Effectiveness of Plaque Removal Using

Indicating-Dye Toothpaste Versus Traditional Toothpaste

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THESIS

Submitted as partial fulfillment of the requirements
for the degree of Master of Science in Oral Sciences
In the Graduate College of the
University of Illinois at Chicago, 2015

Chicago, Illinois

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ACKNOWLEDGEMENTS

I would sincerely like to thank my thesis committee members for their time and support during this research project. I wish to express my utmost gratitude for their encouragement of my studies and introduction to this profession.

I would like to acknowledge Dr. Budi Kusnoto and Dr. Dale Benjamin for sharing their extensive photographic and computer knowledge. Their assistance was incredibly helpful while pursuing this project.

I could not have completed the digital photo analysis without the hard work and ingenuity of David Franz and Ryan Deaton from the UIC Research Resources Center. Their continued support throughout the process was much appreciated.

I would also like to thank Mr. Paul Sagel and Mr. Michael Rubush from Procter & Gamble. Their kind willingness to assist me with the adaptation of the digital plaque imaging analysis used in this study was crucial, and I am extremely grateful.

In addition, thank you to Wendy Swanson at Johnson & Johnson for donating the toothbrushes used in the experiment.

Finally, my sincere appreciation goes to Dr. Lawrence Hier for developing the idea for this study. All of his enthusiasm, suggestions, and donation of resources made this project possible.

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LIST OF ABBREVIATIONS

3D	Three-Dimensional
BBI	Bonded Bracket Index
DMPI	Distal Mesial Plaque Index
DPIA	Digital Plaque Imaging Analysis
FDA	Food and Drug Administration
FD and C	Food, Drug, and Cosmetic
LED	Light Emitting Diode
NPI	Navy Plaque Index
OHI-S	Simplified Oral Hygiene Index
OPRS	Office of the Protection of Research Subjects
OTC	Over-the-Counter
PHP	Patient Hygiene Performance
PPI	Plaque Percent Index
QLF	Quantitative Light-induced Fluorescence
RGB	Red, Green, Blue
SD	Standard Deviation
UV	Ultraviolet
UIC	University of Illinois at Chicago

SUMMARY

Inadequate oral hygiene practices in the general population lead to gingivitis, periodontitis, and tooth decay. There are numerous oral care products on the market which aim to assist the general public to improve plaque removal efficacy at home, such as disclosing tablets, mouth rinses, and toothpastes containing antimicrobials. Plaque-A-Way™ (TJA Health, Joliet, IL) is a newly developed dentifrice which incorporates a disclosing agent into the formulation. The purpose of this study was to compare the plaque removal efficacy of Plaque-A-Way™ to that of a placebo toothpaste.

There are several methods for measuring plaque in the oral cavity. These include conventional plaque indices, which measure the presence, absence, or amount of plaque in designated tooth locations; planimetry, which maps the outline of plaque and calculates the percentage of coverage; quantitative light-induced fluorescence (QLF), which illuminates the oral cavity with ultraviolet light that results in red auto-fluorescence of plaque deposits; and digital plaque imaging analysis (DPIA), which discloses plaque with fluorescein causing deposits to glow yellow-green when exposed to ultraviolet light followed by a computerized photo analysis.

A total of 35 subjects completed this study. After a period of refraining from the completion of oral hygiene, subjects were asked to brush their teeth with either the test toothpaste (Plaque-A-Way™) or a placebo toothpaste at two separate appointments. No special brushing instructions were given to the subjects. A rinse sequence was completed using fluorescein to disclose any remaining plaque after brushing. An

intraoral photo was captured and analyzed for percentage plaque coverage using custom made DPIA software.

The changes of plaque percentages between appointments for the control and test groups were calculated and there were no statistically significant differences found between the two. This suggests that the use of Plaque-A-Way^{TM} did not result in a significant amount of plaque removal compared to the placebo with the testing protocol used. This may have been due to several limitations to this study including: non-specific brushing instructions, small sample size, inconsistent lighting parameters, and investigator subjectivity during photo processing. Despite inconclusive results obtained from the present study, the test toothpaste (Plaque-A-Way^{TM}) demonstrates potential as a valuable over-the counter (OTC) oral hygiene aid for the general public.

1. INTRODUCTION

1.1 Background

Ineffective dental plaque removal has been shown to cause demineralization, caries, gingivitis, and periodontitis (Loe and Silness, 1963; Ower, 2003; Ferreira and Mendes, 2005). These conditions result 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 for removing plaque. The discrepancy in what patients report versus disease actually found is likely due to a variety of factors beyond intentional misrepresentation by patients. For example, poor oral hygiene skill, restricted dexterity, and a lack of dental knowledge, motivation, and ability to accurately evaluate one's oral status all have negative impacts on plaque removal.

In order to address the problem of ineffective dental plaque removal, there is a need to elevate the level of homecare among patients in the general population. Increasing education and technique instruction is one way to achieve that goal. Improving homecare products themselves may be another effective way to address the problem. This is especially important in populations of minorities, patients having low socioeconomic status, and elderly patients, each of which traditionally have decreased access to professional oral care (Kim et al., 2012). The link between higher socioeconomic status and increased oral health knowledge, positive dental attitudes and behaviors was echoed by Schou and Wight (1994).

There are a variety of methods to study the impacts of oral hygiene on the disease process. Clinical plaque analysis has been performed for decades using several manual indices developed by Ramfjord, Quigley and Hein, Silness and Loe, O'Leary, and Elliott, as well as others (Fischman, 1986). However, reviews of such manual indices, including that of Pretty, et al. (2005), found that "traditional plaque indices are problematic due to their integral nature and their failure to detect small, but potentially clinically relevant changes in plaque area." These procedures are also time consuming, subjective, and invasive to patients.

Alternatively, the digital plaque imaging analysis (DPIA) method introduced by Sagel et al. (2000) makes use of clinical photography and computer software to increase speed of data collection, operator consistency, reproducibility of results, the ability to store data for later use and analysis, and most importantly patient comfort. The disclosing agent used in DPIA, which is fluorescein (FD and C No. 8), has been well-documented for intraoral plaque disclosure (Lang et al., 1972; Cohen et al., 1972). To conduct DPIA, long wave UV light, similar to commercially available black lights and dental curing lights, and commonly used in medical, scientific, and law enforcement applications, is used to excite the fluorescein-incorporated plaque on intraoral structures with enough photographic color separation to be analyzed quantitatively pixel by pixel (Sagel et al., 2000). The efficacy, safety, and reliability of DPIA have been tested thoroughly, and it has become a standard plaque analysis procedure at Procter & Gamble (White et al., 2006; Klukowska et al., 2011).

Despite the availability of existing oral care products to aid patients with removing plaque, such as disclosing tablets or mouth rinses, the inadequacy of patients' oral hygiene technique suggests the need for additional products that are simple, easily accessible, and minimally invasive. Visualization of the location of plaque has the potential to increase patients' awareness and encourage them to be more thorough when performing homecare (Block et al., 1972). Plaque-A-Way™, the test product in this study, incorporates a disclosing agent directly into the toothpaste. The dye in Plaque-A-Way™ is an organic food colorant derived from the plant Annato (*Bixa orellana*) and is registered with the FDA. The dye adheres to plaque and stains it green, providing users with a visual indication of the location of plaque on their teeth to improve brushing efficacy.

1.2 Specific Aims

The purpose of this pilot clinical trial was to compare subjects' plaque removal efficacy with the test toothpaste (Plaque-A-Way™) versus the placebo toothpaste by using digital plaque imaging analysis. We were to determine whether the presence of a visual indicating dye in the toothpaste would cause a difference in the way subjects brush their teeth even if the subjects were not told specifically to brush off the green dye. Ultimately the goal would be to increase patient awareness of existing plaque deposits and, therefore, improve the level of plaque removal during homecare. In addition, a simple and objective method of plaque analysis that can be readily used in a smaller scale clinical setting is presented.

1.3 Null Hypothesis

There is no mean difference in plaque reduction between an indicating dye-containing toothpaste and a traditional non dye-containing toothpaste when subjects are not given specific instructions to brush off the stained plaque.

