in the oral cavity. Despite many patients’ claims that recommended hygiene guidelines were followed, the prevalence of tooth decay and periodontal disease is high. Products that disclose residual plaque have the potential to increase patients’ awareness and encourage more thorough homecare, however, no product previously existed on the market which combined a dentifrice and a plaque-indicator. The purpose of this study was to test such a novel product (PlaqueHD™, TJA Health, LLC, Joliet, Illinois, previously known as Plaque-A-Way™, SLH Optimal Health LLC, Boynton Beach, FL) to determine whether or not it promotes more effective plaque removal than do conventional dentifrices, when given proper use directions and hygiene instructions.

Though there are several well-documented methods for measuring intraoral dental plaque, including conventional plaque indices and quantitative light-induced fluorescence (QLF), this study relied on the digital plaque imaging analysis (DPIA). Plaque is disclosed with a fluorescein solution, creating glowing deposits when exposed to ultraviolet (UV) light; the fluorescence is captured with a photo and then analysed by custom computer imaging software.

Subjects were randomized and divided into control or experimental groups. After a short period of hygiene-cessation (no brushing or gum chewing
the evening before and morning of each appointment), they were asked to brush their teeth using the control dentifrice (a generic, fluoridated toothpaste), then rinse with a solution of fluorescein. Photos were taken using the well documented ultraviolet DPIA method. This method uses UV light to detect plaque-bound fluorescein in patients’ mouths. Photos between time points could then be compared. At a follow-up appointment occurring after another period of hygiene-cessation, at least 7-10 days later, subjects brushed with either control or experimental dentifrice, rinsed with fluorescein, and were photographed. If patients were given the experimental dentifrice, they were also given proper-use directions and were instructed to “brush away any green staining” on their teeth. Photos from the two time points were compared using custom software which counts pixels and renders a percentage change per patient for remaining plaque.

A total of 39 qualified subjects were recruited and divided into control and experimental groups, however, after removing the data points for subjects who chose to stop coming to data collection appointments, there were a total of 16 subjects in the control group and 17 in the experimental group.

Results show that subjects using the experimental product had statistically significantly less mean remaining plaque between time points than subjects using the control dentifrice, but only when they were instructed on how to properly use the product.
The results of this study demonstrated that, along with proper oral hygiene instruction, effective plaque removal is significantly aided by the addition of a plaque-indicating dye into a standard dentifrice. Such plaque reduction could reasonably be shown to help reduce periodontal disease and incidence of caries as well.
1. INTRODUCTION

1.1 Background

Ineffective dental plaque removal causes demineralization, caries, gingivitis, and periodontitis (Ferreira and Mendes, 2005; Ower, 2003). This results in physical and cosmetic damage to both soft and hard tissues in the form of bleeding and swollen gums, white spot lesions, enamel discoloration, the need for restorations, and potentially tooth loss. Prevalence of tooth decay and periodontal disease is high despite many patients’ claims of following recommended homecare guidelines. According to the U.S. Department of Health and Human Services, adult prevalence of caries in the United States is 92% and periodontal disease is 8.52%, but that figure does not include gingivitis (National Health and Nutrition Examination Survey, 2014). The discrepancy in patient reporting versus disease prevalence is likely due to additional factors besides intentional misrepresentation. Poor oral hygiene skill and dexterity, and lack of dental knowledge, motivation, and ability to accurately evaluate one’s oral status all have negative impacts on plaque removal. Increasing education and technique instruction is one way to address the problem. However, improving homecare products themselves may also be helpful. This is especially important in populations of minorities, low socioeconomic status, and the elderly that traditionally have decreased access to regular professional oral care (Kim et al., 2012).
Clinical plaque analysis has been traditionally performed using several manual indices developed by Ramfjord, Silness and Loe, Turesky, and Elliott (Fischman, 1986). However, according to Pretty et al, “traditional plaque indices are problematic due to their integral nature and their failure to detect small, but potentially clinically relevant changes in plaque area” (Pretty et al., 2005). These procedures are also time consuming, more subjective, and more invasive to patients. The digital plaque imaging analysis (DPIA) method makes use of routine photography and computer software to increase speed of data collection, operator consistency, reproducibility of results, ability to store data for later use and analysis, and most-importantly patient comfort (Sagel et al., 2000; Klukowska et al., 2011; Bellamy et al., 2011). The disclosing agent, fluorescein disodium salt (FD&C No. 8), has been well documented for intraoral plaque disclosure (Sagel et al., 2000; Klukowska et al., 2011; Bellamy et al., 2011). Long wave UV light (405nm), equivalent to commercially available black light and dental curing lights and commonly used in medical, scientific, and law enforcement applications, is used to excite the fluorescein bound to plaque, gingiva, and enamel with enough photographic color separation to be analyzed quantitatively pixel by pixel (Sagel et al., 2000). The efficacy, safety, and reliability of this method have been tested thoroughly (Smith et al., 2001; Raggio et al., 2010), and it is now a standard plaque analysis procedure at Procter & Gamble Company (Cincinnati, Ohio) (White, 2007; Bellamy et al., 2011). The subjects are only required to rinse with disclosing solution and sit for a few intraoral photographs instead of enduring more intensive gingival probings.
Adding a visible dye to toothpaste to stain remaining plaque, as done in this study, has the potential to increase patients’ awareness and encourage them to be more thorough when performing homecare. The dye in the experimental toothpaste being studied is an organic food colorant, and the toothpaste is listed with the FDA. The purpose of the dye is to adhere to plaque and stain it green to provide the subject with a visual indication of where plaque is located on their teeth so that they may better target their tooth brushing.