2. LITERATURE REVIEW

The complex biofilm present in dental plaque has long been recognized as the cause of caries and periodontitis (Loe and Silness, 1963). The American Dental Association (Council on Dental Therapeutics, 1985) defines plaque as “a highly variable entity resulting from the colonization and growth of micro-organisms on the surfaces of the teeth and oral soft tissues and consisting of a number of microbial species and strains embedded in an extracellular matrix.” The bacterial and salivary components of plaque attach to the tooth surface in layers, starting with pioneer species and progressing to a more diverse array of filamentous and anaerobic bacteria (Marsh and Martin 1992). Compared to food deposits and other oral debris, plaque has a specific adherent architecture and cannot simply be rinsed away (Block et al., 1972). Therefore, mechanical removal of plaque has been the cornerstone of oral hygiene practices for centuries. Studies aiming to further the understanding of periodontal disease should evaluate subjects’ oral hygiene status and technique, and performing a plaque index is an important part of that process (Silness and Loe, 1964).

The soft biofilm of plaque becomes calcified over time, making at-home removal increasingly difficult for patients. Therefore, the general aim of oral hygiene protocols is to direct patients to remove as much of the plaque as possible from the teeth on a daily basis. The successful use of a toothbrush or dental floss to reduce soft deposits depends on the awareness, skill, and motivation of the individual patient (Rustogi et al., 1992). A person must be able to identify plaque deposits and visualize potential problem areas where plaque tends to accumulate. Common sites include interproximal contact regions, carious lesions, irregular gingival contours, occlusal fissures, poorly contoured

restorations, and the area around the junction of the teeth with removable or fixed appliances, including orthodontic bands and brackets (Pretty et al., 2005). Effective visual feedback can have a significant positive effect on patients' oral hygiene technique (Godin, 1976).

2.1 Plaque Disclosure

Even before the plaque-associated etiology of periodontal disease was discovered, Skinner (1914) recognized the role of plaque disclosure in improving oral health status. His approach was to stress the importance of prevention versus restoration. By giving patients a visual tool to improve their oral hygiene, he also gave them some responsibility and control over any subsequent outcomes. Later work by Arnim (1963) recognized that plaque accumulation occurred too quickly to be the sole responsibility of the dental practitioner. This realization led, in part, to a more widespread use of disclosing agents as part of an oral care routine.

Because plaque deposits may be difficult for patients (and sometimes even practitioners) to identify clearly, a disclosing agent can be used to stain the soft material, which includes bacteria-related products and pellicle (Fischman, 1986; Marsh and Martin 1992). The use of disclosing agents chairside and at home has now become a common teaching tool to accompany oral hygiene instructions. Baab and Weinstein (1983) suggested that "patients can be taught accurately to recognize and score plaque in their own mouths using a self-instructional format." Indeed self-evaluation is very important for long-term oral hygiene improvement and the prevention of gingivitis and periodontitis (Lindhe et al., 1984; Baab and Weinstein, 1986).

The overall goal of plaque disclosure is to stimulate a change in the patient's behavior in order to observe progression from gingivitis to health (Edwards, 1975). In addition to a thorough explanation of the etiology of periodontal disease and why proper oral hygiene is important, a visual representation made to a patient can be extremely helpful (Lang et al., 1972; Cohen et al., 1972; Block et al., 1972). The ideal characteristics of plaque disclosants were outlined by Edwards and Sullivan (1973), and include (i) the ability to distinguish between plaque and other oral debris, (ii) sufficient contrast with gingival tissues, (iii) no antimicrobial effects if to be used in plaque growth studies, and (iv) non-harmful, convenient, tolerable, and esthetically acceptable to patients. Commonly cited problems with disclosing agents have been that they stain the tooth pellicle in addition to plaque, the color of red-tinted agents such as erythrosine blends in with the gingiva (especially along the gum line), and the stain of the lips and gingiva can last for several hours (Lang et al., 1972; Block et al., 1972). The gingival margin is an especially important area for proper brushing and should not be obscured. The prolonged staining of soft tissues is displeasing to the patient and using the dyes can also be messy for the dental office staff to clean up.

Reviews of dental disclosing agents by Lang et al. (1972), Cohen et al. (1972), Block et al. (1972), and Tan and Wade (1980) outline a wide variety of solutions originating with iodine, mercurochrome, and organic dyes, followed later by erythrosine, fuchsin, Bismarck brown, and fluorescein. Erythrosine (FD and C Red No. 3) was introduced as a disclosing agent by Arnim (1963) and has become a mainstay of plaque research (Podshadley and Haley, 1968; Warren et al., 1977). Erythrosine is also the

main ingredient in the popular GUM Red-Cote® disclosing tablets and liquid commonly used in clinical trials and for patient education. Similarly to erythrosine, fuchsin stains plaque a strong red-magenta color. However, both erythrosine and fuchsin have been plagued by claims of potential carcinogenicity (Lang et al., 1972; Fischman, 1986).

A two-tone dye was developed by Block et al. (1972), which combines erythrosine and fast green (FD and C Green No. 3) to create a bluish disclosant. This combination is unique in that thinner, newer plaque coverage is stained red, while thicker, older bacterial colonies are stained blue. Block's study claims that the blue color is more distinguishable to patients and there are no issues concerning persistent staining. The study also proposed that this method would be helpful in studying the bacterial composition of different regions and stages of plaque accumulation.

Kieser and Wade (1976) compared the use of food colorings to traditional disclosants and suggested that the food colorings would be more cost-effective, have better taste properties, and possibly have less concerns with carcinogenicity. Kieser and Wade found the food colorings to have similar ability to stain plaque as the other agents mentioned above. They also determined that colors in the blue range were more effective than other colors because of the increased contrast between disclosed plaque and the gingiva.

The use of fluorescein (FD and C Yellow No. 8) as a disclosant was first introduced by Brilliant in 1971 (Cohen et al., 1972). Fluorescein is similar in chemical structure to erythrosine. However, due to its pale yellow color under visible light, it has a lower propensity for unpleasant staining of the oral cavity. When exposed to UV light,

the fluorescein-stained plaque glows bright yellow-green against darker plaque-free tooth and gingival structures. The Plak-Lite® system (Brilliant Enterprises, Inc., Philadelphia, PA) using fluorescein as a disclosing agent was evaluated by Lang et al. (1972). The illumination was in the range of 420-560nm which targets the peak absorption spectrum of fluorescein. The study evaluated subjects via the Silness and Loe plaque index method (1964), and compared disclosure with fluorescein and the Plak-Lite® system to a more traditional disclosure with erythrosine. In addition to less residual visible staining, fluorescein was also reported to have a more pleasing taste than erythrosine and was found to be a more specific disclosant because it does not adhere to the pellicle like erythrosine. Unlike erythrosine, fluorescein also has the added benefit of not being antibacterial, which would make it more acceptable for use in long-term plaque growth studies. Another study comparing plaque disclosure using fluorescein and erythrosine by Cohen et al. (1972) also proposed that disclosure with fluorescein was more successful and possibly more preferred by subjects due to better taste, more vibrant visual disclosure, and less objectionable staining of the oral cavity.

Silva et al. (2004) compared DentPlaque (Axis Biotec, Brazil), a product which incorporates a disclosing agent directly into toothpaste (although not currently available in the United States), to traditional disclosing tablets. The study concluded that while the tablets produced better plaque disclosure than the toothpaste, subjects preferred the taste of the toothpaste over the disclosing tablets and were, therefore, more motivated to use the product. The direct dispensing of a disclosing agent from a toothbrush has been proposed in United States Patent No. 6371674 (Lerner, 2002), but such a product

does not currently appear to be on the market. Finally, Miranda et al. (2014) compared the use of certain pre-brushing mouth rinses containing disclosing agents, but such rinses showed no difference in plaque removal versus a placebo containing just water.

2.2 Conventional Plaque Indices

An increased focus on the identification and quantification of dental plaque both for research purposes and patient education began in the 1950s (Podshadley and Haley, 1968). The need arose to consistently identify plaque in order to study the components of its biofilm as well as its removal efficacy by oral care products, such as toothbrushes, toothpastes, and mouth rinses. As a result, numerous conventional plaque indices have been developed and tested over the years, as outlined in reviews by Mandel (1974) and Fischman (1986). Recommended requirements of an index outlined by Davies (1968) included (i) ease of use with a large population while minimizing time and cost, (ii) clear and reproducible criteria, (iii) suitability for statistical analysis, and (iv) consistent sensitivity across the designated scale with distinction of disease progression. In general, plaque indices attempt to quantify plaque deposits either by defining and scoring coverage zones, or measuring the actual thickness or volume of the soft debris itself (Fischman, 1986). Ideally, the information gained through plaque analysis can be rapidly translated into education and treatment options for a patient (Silberman et al., 1998).

One of the first references to a plaque index in the literature was by Ramfjord (1959). Modified by Shick and Ash (1961) and eventually used as an adjunct with the Periodontal Index (Ramfjord, 1967), Ramfjord's index utilizes a scoring system that

accounts for presence of plaque along the gingival margins and interproximal surfaces of six selected teeth stained with Bismarck brown solution. The scores range from 0 (absence of plaque) to 3 (plaque covering more than two-thirds of the selected site). The site scores are then averaged to derive the overall score. Using a subset of representative teeth allows for quick scoring by an examiner for research purposes, but it may not be the best method for patient education.

Another plaque index commonly used for research purposes was created by Quigley and Hein (1962) and modified by Turesky at el. (1970). Subjects were instructed not to brush their teeth for three days prior to evaluation to increase the presence of biofilm. Plaque disclosure was accomplished with fuchsin and photographs were taken to create a permanent record of the data. The tooth surface subdivisions are slightly different from Ramfjord's index, and there is a longer scale from 0 (no plaque) to 5 (plaque covering two-thirds or more of the crown). Again, the total score is the mean of the site scores and only selected teeth are scored. Scoring sites on the labial, buccal, and lingual surfaces is valuable for anti-plaque studies which evaluate the efficacy of various oral hygiene aids such as toothbrushes, dental floss and topical anti-plaque agents and dentifrices (Fischman, 1986).

The simplified oral hygiene index (OHI-S) developed by Greene and Vermillion (1964) evaluates all soft debris composed of salivary proteins and food as well as bacterial components. The scoring ranges from 0 (no debris or stain) to 3 (soft debris covering more than two-thirds of the tooth surface). The presence of debris is detected by running an explorer along the non-disclosed surfaces of six teeth (four posterior and

two anterior). Fischman (1986) determined that this index gives inadequate weight to deposits along the gingival margin and was deemed less suitable for study groups with lower plaque levels. However, the developers of the index claim that advantages include less discretionary decision-making by the examiner and reduced completion time (Greene and Vermillion, 1964). Additionally, this index has been useful for assessing oral hygiene education programs (Greene, 1967).

Similar to the OHI-S previously described, a plaque index method developed by Silness and Loe (1964) and later updated by Loe (1967) can be performed without disclosure because it measures soft deposits collected by running a probe over the tooth surfaces. The scoring ranges from 0 (no plaque present visually or on the probe) to 3 (heavy accumulation of visible plaque along the gingival margin and into the interdental area). This index is more difficult to use in larger scale trials because the plaque deposits are disturbed during data collection and therefore unable to be evaluated repeatedly by additional examiners (Fischman, 1986; Pretty et al., 2005).

One of the first indices to take all teeth into account was originated by O'Leary (1967a; O'Leary et al., 1972). His method was prompted by the need for a plaque index that could be applied more easily by practitioners chairside, specifically in the military, as compared to a research setting. O'Leary also intended to encourage "the patient to visualize his own progress in learning plaque control" which subsequently increases patient motivation (O'Leary et al., 1972). Each tooth is scored from 0 (no plaque) to 3 (plaque covering more than one-half of the crown), and only the highest scoring tooth in each segment is fed into the overall mean score. This index has been criticized for

giving too little weight to the gingival margin zone (Fischman, 1986). However, Baab and Weinstein (1983) found this method helpful because of its simplicity and because, in their opinion, it does adequately highlight plaque at the gingival margin as well as interproximally.

The patient hygiene performance (PHP) method developed by Podshadley and Haley (1968) and modified by Martens and Meskin (1972) discloses plaque with erythrosine tablets and evaluates six teeth. A score is given to each of the five subdivisions per tooth and then the mean overall score is calculated. Only two scores per subdivision are possible, 0 (no debris present) or 1 (debris definitely present). The PHP was found to be more sensitive interproximally than the OHI-S when evaluating oral hygiene aids (Anaise, 1977) and is useful for patient education (Silberman et al., 1998).

The Navy Plaque Index (NPI) was developed by Elliot et al. (1972) and modified by Rustogi et al. (1992). Plaque disclosure is carried out using fuchsin. Instead of a score range, however, the presence of plaque in an individual zone is given a score of 1 and then all the zones are aggregated to give a final score. There are more designated zones adjacent to the gingival margin compared to the rest of the tooth surface. Therefore, unlike several of the previously described indices, the NPI more adequately considers the importance of plaque along the gingival margin by giving a higher weight to deposits located there (Fischman, 1986).

The distal mesial plaque index (DMPI) originated by Cancro in 1983 and outlined by Fischman (1986) uses more detailed subdivisions and is more time consuming than

other indices. However, it is highly useful for clinical trials related to oral care product testing due to the attention paid to the gingival margin and interproximal areas. Scores range from 0 (absence of plaque) to 3 (plaque covering the entire area).

Numerous additional modifications to the concept of conventional plaque indices have evolved over the last several decades including the hygiene analysis index (Love et al., 1975), global plaque index (Benson et al., 1993), plaque assessment scoring system (Butler et al., 1996), index of oral cleanliness (Bearn et al., 1996), and the University of Mississippi oral hygiene index (Silberman et al., 1998). However, despite these repeated attempts at redefining evaluation zones to improve plaque evaluation, no consensus has been reached in the literature on the best one to use.

There are fewer examples in the literature of plaque indices devised specifically for orthodontic patients. The bonded-bracket index (BBI) developed by Ciancio et al. (1984) is a system that specifically assigns subdivisions of plaque assessment to the teeth, gingiva and orthodontic brackets. Scores range from 0 (no plaque on the bracket or tooth surfaces) to 5 (plaque on tooth, bracket and extension to gingiva). A recent systematic review by Al-Anezi and Harradine (2012) determined that the most common plaque index used for evaluating orthodontic subjects is that of Silness and Loe, but that newer techniques such as planimetry and DPIA would increase validity and reproducibility over traditional indices (although the latter are more technically complex).

The conventional plaque indices described above have been considered complicated, time-consuming, cumbersome and tedious (Butler et al., 1996; Silberman et al., 1998). Other limitations of such plaque indices involve inherent difficulties in

appropriately weighting certain critical areas of the tooth surface, such as the gingival margin. Some indices fail to show a clear change in score even though the amount of plaque has been significantly reduced. Indices are also somewhat subjective and require specific training and strict calibration of all examiners (O'Leary, 1967b; Greene, 1967; Bentley and Disney, 1995; Shaloub and Addy, 2000). This makes it more difficult to conduct larger scale and multicenter trials consistently because often intra-examiner consistency is better than inter-examiner agreement (Shaloub and Addy, 2000). Conversely, methods of analyzing plaque that increase precision, objectivity, and reliability would potentially allow a reduction in time and number of study participants in clinical trials (Pretty et al., 2005). In addition, many of the plaque indices appear more suited for epidemiological studies and not necessarily practical for clinical trials or oral hygiene evaluation in a private practice setting (Warren et al., 1977; Butler et al., 1996; Silberman et al., 1998). Because different indices measure subdivisions that are constructed in different ways, it can be very difficult to accurately compare them to each other to determine which is most valid or useful (Poulsen et al., 1979).

2.3 Planimetry

Planimetric analysis involves the calculation of a Plaque Percent Index (PPI) (Lang et al., 1972), which is accomplished by disclosing the plaque, taking a photo, tracing and determining the area covered by plaque and then dividing by the total tooth area. Planimetric analyses differ from traditional plaque indices because the areas of plaque deposits are mapped and quantified, either manually or with the aid of a computer, making the use of interval rather than ordinal scale data analysis possible

(Pretty et al., 2005). One key advantage of planimetry over traditional plaque indices is that in planimetry, a photographic record is captured and saved, such that the same sample may be used for repeated or future analysis (Addy et al., 1999). This is more favorable than conventional plaque indices where the disclosed plaque sample may be disturbed or degraded during data collection, and where such sample is only available while the subject is present. Planimetry is still time-consuming. However, it has potential as a highly efficient and reliable method with increased objectivity, reliability, precision, and sensitivity when compared to traditional plaque indices (Soder et al., 1993; Pretty et al., 2005). A study by Renton-Harper et al. (1999) compared previously calculated plaque coverage area percentages to one of the most commonly used conventional plaque indices and confirmed that planimetric areas can also be reliably converted to other indices for research purposes.