1.2 Specific Aims

The goal of this study is to compare subjects’ plaque removal ability with the dye-containing toothpaste versus the placebo by using digital plaque imaging analysis. We questioned whether the presence of a visual indicating dye, coupled with instructions on how to correctly use the toothpaste containing it, would cause a difference in the way subjects brush their teeth. Ultimately, the goal was to increase patient awareness of existing plaque deposits and, therefore, improve the level of plaque removal during homecare.

1.3 Null Hypothesis

There is no mean difference in plaque reduction between an indicating-dye-containing toothpaste and a traditional toothpaste when subjects are given proper-use instructions to brush off stained plaque.
2. REVIEW OF LITERATURE

2.1 Oral Biofilm and Plaque Mediated Disease

According to Health Services Research Information Central (HSRIC), there are more than 700 species of microorganisms that can be found in the oral cavity (Health Services Research Information Central, 2015), many of which adhere to various oral hard and soft tissue surfaces in the form of a biofilm (Harnacke et al., 2015). These oral biofilms are complex, structured microbial communities that form mature colonies within three weeks to two months (Zijnge et al., 2010). As this biofilm matures, its microbial composition changes, affecting its pathogenicity as well (Harnacke et al., 2015).

Initial studies on dental plaque biofilms were completed by Listgarten et al. in 1976, in which they deduced the architecture of biofilm using light and electron microscopy. They determined that the initial colonizers were gram-positive cocci, which proliferated within one day of attachment. After one week, the structure of the plaque became more filamentous, attracting new species and subsequently replacing the previously dominant coccoid structure. At about three weeks, the plaque’s maturity seemingly plateaued, as further changes were minor in nature (Listgarten et al., 1975; Listgarten, 1976; Zijnge et al., 2010). Many studies have also confirmed that one of the earliest colonizers, *Streptococcus mutans*, is the most cariogenic oral bacteria in the plaque biofilm, and is able to ferment dietary carbohydrate to produce acid and water-insoluble, sticky glucans. It has been
shown that the latter contributes to the adherent nature of dental plaque (Moye et al., 2014). Other studies, performed by Palmer et al. in 2003, used fluorescence in-situ hybridization (FISH), a method of using fluorescent probes to bind to specific portions of a cell’s chromosome, to show that the initial biofilm formation was from a co-aggregation of *Streptococcus* and *Actinomyces* species (Palmer et al., 2003). Furthermore, it was later shown, using the same technique, that as the plaque matured, there was an increased presence of *Fusobacterium nucleatum* (Al-Ahmad et al., 2007). *F. nucleatum*, an aggressively invasive anaerobe, has long been associated with periodontal disease, though several studies have linked its presence in the oral cavity in periodontally involved patients to stillbirths (Han et al., 2010), as well as colorectal carcinoma (Castellarin et al., 2012).

Most recently, in 2010, Zijnge et al. used FISH to localize the most abundant species in biofilm and to help identify the bacterial species associated with periodontitis (as well as other oral diseases). Their data demonstrated convincingly the dominance of *Actinomyces, T. forsythia, F. Nucleatum, Spirochetes*, and *Synergistetes* (Zijnge et al., 2010). With *Synergistetes*, they proposed a potentially important host-pathogen interaction due to the proximity with which it is found to host immune cells. They were also able to identify the differences in supra- and sub-gingival plaque aggregation, and how these differences contribute to different pathogenicity (Zijnge et al., 2010). When dental plaque biofilm remains attached to teeth and gums, and is not removed, it can
lead to oral disease including caries, gingivitis, and periodontal disease, resulting in cosmetic damage, eventually leading to permanent physical damage, and potentially tooth loss (Loe and Silness, 1963; Feirreira and Mendes, 2005), not to mention the social repercussions of poor oral health and associated halitosis (Rayman and Almas, 2007).

Although oral hard and soft tissue damage may occur due to the chronic presence of microbial biofilm (Loe and Silness, 1963), recent research has demonstrated that the inflammation caused by oral biofilm may also increase the risk of systemic diseases, such as cardiovascular disease (Kholy et al., 2015; de Oliveira et al., 2010). Multiple studies have appraised the association between oral disease – specifically periodontal disease – and cardiovascular disease, but there is little evidence to directly relate oral plaque levels and the incidence of cardiovascular disease (Anderson et al., 1991; World Health Organization, 2015; Centers for Disease Control, 2015; Watson et al., 2012; Loos et al., 2000). What has been found, however, is that the risk of cardiovascular disease increases with elevated C-reactive protein (CRP), a marker for systemic inflammation (Watson et al., 2012). This is especially true for chronic inflammation (Ridker et al., 1997), such as that found with periodontal disease (Loos et al., 2000). This relationship is what has prompted intense research aimed at correlating periodontal disease and cardiovascular disease, and why the American Heart Association (AHA) has stated that “periodontitis and heart disease share risk factors…and both contribute to inflammation in the body” (American Heart
Therefore, the promotion of plaque biofilm reduction is essential in improving systemic health and overall well-being.

2.2 **Oral Hygiene and Oral Hygiene Instructions**

Because of the complexity and adherent nature of oral biofilm, plaque deposits cannot simply be rinsed away like food deposits or other forms of oral debris (Block et al., 1972). Plaque must be mechanically removed frequently, either at home or professionally. If plaque is not removed daily, over time, soft plaque will calcify into hard deposits, making it increasingly difficult to remove without professional help. This further emphasizes the daily need for successful plaque removal, lest one suffers the pathological and social consequences of heavy plaque deposits. However, many people lack the awareness necessary to effectively remove plaque (Rustogi et al., 1992).

The most common oral hygiene aids include toothbrushes (both manual and electric), dental floss, various dental picks, interproximal brushes, and rubber tips (Warren and Chater, 1996). Mechanical plaque removal is commonly combined with adjunct chemical agents, namely fluoridated toothpastes, mouth rinses, or antimicrobial gels. Many of these agents may also have oxygenating agents, non-ionic agents such as triclosan, or anti-attachment agents (Gaffar et al., 1997). However, as mentioned before, good oral care is not simply a function of access to oral hygiene aids; factors such as dexterity, motivation,
socioeconomic status and even dental knowledge play a large role in effective plaque removal (Mbawalla et al., 2010).

It is important to improve the level of oral homecare in order to address ineffective plaque removal, however, it is not so simply done. Oral hygiene instructions are a crucial component in patient education, as they allow the provider to assess knowledge gaps in their patient, fill them with pertinent information, and alter poor hygiene habits in a nurturing way. There are a few notable ways to achieve this goal: improved patient education and technique instruction have been traditional methods practiced by most healthcare providers. However, less emphasis has been placed on the improvement and development of common oral hygiene products conducive to plaque removal efficiency.

2.3 **Toothpaste Components and Triclosan**

According to the American Dental Association (ADA), the common toothpaste components are: fluoride; mild abrasives such as calcium carbonate or silica gels; humectants to maintain hydration, such as glycerol or glycol; flavorants; thickening agents and binders; and detergents such as sodium lauryl sulfate. Some toothpastes may also contain potassium nitrate or strontium chloride to promote the reduction of tooth sensitivity (American Dental Association, 2015b). Many toothpastes contain additional antimicrobial agents to reduce microbial load and act as adjuncts to mechanical plaque removal (Teles and Teles, 2008; Otten et al., 2011). However, selected earlier studies and
recent systemic reviews have also reported no significant positive effect of added antimicrobial agents in toothpastes (Moran et al., 1989; Teles and Teles, 2008; Salzer et al., 2014).

Otten et al. (2001), on the other hand, reported that because plaque removal can be ineffective, especially in hard reaching locations, using a fluoridated toothpaste with an antimicrobial agent may create an intra-oral reservoir of the components needed to promote plaque destabilization. They also reported that toothpastes containing larger aggregates of antimicrobials were not as effective as those containing stannous fluoride alone, likely because the larger molecules were not as readily absorbed in the dental plaque (Otten et al., 2011).

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) is a broad spectrum antibacterial agent that has been used for several decades in a myriad of household products, including soaps, cosmetics, and toys. It has been found to be effective against Gram-negative, Gram-positive and Mycobacteria, making it quite a comprehensive bactericidal agent (Levy et al., 1999). Its inert nature, ability to withstand a wide range of temperatures, extensive use, lack of established toxicity, and its antimicrobial properties contribute to triclosan being an ideal additive in toothpastes for plaque control (Bhagarva and Leonard, 1996). Its ability to suppress plaque formation has also been reported (Moran et al., 2001).
Another desirable trait of triclosan is that it is active at very low concentrations, meaning very little needs to be added to a product for it to display antimicrobial activity. This is due primarily to its mechanism of action in which it potently inhibits bacterial lipid biosynthesis by inhibiting the enzyme enoyl-acyl carrier protein reductase (ENR). ENR catalyzes the final step of lipid biosynthesis in bacteria, however, many bacteria are resistant to the drugs previously used to inhibit it. This means that bacteria exposed to triclosan cannot complete construction of their cell wall and subsequently lyse (Levy et al., 1999). Unfortunately, there are ways in which bacteria can be resistant to even triclosan, primarily through three separate single amino-acid substitutions which ultimately result in a change in the protein’s binding site, preventing triclosan-mediated inhibition of activity (Levy et al., 1999; Heath and Rock, 2000).

Much research has been done recently on triclosan’s efficacy as an antimicrobial agent, with several focusing on how to best administer triclosan via toothpaste into the mouth to promote its sustained release in the oral cavity. One notable paper by Kokisch et al. (2004) described triclosan-loaded microspheres which can be added to toothpaste. Her team described the inadequacy of many previous triclosan-delivery systems (ostensibly hinting at an explanation of why so many studies have conflicting results on its efficacy), and how sub-therapeutic levels are quickly reached in the mouth post-application because of salivary flow, masticatory action or swallowing. They engineered chitosan microspheres which
could increase retention on the oral mucosa, in dental crevices and gaps, and in areas where slow release would be most beneficial (Kokisch et al., 2004).

Just as triclosan became ubiquitous in nearly every household product, environmental studies began to emerge describing triclosan’s prevalence not just in soap, deodorant and toothpaste, but also in human milk, aquatic environments, and lake sediments (Singer et al., 2002). These findings spawned concern in the scientific community and popular press, and environmental studies began to be performed to investigate potential human exposure. It was found that triclosan contaminates human milk and lipid deposits, is able to survive several degradation steps in water treatment facilities, and is able to contaminate fish and other wildlife that flourish in aquatic environments (Adolfsson-Erici et al., 2002; Singer et al., 2002). This may be detrimental to human health because it may foster the development of resistant bacteria in the environment and may also be connected with an increase in allergies (Dann and Hontela, 2011).

Currently, the only ADA-accepted toothpaste that contains triclosan is Colgate Total™ (Colgate-Palmolive, New York, New York), and in a 2014 press conference, the ADA Council on Scientific Affairs stated that it evaluates the safety of Colgate Total frequently. They currently state that “there is no clinically relevant scientific evidence indicating that the [ADA] Seal should be removed from…Colgate Total” (American Dental Association, 2015a). Furthermore, the
FDA posted a Consumer Update in 2013 stating that there is insufficient evidence to suggest how consumers use or how companies produce products that contain triclosan (Food and Drug Administration, 2013). Furthermore, a Cochrane review compiled in 2013 by Riley and Lamont assessed the effects of triclosan-containing toothpaste (of which there is only one on the market). They found that there was moderate-quality evidence that triclosan-containing toothpaste reduce the oral presence of plaque, reduce gingivitis and gingival bleeding, and may lead to a reduction in dental caries. Furthermore, they state that there appear to be no safety concerns with toothpastes containing triclosan (Riley and Lamont, 2013). Whether or not this Cochrane review cleared up the debate about triclosan, or muddied it further, is difficult to assess.