2.4 Quantitative Light-induced Fluorescence

Quantitative light-induced fluorescence (QLF) is another method that has applications both in research and patient education. Initially used to identify carious lesions and tooth decalcification, QLF with blue light at 408nm has since been shown to effectively highlight plaque deposits by causing them to appear red-orange against the yellow-green tooth background (Amaechi and Higham, 2002; Raggio et al., 2010). The obligate anaerobic bacteria contained in mature plaque are responsible for the red auto-fluorescence. QLF has been determined to be a reliable method for identifying plaque deposits targeted for removal by practitioners, and it may potentially be used for patient education (Pretty et al., 2005). Advantages include ease of intraoral use, reduced

distortion and reflection, and advances are being made to increase positional reproducibility.

2.5 Computerized Plaque Imaging

Based on the method developed by Sagel et al. (2000), digital plaque imaging analysis (DPIA) makes use of fluorescein to disclose plaque deposits. When exposed to long wave UV light, fluorescein is a bright yellow-green, which contrasts with the surrounding tooth structure which appears darker and blue in color. An intraoral photo of the properly illuminated subject is captured and fed into a computer program that classifies each pixel in the image as plaque, tooth, gingiva, etc., using a least squared distance algorithm. Similar to planimetry, a plaque coverage percentage is calculated automatically and then aggregate sample data may be further analyzed. Computer analysis removes much of the subjectivity that has hindered conventional plaque indices. Drawbacks include insufficient application to posterior and lingual surfaces, and patient positioning reproducibility can be an issue as well (Matthijs et al., 2001; Mohan et al., 2012).

The DPIA method is now used frequently by researchers at Procter & Gamble to test oral care products such as toothpaste and mouth rinses, and a version using illumination with a white light only (non-UV) has been shown to be effective as well (Bellamy et al., 2008). Klukowska et al. (2011) has successfully adapted the DPIA method for studying orthodontic patients which is more complicated due to the presence of fixed appliances on the teeth.

Other contemporary plaque imaging techniques were created by Smith et al. (2001) and Carter et al. (2004) in an attempt to reduce inter-operator error. Their procedures followed a similar progression as Sagel's original DPIA method (i.e., plaque disclosure, digital photo capture, software manipulation and computerized pixel analysis). The main differences among them are that Smith used erythrosine and Carter used methylene blue as the disclosants instead of fluorescein, and both Smith and Carter incorporated white lighting versus the UV illumination used by Sagel.

In any event, these digital plaque assessment methods all attempt to better quantify a percentage of total plaque coverage as compared to using tooth subdivisions to assign ordinal scores as in traditional manual plaque indices. Quantifying a percentage of total plaque coverage is important because with the conventional index scoring methods, the same score may be obtained with different total amounts of plaque present. In addition, teeth of different sizes may be assigned different scores despite having the same amount of total plaque present (Carter et al., 2004).

2.6 Oral Hygiene Products for Plaque Removal

Commonly available over-the-counter (OTC) oral hygiene aids used for mechanical plaque removal include toothbrushes, dental floss, disclosing tablets, rubber tips, interdental picks and proxabrushes (Warren and Chater, 1996; Schiff et al., 2006). Examples of chemical agents used to reduce dental plaque include toothpastes, mouth rinses, and topical gels or foams which often contain antimicrobials such as fluoride, oxygenating agents, anti-attachment agents, and non-ionic agents like triclosan (Gaffar et al., 1997).

3. MATERIALS AND METHODS

3.1 Institutional Review Board Approval

This study was approved by the University of Illinois at Chicago (UIC) Institutional Review Board, Office of the Protection of Research Subjects (OPRS), on February 14, 2013, IRB Protocol #2013-0113 (Appendix A).

3.2 Study Design

This was a pilot study to compare the effects on dental plaque removal of a test toothpaste versus a placebo. From here on, the test toothpaste is defined as Plaque-A-Way™ and contains a green disclosing dye. The placebo toothpaste has the same basic formulation as the test toothpaste. However, it does not contain the green disclosing dye and is white in color. The participants were divided into two groups, a control (Group A) and an experimental group (Group B). A crossover design shown in Table I was used where the control group brushed with the placebo at both Appointment 1 and Appointment 2, and the experimental group brushed with the placebo toothpaste at Appointment 1 and then the test toothpaste at Appointment 2. The subjects were asked to perform toothbrushing in their customary way. When applying the test toothpaste No instruction was given to brush off the green dye on their teeth surfaces. Statistics were then used to compare the two study groups to each other at both Appointment 1 and Appointment 2, and also compare the individual groups between appointments.

TABLE I

STUDY CROSSOVER DESIGN

Group	Appointment 1 Toothpaste	Appointment 2 Toothpaste
Control (Group A)	Placebo	Placebo
Experimental (Group B)	Placebo	Test (Plaque-A-Way^{TM})

3.3 Inclusion and Exclusion Criteria

Inclusion criteria were English-speaking adults 18 years of age or older; in good general health (via self-assessment); with all 12 anterior teeth present (canine to canine in both dental arches); and able to commit to two 30 minute visits. Exclusion criteria were pregnant or nursing subjects; dental students and clinical faculty or staff; antibiotics taken within two weeks of the data collection appointments; symptoms of dry-mouth or significant food allergies; dental restorations or fixed appliances of any kind in the anterior region (canine to canine in both dental arches); visible caries or staining present in the anterior region; and new restorations or prophylaxis anywhere in the oral

cavity within 30 days prior to the first appointment or planned within the duration of study participation.

3.4 Subject Enrollment

Subjects were recruited from around the campus through flyers and an advertisement posted in the UIC online event calendar. The inclusion and exclusion criteria were assessed of interested participants in person or via phone to confirm general eligibility. Eligible participants were then scheduled for the first data collection appointment and given pre-appointment instructions via phone or email. Subjects were instructed to refrain from brushing, flossing, or using other oral hygiene aids and chewing gum the evening prior and morning of their scheduled data collection appointment.

A key code list was created with randomized order subject identification numbers divided into the two study groups, control and experimental. Qualifying subjects were assigned to one of the two groups by adding the subject's name to the next random subject number on the key code list during study enrollment. A total of 37 subjects were enrolled in the study and assigned subject numbers. Two subjects (Subjects 19 and 31) were dropped due to failure to be present at the first appointment. Thirty-five subjects completed both appointments and were included in the data analysis. The control group consisted of 18 participants and the experimental group consisted of 17. The sample consisted of 24 females and 11 males with ages ranging from 18 to 61 years.

3.5 Data Collection Outline

3.5.1 Appointment 1

Subjects reported to the Orthodontics Department Clinic, Room 131, where they were provided with a subject information sheet and informed consent form, and any questions were answered. A signed copy of the informed consent form was retained for documentation purposes. Inclusion and exclusion criteria and completion of pre-appointment instructions were verified verbally and visually. At the first appointment all subjects in both study groups were asked to brush their teeth using the placebo toothpaste for one minute. Brushing took place at the hallway brushing station in front of the mirror. The subjects were then escorted to an administrative office and oriented to the image capture steps, cheek retraction, and head positioning with a brief demonstration and practice. Next they were asked to complete the disclosing sequence with fluorescein and phosphate buffer. The subjects were then positioned in the chin rest with cheeks retracted and teeth slightly apart, the room lights were turned off, and a frontal intraoral image of the upper and lower teeth was captured.

3.5.2 Appointment 2

At the second appointment, the brushing, disclosing, and photographic procedures above were repeated. The only difference was that the control group was instructed to brush with the placebo toothpaste again and the test group brushed with the test toothpaste (Plaque-A-Way^{TM}) instead of the placebo. Immediately following the intraoral photograph, there was a study debrief session where the subjects were informed of the special dye in the test toothpaste, its purpose in the study, and whether

they used it or not. They were given the opportunity to withdraw their photographs if they wished. They were also permitted to brush their teeth again if any of the green toothpaste or fluorescein remained.

3.6 Technical Details

3.6.1 Brushing Sequence

Subjects were provided with a manual toothbrush and a 1 mL single-use syringe of the assigned toothpaste. The specific brushing instructions were: “Brush your teeth in the mirror for one minute.” The process was timed with a stopwatch, but there was otherwise no intervention by the investigator.