2.4 Plaque Disclosing Products

One method of improving existing oral hygiene products, without adding antimicrobial agents such as triclosan, involves the incorporation of plaque-adherent dye capable of disclosing existing dental plaque on the teeth. Such products are available in the form of disclosing rinses and tablets. However, these products are mostly available in a dental-office setting rather than as over-the-counter (OTC) products. For plaque removal products to be effective and acceptable, they should be simple, readily accessible, and be as non-invasive as possible. As noted by Block et al. in 1972, visualization of plaque has both the potential to increase patient awareness, as well as the ability to encourage them to be more thorough when removing plaque. An OTC product that allows such
visualization, without requiring extra steps, could significantly improve oral hygiene and homecare by reducing dental plaque and plaque-mediated diseases.

Selected products have been studied before, but results have been controversial. In 2004, Silva et al. compared the use of DentPlaque (Axis Biotec, Brazil), a toothpaste with an incorporated disclosing solution, to the use of disclosing tablets and traditional toothpaste. They found that subjects preferred using DentPlaque, even though the tablets disclosed plaque better, and were more motivated to use such a product. A proposed product that, unfortunately is not available on the market, is a toothbrush that dispenses disclosing agent directly onto the teeth, however all references to such a product are that it is simply a patented idea (Lerner, 2002). When Miranda et al. (2004) compared the plaque-removal efficacy of using disclosing mouth rinses to placebos (water), it was found that there was no significant difference in plaque removal when using either product prior to brushing. A recently marketed product, PlaqueHD™ (TJA Health, LLC, Joliet, Illinois), has been previously evaluated for inherent plaque removal efficacy (under the name Plaque-A-Way™ (SLH Optimal Health, LLC, Boynton Beach, FL)). The objective of the current randomized, controlled clinical study was to examine if the use of PlaqueHD™, when given proper use and oral hygiene instructions, increased plaque removal efficacy.
3. MATERIALS AND METHODS

3.1 Pilot Study Design and Treatment Protocol

A pilot human study previously performed at UIC established an optimal methodology and study protocol for accurate and reproducible data collection. The previous pilot study and the current study used similar methodology (Figure 1). Any apparent differences are noted in the text.
Figure 1. Appointment flowchart

Screening
- Recruitment via fliers and mass-email communication
- Phone/Email interview using short questionnaire
- Participants who fit the inclusion and exclusion criteria were scheduled for two appointments and randomized into control or experimental groups

First Appointment
- Confirm answers to questionnaire
- Review informed consent
- Confirm appropriate hygiene cessation
- Practice photo acquisition steps (cheek retractor placement, chin and mouth positioning, etc)
- Brush teeth with control toothpaste
- Rinse with 25mL phosphate buffer for 10 seconds, rinse with 10mL 1240-ppm fluorescein in buffer for 60 seconds, rinse 3 times for 10 seconds each with 25 mL phosphate buffer
- Take intraoral photo
- Dimiss participant

Second Appointment
- Confirm appropriate hygiene cessation
- Control group brushed with control toothpaste, Experimental group brushed with PlaqueHD
- Rinse with 25mL phosphate buffer for 10 seconds, rinse with 10mL 1240-ppm fluorescein in buffer for 60 seconds, rinse 3 times for 10 seconds each with 25 mL phosphate buffer
- Take intraoral photo
- Dimiss participant
Participants were recruited with flyers and an online classified ad, and after passing an initial phone-screening, were divided into two groups – control and experimental – based on a computer-generated order. Study qualification was based on the following criteria:

- Inclusion criteria consisted of: Participants must be 18 years of age or older, in good general health (self-assessment), and must have all 12 anterior teeth present, canine to canine.

- Exclusion criteria consisted of: participants should not be pregnant or nursing, not be a dental student or faculty or staff member, not have taken antibiotics within two weeks prior to testing, not have dry-mouth symptoms or significant food allergies, not have dental restorations or caries (canine to canine in both arches), and not have had anterior dental work or prophylaxis performed within 30 days prior to testing.

Qualified participants were required to visit the College of Dentistry, Department of Orthodontics, twice over 7 to 10 days for the study. Participants in the control group used the control toothpaste at one visit and the experimental (PlaqueHD™) at the second visit (Table I). PlaqueHD™ is an FDA-registered toothpaste (Appendix A) which contains the FDA-registered Annato (Bixo orellana) seed-extract dye, plus FD&C Blue No. 1, which colors the toothpaste green and adheres to dental plaque (Appendix B) (SLH Optimal Health, LLC, 2012). Both fluoridated control and experimental toothpastes had similar chemical compositions, apart from the presence of plaque-indicating dye in the
experimental toothpaste. A total of 37 subjects were recruited for the pilot study, and 35 completed both appointments.

Prior to the first visit, participants were instructed to refrain from brushing, flossing, or using other oral hygiene aids and chewing gum the evening prior and the morning of the visit. At the first visit, informed consent was administered to confirm that participants understood the risks and benefits and participants had an opportunity to ask questions. They were then asked to brush their teeth with the control (no dye) toothpaste using a provided manual toothbrush (Henry Schein Inc., Melville, NY) for one minute in front of a mirror. They were then asked to complete the following procedure for plaque disclosing: rinse for 10 seconds with 25mL of phosphate buffer; rinse for 1 minute with 5.0mL of 1240-ppm fluorescein (FD&C yellow No. 8) in phosphate buffer; and rinse 3 times for 10 seconds each with 25mL of phosphate buffer (Klukowska et al., 2011). The phosphate buffer consisted of 3.62g monosodium phosphate and 0.349g disodium phosphate in 2L of water at a pH of 5.5. Participants expectorated the solution after each rinse.

After rinsing, a digital frontal intraoral image of the participant’s upper and lower teeth in edge-to-edge occlusion was captured. Images were taken using a Canon Rebel Xi camera (Canon, Melville, NY) with a Tamron 90mm fixed-focal-length lens (Tamron, Commack, NY) and a mounted black-light emitting flash (Digi-Slave L-Ring Ultra II UV, SR Electronics, Dallas, TX). The flash-ring was
powered by batteries. Images were oriented to be as perpendicular to the incisors as possible, and centered on the maxillary midline such that the anterior teeth were in focus (Figure 2). Participants were asked to hold cheek retractors during image capture to increase the visibility of their teeth and gingiva (Figure 3). The room was darkened prior to image capture and images were then saved to a desktop computer in the Department of Orthodontics.

Figure 2. Photographic set up
At the second visit, participants in the control group repeated all of the above steps exactly, while participants in the experimental group brushed with experimental PlaqueHD™ toothpaste. The brushing, disclosing and photographic procedures were identical to those that took place during the first appointment.

No instructions were given to participants, in either group, at either visit, apart from requesting that they brush their teeth with the provided toothpaste for one minute in front of a mirror. All steps were timed by the investigator using a stopwatch.
TABLE I. STUDY DESIGN

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<th>Appointment 2 Toothpaste</th>
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<td>Control</td>
<td>Control</td>
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<tr>
<td>Experimental Group</td>
<td>Control</td>
<td>ControlHD™</td>
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3.2 **Primary Study Design and Treatment Protocol**

Participants were recruited with flyers and a classified ad and were selected to represent the general population as best as possible. 39 participants were recruited after passing an initial screening process via phone-call and then scheduled for two appointments at the Clinical Research Center (CRC) at the College of Dentistry, unlike the pilot study which required participants to be present at the Department of Orthodontics. A total of 33 participants completed both appointments of the study and were used in data analysis.

Qualified participants were randomly assigned to one of two groups – control and experimental. All participants were required to make two visits to the
College of Dentistry. Participants in the control group used the control toothpaste at one visit and the experimental (PlaqueHD™) at the second visit. Both fluoridated control and experimental toothpastes had similar chemical compositions, apart from the presence of plaque-indicating dye in the experimental toothpaste.

Prior to the first visit, participants were instructed to refrain from brushing, flossing, or using other oral hygiene aids and chewing gum the evening prior and the morning of the visit. At the first visit, informed consent was administered to confirm that participants understood the risks and benefits and had an opportunity to ask questions. Participants were then asked to brush their teeth with the control toothpaste using a provided manual toothbrush (Henry Schein Inc., Melville, NY) for one minute in front of a mirror. This was the same toothbrush type that was used in the pilot study. They were then asked to complete the same plaque disclosing procedure after brushing described in the pilot study.

After rinsing, a digital frontal intraoral image of the participant’s upper and lower teeth in edge-to-edge occlusion was captured. Images were taken using the same equipment, with the same settings, as in the pilot study, however, the flash-ring was powered by a direct wall-socket connection rather than through battery power, to maintain constant flash emittance power. Image capture and storage were identical to the pilot study.
At the second visit, participants in the control group repeated all of the above steps exactly, while participants in the experimental group brushed with experimental PlaqueHD™ toothpaste. The brushing, disclosing and photographic procedures were identical to those that took place during the first appointment, however, specific instructions were given on how to use the experimental product, i.e. “brush for one minute in front of the mirror and concentrate on removing any green stains.”

3.3 **Data Analysis and Statistics**

Using a custom computer software, the twelve anterior teeth in each digital photo were masked by the operator to define the area of analysis (Figure 4). The masked images were then run through the software to generate a pixel map representative of “tooth,” “plaque,” and “other” pixels (Figure 5). The overall plaque coverage score was calculated with: (plaque pixels)/(plaque + teeth pixels) x 100%. Statistical analysis assessed results of plaque reduction.
Figure 4. Masked anterior teeth with defined area of analysis

Figure 5. Masked image with generated pixel map
Using SPSS (IBM SPSS for Windows, Version 22.0 Armonk, NY: IBM Corp.), statistics were performed to test the mean plaque reduction in the control and experimental groups.

3.4 **IRB/ACC Approval**

Protocols for both the pilot and the primary study were approved by the University of Illinois at Chicago (UIC) Institutional Review Board (IRB), Office of Protection of Research Subjects (OPRS), on February 14\(^{th}\), 2013 and February 12\(^{th}\), 2014, respectively (IRB Protocol #2013-0113, Appendix C).
4. RESULTS

Data from the pilot study, obtained without specific hygiene instructions for using PlaqueHD™, showed no mean significant differences in plaque reduction between the control and experimental toothpaste (p-value>0.05).

Subsequently, thirty-nine subjects were initially recruited and divided into control (20 subjects) and experimental (19 subjects) groups for the primary study. Six subjects dropped out due to scheduling conflicts leaving 16 subjects in the control group and 17 in the experimental group. All remaining participants completed two visits; no complaints or adverse effects after using the experimental toothpaste were reported.

In the primary study, the data was obtained after instructions about removing visible stains on the teeth were given to subjects in the experimental group. There was no statistically significant mean difference between the control group and experimental group at the baseline initial appointment when using the control dentifrice, \( t(37) = 0.737; p\text{-value}=0.466 \). No statistically significant differences were noted in mean plaque reduction between the first and second visits for the control group \( t(15) = -1.377; p\text{-value}=0.189; \text{Table II} \).