The test toothpaste, Plaque-A-Way^{TM}, is an FDA registered product which contains a yellow-orange vegetable dye made from Annato (*Bixa orellana*) seed extract, plus FD and C Blue No. 1, giving the toothpaste a green color that adheres to intraoral plaque deposits. A sample intraoral photo of Plaque-A-Way^{TM} in use is shown in Figure 1. The Drug Facts are contained in Appendix B. The placebo toothpaste is the same base product, but it does not contain the green dye components and is white.

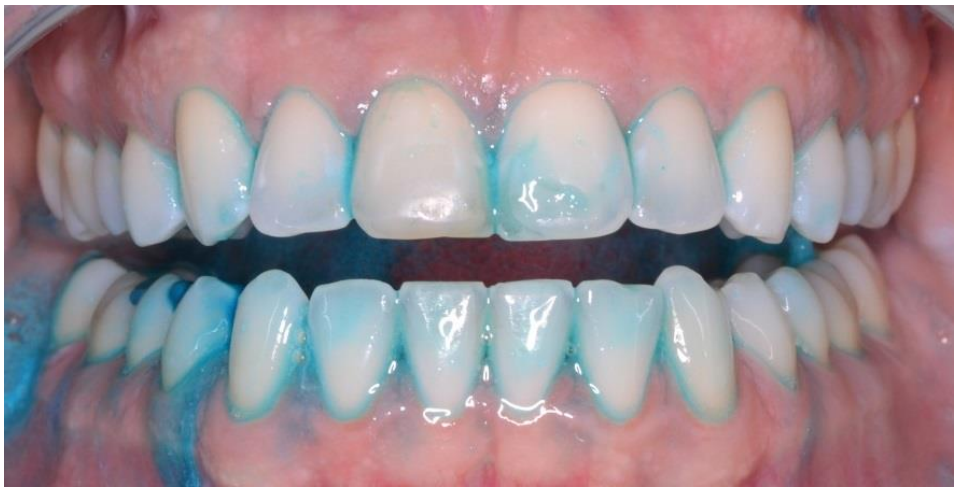


Figure 1. Sample photo of Plaque-A-Way™ disclosing plaque on teeth.

3.6.2 Disclosing Sequence

The disclosing procedure was taken from Procter & Gamble's DPIA experiments (Klukowska et al., 2011). The phosphate buffer was composed of 3.62 g monosodium phosphate and 0.349 g disodium phosphate in 2 L of water, pH 5.5. The disclosing solution consisted of 1240-ppm fluorescein (FD and C yellow No. 8) in phosphate buffer. Subjects were instructed to rinse for 10 seconds with 25 mL of phosphate buffer, rinse for 1 minute with 5.0 mL of 1240-ppm fluorescein in phosphate buffer, and then rinse 3 times for 10 seconds with 25 mL of phosphate buffer. Subjects expectorated the solution after each rinse.

3.6.3 Photographic Setup

The photographic setup was modified from Sagel et al. (2000). The camera used was a Canon EOS Rebel T3 (Canon, Melville, NY). A Tamron 90mm macro lens

(Tamron, Commack, NY) was attached to the camera and a Digi-Slave 3200 LED UV ring flash (SR Electronics, Dallas, TX) was then added to the end of the lens.

The location was an administrative office with the windows blacked out. The photographic setup is shown in Figure 2. Two tripods were placed opposite each other on a desk and the positions marked with tape for consistency. The camera assembly was attached to one tripod. A chin rest for patient positioning was attached to the other. The distance from the end of the ring flash to the anterior edge of the chin cup was measured to be 15 inches.

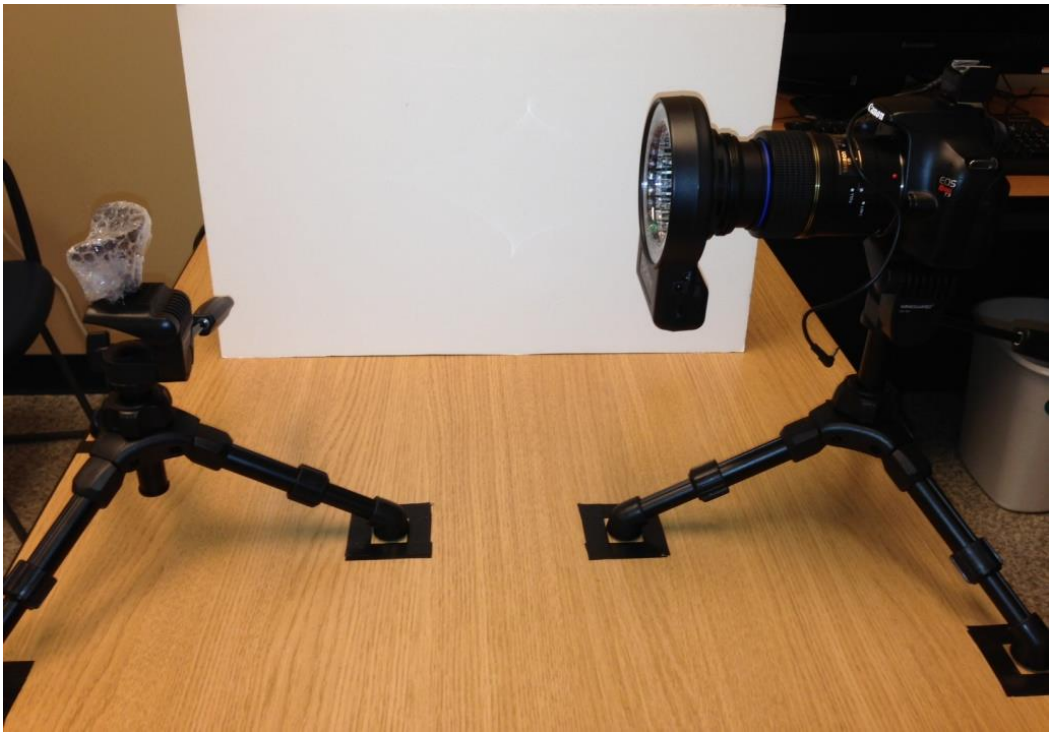


Figure 2. Photographic setup.

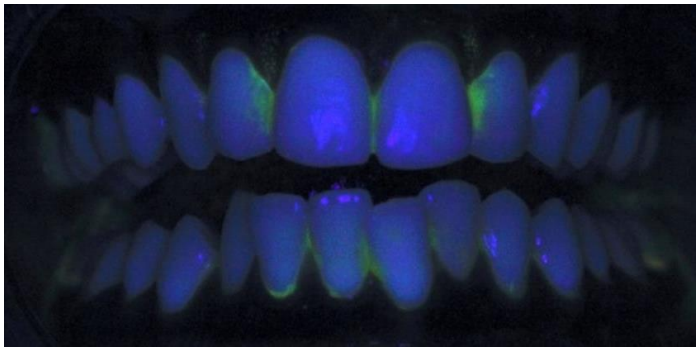
3.7 Data Processing and Analysis

Upon completion of data collection, the subjects' photos were stored under a filename string including only their de-identified subject number, study group, appointment number, and age for sorting purposes. All other personal data used for screening and contact purposes was destroyed.

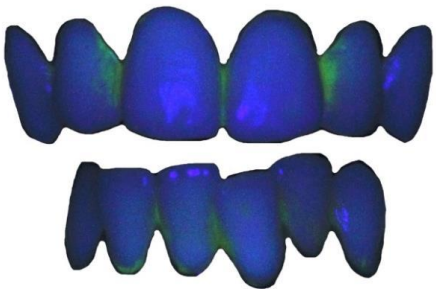
Photos were assessed by a computer analysis method modified from Sagel et al. (2000). The photo processing and upload sequence is shown in Figure 3. Using Photoshop (Adobe, San Jose, CA), the 12 anterior teeth in each intraoral digital photo (Figure 3A) were cropped around the gingival margin and incisal edges using the freeform pen tool and eraser tool to define the area of analysis (Figure 3B).

Data analysis software was developed by David Franz at the UIC Research Resources Center. All cropped digital photos were uploaded into the software program interface shown in Figure 3C.