Statistically significant mean plaque reduction between the initial baseline appointment and the second appointment for the experimental group using
*PlaqueHD™*, [\( t(16) = (2.718); p\text{-value}=0.015; \) Table III]. The data in Figure 5 show that participants using the experimental toothpaste had more plaque elimination than those using the control toothpaste (51.3% mean change versus 8.3% mean change), with the mean change in the control group not deemed of statistically significant value (\( p=0.189 \)).

The data also showed a statistically significant mean difference in plaque removal between the control group and the experimental group after the second appointment, [\( t(31) = (2.241); p\text{-value}=0.032 \)].

The data analysis is reported using parametric Student paired samples \( t \)-test and independent \( t \)-test. The Shapiro-Wilk Normality test results indicated that the raw data are not distributed on a normal curve, therefore corresponding non-parametric tests were run, as well. Similar results were found with parametric and non-parametric tests, so parametric data were reported. Statistical significance was set at 0.05.

**TABLE II. COMPARISON OF MEAN PLAQUE COVERAGE (%) OF THE CONTROL GROUP BETWEEN APPOINTMENTS**

<table>
<thead>
<tr>
<th>Appointment</th>
<th>N</th>
<th>Mean, SD</th>
<th>Mean Diff., SD</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>1</td>
<td>16</td>
<td>21.53 ± 23.39</td>
<td>1.78 ± 5.15</td>
<td>0.97</td>
<td>4.52</td>
</tr>
<tr>
<td>2</td>
<td>16</td>
<td>19.75 ± 22.76</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### TABLE III.
COMPARISON OF MEAN PLAQUE COVERAGE (%) OF THE EXPERIMENTAL GROUP BETWEEN APPOINTMENTS

<table>
<thead>
<tr>
<th>Appointment</th>
<th>N</th>
<th>Mean, SD</th>
<th>Mean Diff., SD</th>
<th>95% Confidence Interval</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>17</td>
<td>27.33 ± 28.61</td>
<td>14.02 ± 21.27</td>
<td>3.09</td>
<td>24.96</td>
</tr>
<tr>
<td>2</td>
<td>17</td>
<td>13.31 ± 17.56</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
5. DISCUSSION

5.1 Discussion

It has been shown that self-performed mechanical plaque removal in adults is frequently ineffective (Van der Weijden et al., 2005; Schäfer et al. 2003) and that providing patients with a means to assess their hygiene habits at home can significantly improve plaque removal efficacy. It seems obvious, therefore, that by simplifying oral hygiene, patients are likely to have improved homecare.

Patients and providers have long been aware of various plaque indicating products, but their use outside of dental offices is typically limited. Disclosing tablets and disclosing solutions are commonly used in dental offices. However, to our knowledge, no product that incorporates a plaque-indicating dye in toothpaste was previously available on the market for direct-to-consumer use.

Toothpaste combined with a plaque-disclosing agent is also more likely to work for patients than using multiple products concurrently to achieve the same result because fewer steps are involved. This type of toothpaste may improve oral hygiene habits by showing patients where they are deficient in removing plaque. Choo et al. (2001) explained that without such reinforcement, patients are likely to revert back to old, possibly ineffectual, habits. This is especially important in children, where establishing an easy, visual method of performing good oral hygiene is important for a lifetime of reduced oral disease. Studies have shown that, in adults, effective plaque removal was based not only on the
ability to remove plaque but also an understanding of how to do so (Renz et al., 2007). By combining proper oral hygiene instructions and a toothpaste that discloses plaque, adults and children alike should be more efficient at mechanically removing dental plaque.

Furthermore, public acceptance of such a product is likely to be high due to recent worries over antibacterial agents such as triclosan, which has otherwise been used to reduce intraoral plaque. Public concern over the efficacy of triclosan, as well as its recently exposed potential health and environmental dangers, have caused many companies to remove triclosan from their products (San Francisco Chronicle, 2013; New York Times, 2011). Many consumer advocate groups are even calling on the FDA to rethink their neutral stance on the inclusion of triclosan in household products. The advantage of an FDA-approved, non-toxic product such as PlaqueHD™, whose main anti-bacterial action is through improved mechanical plaque removal, would therefore be much more readily accepted by the public.

5.2 Importance of Oral Hygiene and Proper Use Instructions

As dentists, we seek to improve the oral health of our patients. Regular examinations, thorough oral hygiene instructions, and reinforcement of learned concepts are effective chair-side, but how can we best maintain these practices at home? Traditionally, patients have been provided with adjunct products to use for home learning and refinement, but the success of these products is based
entirely on their correct use. By adding a plaque-disclosing agent to fluoridated toothpaste, oral hygiene habits and efficiency may improve because immediate plaque removal is noticeable, compared with a toothpaste without a disclosing agent. We can therefore improve our patients’ homecare by having them use a product that provides immediate feedback on their hygiene. This would then motivate patients to improve their homecare habits, subsequently removing disease-causing plaque more effectively and consistently.

In our pilot study, no significant difference in plaque removal between a control toothpaste and PlaqueHD™ were noted when no oral hygiene instructions were given. But when participants were given instructions on how to use a plaque-disclosing toothpaste, a significant improvement in plaque removal efficacy was noted. We believe that the increase in plaque removal efficiency was because users could visualize the presence of plaque and effectively remove it while brushing. The long-term use of such a product is likely to promote better oral hygiene habits and mechanical plaque removal, thus reducing oral diseases.
5.3 Limitations of the Study

Of the 100 subjects screened, only half qualified for appointments, while only 43 scheduled for appointments. Among these, 33 continued and completed the study. Although the sample size appears to be low, it was not found to affect the results since both parametric and non-parametric data demonstrated significant results.

5.4 Future Research

Further studies with increased subject sample size are warranted. Studies including patients with fixed orthodontic appliances would also be encouraged because these patients are typically at higher risk for plaque-mediated disease due to inefficient plaque removal around appliances. Furthermore, due to the relationship between plaque-induced oral disease, inflammation-related CRP, and potential CRP-related cardiovascular disease, research concerning this association is warranted.

5.5 Conclusions

We believe that the ability to visualize plaque on tooth surfaces may be an important factor contributing to proper oral hygiene and plaque removal. This study demonstrates that using a toothpaste with plaque-indicating dye, along with proper brushing instructions, significantly increases plaque removal efficacy compared to using a standard fluoridated toothpaste.
Cited Literature


APPENDIX A

2012
CERTIFICATE OF ELECTRONIC DRUG LISTING

This certifies that:
SLH Optimal Health LLC
10075 S Jog Rd Ste 301
Boynton Beach, FL 33437
USA
has listed the referenced drug product with the U.S. Food and Drug Administration pursuant to part 207 of Title 21, US Code of Federal Regulations, such listing having been verified as currently effective on the date hereof by Registrar Corp:

Product Trade Name: Spearmint and Peppermint PLAQUE A WAY Fluoride Anticavity Toothpaste Gentle Formula 116g
Product Code: 000
Package Code: 04
NDC Number: 42288-000-64
Registrar Contact: Registrar Corp
144 Research Drive, Hampton Virginia, 23666 USA
Telephone: +1-757-224-0177 • Fax: +1-757-224-0179

Registrar Corp will confirm that such listing remains effective upon request and presentation of this certificate until the expiration of each year from the date hereof, unless terminated after notice of the certificate. Registrar Corp makes no other representations or warranties, nor does this certificate make any representations or warranties to any person or entity other than the named certificate holder, for whose sole benefit it is issued. Registrar Corp assumes no liability to any person or entity in connection with this certificate. Neither employs or solicits Registrant, nor will hold harmless and indemnify Registrar Corp from any and all claims of conversion with this product, its labeling, FDA drug listing, occurrence or use. Registration of a drug conversion or drug wholesale, or assignment of a registration number, or assignment of a NDC number does not in any way denote approval of the same by the U.S. Food and Drug Administration. Any representation that creates or implies an impression of official approval because of drug listing or the conversion of any NDC number is misleading and constitutes fraud.

Registrar Corp
144 Research Drive, Hampton, Virginia, 23666, USA
Telephone: +1-757-224-0177 • Fax: +1-757-224-0179
info@registrarcorp.com • www.registrarcorp.com

Russell K. Stahman
Executive Director
Registrar Corp
Dated: September 25, 2012
2012
CERTIFICATE OF REGISTRATION

This certifies that:
SLH Optimal Health LLC
10075 S Jog Rd Ste 301
Boynton Beach, FL 33437
USA
is registered with the U.S. Food and Drug Administration pursuant to part 207 of Title 21, US Code of Federal Regulations, such registration having been verified as currently effective on the date hereof by Registrar Corp.

DUNS® Number: 03-108-1015
Labeler Code: 42288
Registrant Contact: Registrar Corp
144 Research Drive, Hampton Virginia, 23666 USA
Telephone: +1-757-224-0177 • Fax: +1-757-224-0179

Registrar Corp will confirm that such registration remains effective upon request and presentation of this certificate until the expiration of one year from the date hereof, unless terminated after issuance of this certificate. Registrar Corp makes no other representations or warranties, nor does this certificate make any representations or warranties to any person or entity other than the named certificate holder, for whose sole benefit it is issued. Registrar Corp assumes no liability to any person or entity in connection with the foregoing. Registration of a drug establishment or drug wholesaler, or assignment of a registration number, or assignment of a NDC number does not in any way denote approval of the firm or its products by the U.S. Food and Drug Administration. Any representation that creates an impression of official approval because of registration or possession of registration number or NDC number is misleading and constitutes misbranding. The U.S. Food and Drug Administration does not issue a certificate of registration, nor does the U.S. Food and Drug Administration recognize a certificate of registration. Registrar Corp is not affiliated with the U.S. Food and Drug Administration.

Registrar Corp
144 Research Drive, Hampton, Virginia, 23666, USA
Telephone: +1-757-224-0177 • Fax: +1-757-224-0179
info@registrarcorp.com • www.registrarcorp.com

Dated: 12/23/2012

[Signature]
Russell K. Statman
Executive Director
Registrar Corp.
## APPENDIX B

### DRUG FACTS

<table>
<thead>
<tr>
<th>Active ingredient(s)</th>
<th>Purpose</th>
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<tbody>
<tr>
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</tbody>
</table>

**Use(s)**
Add to the prevention of dental caries.

**Warnings**

**Do not use**

**Ask a doctor before use if you have**

**Ask a doctor or pharmacist before use if you are**

**When using this product**

**Step used and ask a doctor if**

**If pregnant or breast feeding**
Keep out of reach of children.
Keep out of reach of children under 6 years of age. If more than used for brushing is accidentally swallowed, get medical help or contact a Poison Control Center right away.

**Directions**

**Adults and children 12 years and older**
Brush teeth thoroughly, preferably after each meal or at least twice a day, or as directed by a dentist or doctor.

**Children under 6 years of age**
Brush children under 6 years of age in good brushing and rinsing habits (by minimizing swallowing). Supervise children as necessary until capable of using without a supervision.