3A



3B



3C

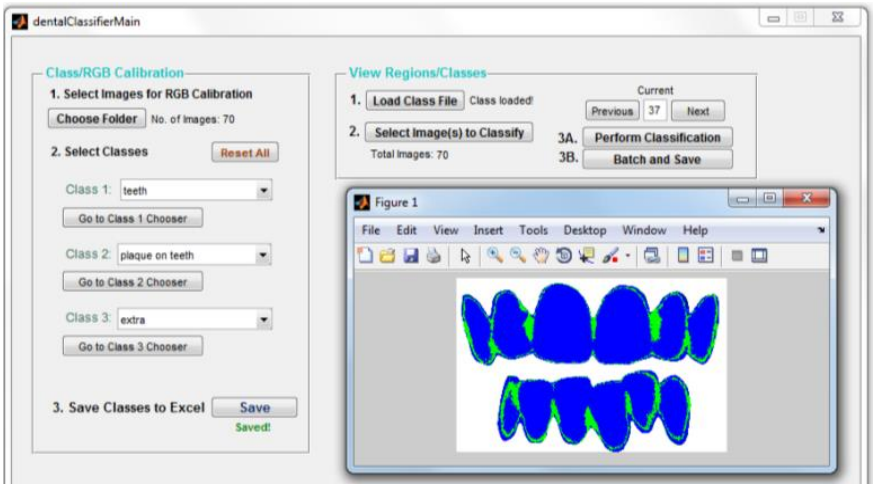
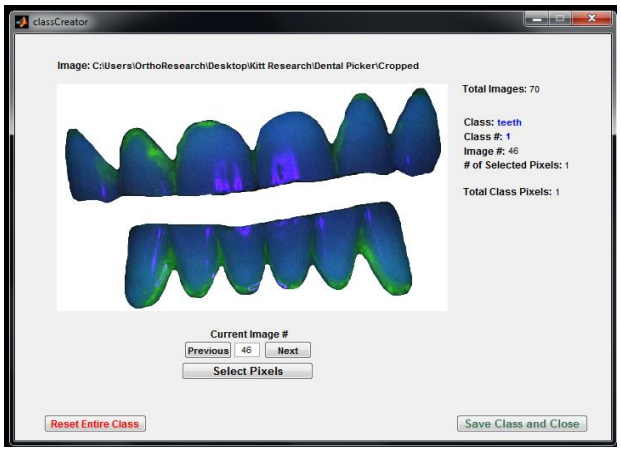


Figure 3. Photo processing and upload sequence.
(A) Uncropped subject photo. (B) Cropped photo. (C) Software interface.

Once the photos were loaded into the software, three classes of pixels were defined (Teeth, Plaque, and Extra) via the process shown in Figure 4. Using the class creator interface shown in Figure 4A, the investigator selected five representative pixels from each photo with the mouse cursor (Figure 4B). This process was repeated for each class which generated the corresponding RGB (red, green, blue) color definitions.

4A



4B

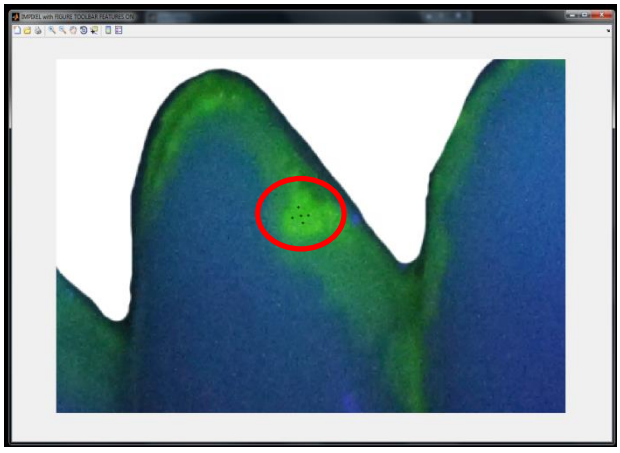


Figure 4. Pixel class definition process.
(A) Class creator interface. (B) Close up of five plaque pixel selections (black dots)

Next, the resulting pixel class definitions were applied by the software to automatically analyze the batch of digital photos, assigning every pixel in each photo to one of the three classes based on the RGB least squared distance color space algorithm presented in Sagel et al. (2000). The data output consisted of both a visual representation for each photo a numerical exportation to Excel (Microsoft, Redmond,

WA). The pixels designated as Extra were subtracted from the total number of pixels to isolate only the area inside the gingival margin. The ratio of (Plaque pixels)/(Plaque + Teeth pixels) x 100% was calculated to give an overall subject plaque percentage.

3.8 Statistical Analysis

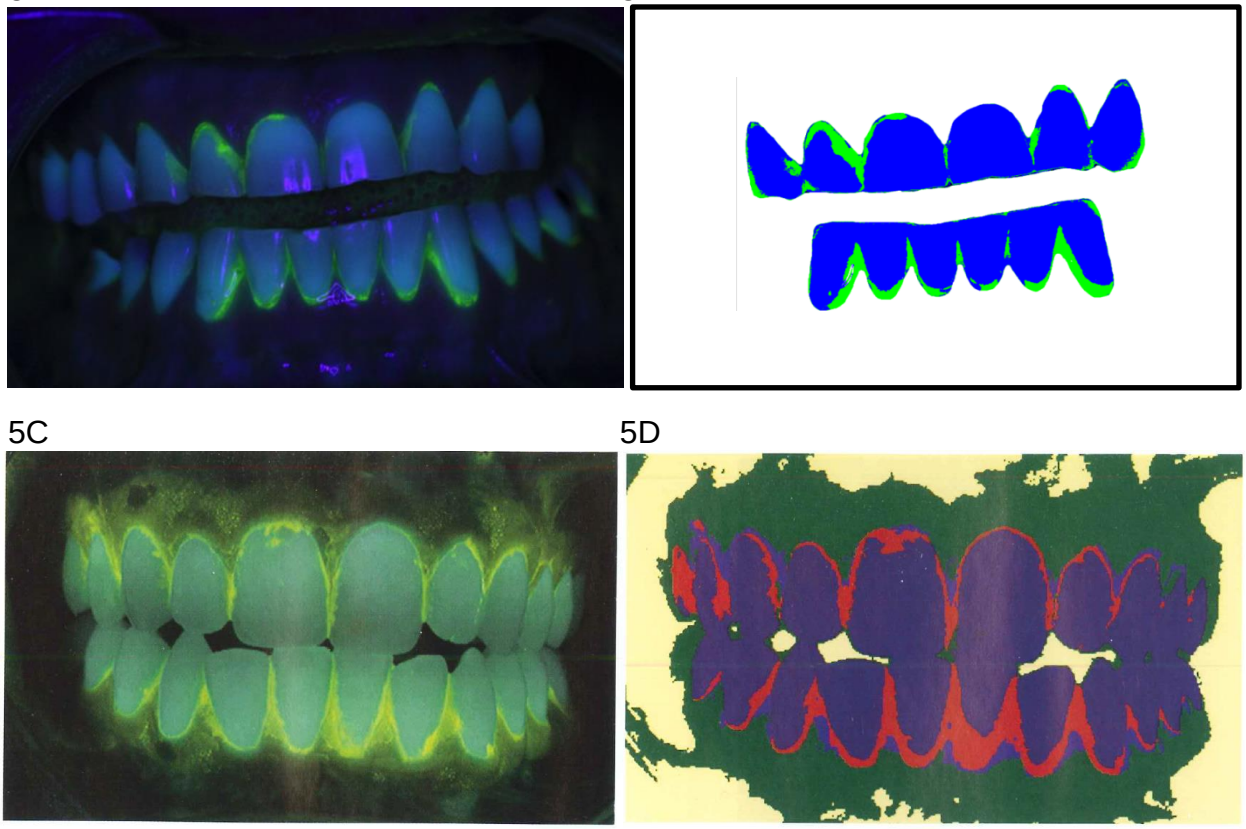
Student paired *t*-tests were performed to test the mean paired differences within each group and independent *t*-tests were used to test the mean differences between groups. Statistical significance was set at $p<0.05$. SPSS version 22.0 (Chicago, IL) was used for data analysis.

4. RESULTS

4.1 Photographic Analysis

There were 70 digital photographs used for analysis, 35 from Appointment 1 and 35 from Appointment 2. The photos were downloaded from the camera onto a desktop computer and each saved with a unique de-identified filename that included subject number, study group, appointment number, and age. The image of the anterior teeth in all of the photos were then cropped using the computer program Photoshop (Adobe, San Jose, CA). The cropped photos were loaded into the custom DPIA software, class definitions were created, and then pixel composition analyzed. The complete Excel data output summary from the present experiment is shown in Table II. A sample photo from the present study and the corresponding visual representation of the classification output were compared to the work of Sagel et al. (2000) and displayed in Figure 5. Copyright permission to use the reproduced images is contained in Appendix C.

5A 5B



5C 5D

Figure 5. Comparison of photos and corresponding classification output between the present study and the work of Sagel et al.

(A) Sample clinical photo from the present study.
(B) Corresponding classification output of the photo shown in (A) processed via the software in this study.
(C) Sample clinical photo from Sagel et al.
(D) Corresponding classification output of the photo shown in (C) processed by the software used by Sagel et al.

TABLE II

RESULTS OF PIXEL ANALYSIS BY DPIA SOFTWARE

Group	Subj	Age	Sex	Appt	Extra (# Pixels)	Teeth (# Pixels)	Plaque (# Pixels)	Total (# Pixels)	Plaque (%)	Appt	Extra (# Pixels)	Teeth (# Pixels)	Plaque (# Pixels)	Total (# Pixels)	Plaque (%)
A	1	22	F	1	15827	688005	193143	896975	21.92	2	4688	819088	132994	956770	13.97
A	3	24	M	1	24485	938705	390474	1353664	29.38	2	2368	1083018	177273	1262659	14.07
A	6	51	F	1	4952	732141	427063	1164156	36.84	2	3008	820781	325660	1149449	28.41
A	7	20	F	1	6883	1002102	305858	1314843	23.38	2	3042	651284	820342	1474668	55.74
A	8	22	F	1	29473	681517	255310	966300	27.25	2	3687	592883	336147	932717	36.18
A	10	21	F	1	121	868229	205852	1074202	19.17	2	880	896515	99278	996673	9.97
A	11	24	M	1	2263	794567	203165	999995	20.36	2	10326	624716	368973	1004015	37.13
A	15	52	F	1	2447	955451	151962	1109860	13.72	2	38	962417	230502	1192957	19.32
A	16	18	F	1	125	674121	251208	925454	27.15	2	1170	823021	51480	875671	5.89
A	17	59	F	1	777	977659	170672	1149108	14.86	2	211	699861	383066	1083138	35.37
A	22	27	F	1	2212	756086	120439	878737	13.74	2	1550	758272	110884	870706	12.76
A	23	61	F	1	50438	894300	99273	1044011	9.99	2	9626	422041	526871	958538	55.52
A	26	28	F	1	46	864139	261858	1126043	23.26	2	332	969685	100202	1070219	9.37
A	30	24	F	1	6952	889678	212564	1109194	19.28	2	198	966917	144357	1111472	12.99
A	32	48	F	1	62	568965	369893	938920	39.40	2	965	765521	124146	890632	13.95
A	34	43	F	1	6706	759997	161250	927953	17.50	2	1102	751908	129459	882469	14.69
A	35	20	M	1	70355	986117	468298	1524770	32.20	2	8457	1244280	245862	1498599	16.50
A	36	42	F	1	48803	812127	333642	1194572	29.12	2	12563	870666	298673	1181902	25.54

Plaque % = (Plaque pixels)/(Plaque pixels + Teeth pixels) x 100%

TABLE II (continued)

RESULTS OF PIXEL ANALYSIS BY DPIA SOFTWARE

Group	Subj	Age	Sex	Appt	Extra (# Pixels)	Teeth (# Pixels)	Plaque (# Pixels)	Total (# Pixels)	Plaque (%)	Appt	Extra (# Pixels)	Teeth (# Pixels)	Plaque (# Pixels)	Total (# Pixels)	Plaque (%)
B	2	37	F	1	7669	780728	248424	1036821	24.14	2	92	767614	306664	1074370	28.55
B	4	26	M	1	24311	714566	274839	1013716	27.78	2	9390	892431	278267	1180088	23.77
B	5	23	M	1	1458	756433	433594	1191485	36.44	2	0	1	0	1	0.00
B	9	25	M	1	102	856437	160779	1017318	15.81	2	184	955783	46094	1002061	4.60
B	12	25	F	1	1137	726200	198943	926280	21.50	2	1182	705524	222547	929253	23.98
B	13	28	M	1	17545	1009996	216352	1243893	17.64	2	1034	978586	239019	1218639	19.63
B	14	26	F	1	1680	762615	106686	870981	12.27	2	1471	535454	359012	895937	40.14
B	18	54	F	1	23597	616933	570870	1211400	48.06	2	43007	987853	58340	1089200	5.58
B	20	18	F	1	1098	878818	142428	1022344	13.95	2	180	928999	71998	1001177	7.19
B	21	23	M	1	268	1016800	274918	1291986	21.28	2	1598	1219002	86701	1307301	6.64
B	24	50	F	1	1499	591233	255728	848460	30.19	2	961	730520	115850	847331	13.69
B	25	47	F	1	8061	777114	192121	977296	19.82	2	6028	784473	192175	982676	19.68
B	27	30	F	1	10555	917178	219202	1146935	19.29	2	8325	817767	234304	1060396	22.27
B	28	24	F	1	17901	593072	396026	1006999	40.04	2	1478	767055	189076	957609	19.78
B	29	58	M	1	2586	1050454	175667	1228707	14.33	2	4746	706332	468558	1179636	39.88
B	33	32	M	1	2258	890414	254906	1147578	22.26	2	3381	902597	306538	1212516	25.35
B	37	31	M	1	1002	748359	475837	1225198	38.87	2	3386	907106	209588	1120080	18.77

Plaque % = (Plaque pixels)/(Plaque pixels + Teeth pixels) x 100%

4.2 Initial Comparison of Groups

The percentage of plaque coverage remaining between the groups at Appointment 1 after both brushed with the placebo toothpaste was analyzed with an independent t-test and is displayed in Table III. The control group had a mean of 23.25% plaque coverage and the experimental group had a mean of 24.92%. No significant difference was found between the two groups at Appointment 1 ($p>0.05$); therefore, the groups were determined to be similar.

TABLE III

COMPARISON OF PLAQUE COVERAGE (%) BETWEEN GROUPS AT APPOINTMENT 1

Group	N	Mean ± SD	Mean Diff	95% CI		p-value
				Lower	Upper	
Control	18	23.25 ± 8.16	-1.67	-8.10	4.76	0.601
Experimental	17	24.92 ± 10.45				

4.3 Change in Plaque Coverage Within Groups

Paired samples t-tests were used to analyze the mean plaque percentages within each group. The change in plaque coverage between Appointment 1 and Appointment 2 for the control is shown in Table IV. When the subjects brushed with the placebo toothpaste initially at Appointment 1, the mean percentage of remaining plaque was

23.25%. For the same participants, after brushing with the placebo toothpaste at Appointment 2, the mean plaque percentage was 23.19%. There was no significant difference ($p>0.05$) for the control group between the two appointments.

TABLE IV

COMPARISON OF PLAQUE COVERAGE (%) OF THE CONTROL GROUP BETWEEN APPOINTMENTS

Appointment	N	Mean ± SD	Mean Diff	95% CI		p-value
				Lower	Upper	
1	18	23.25 ± 8.16	-0.064	-9.40	9.27	0.989
2	18	23.19 ± 15.17				

The difference in plaque coverage after brushing of the experimental group between Appointment 1 (subjects brushed with the placebo) and Appointment 2 (subjects brushed with the test toothpaste) is shown in Table V. The mean plaque coverage percentage using the placebo toothpaste was 24.92% whereas that using the test toothpaste was 18.79%. The mean plaque percentage difference was -6.12. However, the difference was not statistically significant ($p>0.05$).

TABLE V

COMPARISON OF PLAQUE COVERAGE (%) OF THE EXPERIMENTAL GROUP BETWEEN APPOINTMENTS

Appointment	N	Mean ± SD	Mean Diff	95% CI		p-value
				Lower	Upper	
1	17	24.92 ± 10.45	-6.12	-15.63	3.37	0.190
2	17	18.79 ± 11.57				

4.4 Comparison of Toothpastes

Using an independent t-test, the percentage plaque coverage after brushing at Appointment 2 between the control group using the placebo toothpaste and the experimental group using the test toothpaste were compared and the results are shown in Table VI. The mean percentages of plaque coverage between the control and the experimental groups were 23.19% and 18.79% respectively. While there was less percent plaque associated with the test toothpaste, the difference was not statistically significant ($p>0.05$).

TABLE VI

COMPARISON OF PLAQUE COVERAGE (%) BETWEEN GROUPS AT APPOINTMENT 2

Group	N	Mean ± SD	Mean Diff	95% CI		p-value
				Lower	Upper	
Control	18	23.19 ± 15.17	4.39	-4.93	13.71	0.344
Experimental	17	18.79 ± 11.57				

5. DISCUSSION

5.1 Interpretation of the Results

The study groups could be considered reasonably similar with regards to plaque coverage after Appointment 1 where both groups brushed with the placebo toothpaste because there was no significant difference shown in the mean plaque percentages. Both the control and the experimental groups showed an overall reduction in mean plaque percentage following Appointment 2 (-0.064 and -6.12 respectively). The total difference in remaining plaque between the control and the experimental group after brushing at Appointment 2 was 4.39%. However, neither plaque reduction comparison was statistically significant.

The overall reduction in plaque percentages in both groups may be attributed to several factors, including subjects' awareness of being observed and photographed and familiarity with the study procedures when they repeated the steps at Appointment 2. A study using Plak-Lite® by Friedman et al. (1974) highlights the fact that simply participating in a brushing study calls subjects' attention to the presence of plaque, and can have an impact on the results by altering their awareness or inspiring a more meticulous or frequent brushing technique. The greater overall reduction in plaque percentage of the experimental group vs. the control may also indicate that the presence of the green dye in the test toothpaste did help those subjects identify areas of more plaque accumulation than the subjects that brushed with the white placebo toothpaste the second time. However, the statistical analysis cannot guarantee that it was due to the test effect and not chance in this study.

5.2 Subject Selection

Subjects were selected to represent the general population as best as possible in a comparable manner as studies at Procter & Gamble (Sagel et al., 2000; Klukowska et al., 2011). Major exclusions were to protect minors, pregnant or nursing women, or those subjects not in good health. Dental students and clinical faculty were excluded due to their presumed heightened awareness of oral hygiene. Based on the photographic method involved, a full complement of anterior teeth with no caries, restorations, or major stains was required. The criteria regarding no dental procedures within a month, antibiotics within two weeks and lack of dry mouth symptoms were to prevent significant impact on intraoral plaque during the study.

5.3 Test Toothpaste

The purpose of the green dye that is present in the test toothpaste (Plaque-A-Way™) is to adhere to plaque deposits on the teeth allowing better visualization and therefore encouraging users to improve their brushing efficacy. In the past, plaque disclosure by an indicating dye had only been incorporated into chewable tablets and a mouth rinses in the United States (Cohen et al., 1972; Miranda et al., 2014). Both require an extra step in the oral hygiene routine which takes more time for the user, and they are often messy, leaving the mouth bright pink or purple for some time after use. The green dye in Plaque-A-Way™ is persistent enough to highlight areas of plaque accumulation, but it is easily brushed away and mostly unnoticeable by the end of each brushing session. Because the dye is incorporated into the toothpaste itself, the proper brushing technique and plaque removal lessons learned by users can be accomplished

efficiently every time they brush their teeth. In April 2014, the market name of the test toothpaste was changed to Plaque HD®.

5.4 Brushing Instructions

The intention of the instructions: “Brush your teeth in the mirror for one minute,” was to avoid calling specific attention to the presence of the green dye in the test toothpaste. Initially, we did not want to introduce a psychological reason for subjects to change the way they brushed their teeth between appointments in one group and not the other (Friedman et al., 1974). It was expected that the subjects in the experimental group may notice the change in color of the toothpaste (green vs. white) the second time they brushed, but no questions about it were answered until the debrief session following the completion of the two appointments. In retrospect, the plaque-indicating dye in the test toothpaste is the primary mechanism by how it functions. The visual indication of plaque deposits is what allows users to improve their plaque removal efficacy. Therefore, the presence of the dye need not be avoided in the brushing instructions and should be mentioned either before or during brushing.

Additionally, despite instructing subjects to look in the mirror while brushing, it was noticed after the study started that many subjects were not actually looking in the mirror. Possibly that was not part of their normal brushing routine. For consistency, we decided not intervene or redirect the subjects at the time. However, it can be assumed that if the subjects in the experimental group were not looking in the mirror while brushing, the dye in test toothpaste may not have been as effective as if they had been watching.

5.5 Photographic Setup

The photographic setup was modified from that outlined in Sagel et al. (2000) in order to simplify the necessary equipment, reduce costs, and be more easily replicated in a small-scale clinical setting. We chose a readily available digital camera and lens to simulate what many practitioners would already use for clinical photos. Similar to a non-UV model that many clinicians may be familiar with, a ring flash was chosen instead of the complex flash assembly shown in Sagel's article for its ease of setup by attaching directly to the lens. Batteries were used to supply the ring flash LEDs and after analyzing the photos, the inconsistency in flash intensity was noticed. This was likely due to declining battery power over time. It would have been better to use a wall outlet-based power supply to keep the flash level constant throughout the duration of the study. In addition, although convenient, the ring flash had a tendency to produce a purple halo in the most reflective areas of the teeth and gingiva. This was partially compensated for during pixel analysis by including purple pixels as the Teeth category definition. However, it did introduce a source of inaccuracy. This complication would have been mitigated by using two separate flashes at a 45 degree angle as in the original DPIA method to reduce direct reflection of the flash into the camera.

The subject positioning in the chin rest was difficult to replicate at each appointment. The distance from the rest assembly to the camera was kept constant, but specific head orientation was not exactly the same between subjects or at each visit. The subjects' original photos from Appointment 1 were consulted during positioning for

the second photo at Appointment 2, but using a forehead rest may have provided additional stability.

Because of the UV lighting aspect, manual camera focus had to be employed. This introduced focus inconsistencies that were not apparent until photo processing. One of the photos was not focused well enough for pixel analysis to be performed by the DPIA software (Table II, Subject 5, Appointment 2). Focal depth was also a minor problem since some subjects' arches were more anteriorly tapered than others, which made it difficult to keep the line of all anterior teeth from canine to canine in focus at once. It would be helpful to find a method of using auto focus to improve accuracy and consistency.

5.6 Rinse Components

The use of the sodium phosphate buffer solution was important to regulate subjects' intraoral pH during rinsing. The adsorption of fluorescein into the plaque material is pH dependent (Lang et al., 1972). Keeping the pH close to 5.5 was also critical to avoid initiation of demineralization of the enamel that may occur at a lower pH, potentially causing harm to the subjects' tooth enamel.

Fluorescein's glowing quality when excited by UV light provides improved contrast between tooth structure, plaque, and gingiva versus visible light dyes. However, pooling of the fluorescein solution tends to occur and collection in between the tooth contacts and along the gingival sulcus can falsely indicate the presence of plaque when there is none. This is a drawback to the method we used, and it was not

possible to eliminate this issue in the study procedures and photo analysis. The study by Lang et al. (1972) using fluorescein recommended having subjects rinse with water for 30 seconds following the plaque disclosure step to reduce pooling. That is a potential area for improvement in future studies as long as the presence of disclosed plaque is not excessively diminished.

5.7 Photographic Processing

We found that fluorescein-stained deposits on gingiva and other areas of the oral cavity are difficult to distinguish from those on the teeth during photo analysis. Because the area of interest in this study was plaque on tooth structure, including immediately adjacent to the gingival margin, we decided to crop the photos in order to mask the teeth and remove some of the more ambiguous parts of the photos similar to the method outlined in Klukowska et al. (2011). Areas including the bulk of the gingiva, lips, cheeks, tongue and retractors were eliminated. This was a fairly cumbersome and subjective process using the freeform pen and eraser tools and likely introduced some error. More experience and training with Photoshop or a more efficient tool to crop the photos would increase the integrity of the data.

5.8 Digital Plaque Imaging Software

Once the cropped photos were uploaded into the DPIA software, three classes were created to categorize each pixel: Teeth, Plaque, and Extra. The Extra category was used because the cropping was not an exact process and a small area outside the gingival margin and incisal edges of the teeth was included to be sure all tooth structure

and plaque covered tooth structure was available for data analysis. However, the Extra pixels were not to be used in the plaque percentage calculation, so they were subtracted from the total pixels in the cropped photos during Excel output by the DPIA software (Table II).

In order for the DPIA software to automatically classify every pixel in each photo, the three classes had to first be defined by assigning RGB color profiles. This was a visual process completed by the investigator, which involved cycling through each digital photo and selecting five pixels from each of the three classes in each photo through the DPIA software interface. The pixel selection by the investigator was subjective. Meaning a Teeth pixel appears blue, a Plaque pixel (stained by fluorescein) appears green, and an Extra pixel around the edges appears black. For this study, the most representative pixels of each class were selected despite there being a noticeable gradient of colors to choose from. This