**Children under 2 years of age**
Consult a dentist or doctor.

**Other information**

**Inactive ingredients**
WATER, CALCIUM PHOSPHATE, SODIUM, GLYCERIN, SODIUM LAURYL SULFATE, ANISATTU, B BOX (RELLANA) SEED EXTRACT, SODIUM HYDROXIDE, NATURAL AND ARTIFICIAL FLAVORS, DISSOGRAM EDTA, CELLULOSE GUM, SODIUM LAURILSE, TETRASODIUM PYROPHOSPHATE, FD AND C BLUE NO. 1
APPENDIX C

University of Illinois
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 972)
3651 Administrative Office Building
1727 West York Street
Chicago, Illinois 60612-7327

Approval Notice
Continuing Review

January 17, 2014

Benjamin Belavsky
Orthodontics
801 S. Paulina Ave
M/C 841

RE: Protocol # 2013-0113
“Effectiveness of Plaque Removal of Two Different Toothpastes”

Dear Dr. Belavsky:

Your Continuing Review was reviewed and approved by the Expedited review process on January 16, 2014. You may now continue your research.

Please note the following information about your approved research protocol:

Approved Subject Enrollment #: 100 (7 enrolled to date)
Additional Determinations for Research Involving Minors: These determinations have not been made for this study since it has not been approved for enrollment of minors.
Performance Sites: UIC
Sponsor: None
Research Protocol(s):
   a) Protocol "Effectiveness of Plaque Removal," Version 4, 10/1/2013
Recruitment Material(s):
   a) Study Debrief Sheet "Effectiveness of Plaque Removal" Version 1 - 2/1/13
   b) Flyer "Effectiveness of Plaque Removal," Version 4 - 10/1/13
   c) UIC Event Calendar Ad "Effectiveness of Plaque Removal", version 3 - 10/1/13
Informed Consent(s):
   a) Alteration of Informed Consent granted under 45 CFR 46.116(d) for screening and deception
   b) Waiver of Documentation of Signed Informed Consent granted under [45 CFR 46.117 (c)] for screening
   c) Subject Information Sheet "Effectiveness of Plaque Removal," Version 4 - 10/1/13
   d) Consent "Effectiveness of Plaque Removal," Version 3 - 9/20/13

Phone 312-996-1711   http://www.uic.edu/depts/oocr/OPRS/   FAX: 312-413-3929
Your research meets the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific categories:

(1) Clinical studies of drugs and medical devices only when condition (a) or (b) is met.
   (a) Research on drugs for which an investigational new drug application (21 CFR Part 312) is not required. (Note: Research on marketed drugs that significantly increases the risks or decreases the acceptability of the risks associated with the use of the product is not eligible for expedited review.
   (b) Research on medical devices for which (i) an investigational device exemption application (21 CFR Part 812) is not required; or (ii) the medical device is cleared/approved for marketing and the medical device is being used in accordance with its cleared/approved labeling.

(2) Collection of data from voice, video, digital, or image recordings made for research purposes.

(7) Research on individual or group characteristics or behavior (including but not limited to research on perception, cognition, motivation, identity, language, communication, cultural beliefs or practices and social behavior) or research employing survey, interview, oral history, focus group, program evaluation, human factors evaluation, or quality assurance methodologies.

Please note the Review History of this submission:

<table>
<thead>
<tr>
<th>Receipt Date</th>
<th>Submission Type</th>
<th>Review Process</th>
<th>Review Date</th>
<th>Review Action</th>
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<tbody>
<tr>
<td>01/07/2014</td>
<td>Continuing Review</td>
<td>Expedited</td>
<td>01/16/2014</td>
<td>Approved</td>
</tr>
</tbody>
</table>

Please remember to:

- Use your research protocol number (2013-0113) on any documents or correspondence with the IRB concerning your research protocol.

- Review and comply with all requirements on the enclosure, “UIC Investigator Responsibilities. Protection of Human Research Subjects” (http://trigger.uic.edu/deps/ovcr/research/protocolreview/irb/policies.0924.pdf)

Please note that the UIC IRB has the prerogative and authority to ask further questions, seek additional information, require further modifications, or monitor the conduct of your research and the consent process.

Please be aware that if the scope of work in the grant/project changes, the protocol must be amended and approved by the UIC IRB before the initiation of the change.

We wish you the best as you conduct your research. If you have any questions or need further help, please contact OPRS at (312) 996-1711 or me at (312) 996-0865. Please send any correspondence about this protocol to OPRS at 203 AOE, M/C 672.

Sincerely,

Tricia Eifler, BS, CIP  
IRB Coordinator, IRB #1  
Office for the Protection of Research Subjects
Enclosure(s):

1. Informed Consent Document(s):
   a) Subject Information Sheet "Effectiveness of Plaque Removal," Version 4 - 10/1/13
   b) Consent "Effectiveness of Plaque Removal," Version 3 - 9/20/13

2. Recruiting Material(s):
   a) Study Debrief Sheet "Effectiveness of Plaque Removal" Version 1 - 2/1/13
   b) Flyer "Effectiveness of Plaque Removal," Version 4 - 10/1/13
   c) UIC Event Calendar Ad "Effectiveness of Plaque Removal", version 3 - 10/1/13

cc: Carlotta A. Evans, Orthodontics, M/C 841
VITA

NAME: Benjamin Zalman Belavsky

EDUCATION:
B.S., Integrative Biology, University of Illinois at Urbana-Champaign, Urbana, Illinois, 2009
M.S., Oral Sciences, University of Illinois at Chicago, Chicago, Illinois, 2016
Certificate, Orthodontics, University of Illinois at Chicago, Chicago, Illinois, 2016

AWARDS/SCHOLARSHIPS:
American College of Dentists Student Leadership Award, 2013
Adalbert L. Vlazny Scholarship, 2012
Amy J. Cummins Scholarship, 2011
University of Illinois at Urbana-Champaign Dean’s List, 2006/2007

PROFESSIONAL MEMBERSHIP:
American Association of Orthodontists
American Dental Association
Illinois Society of Orthodontists
Omicron Kappa Upsilon
Chicago Dental Society
Alpha Omega International Dental Fraternity

PUBLICATIONS: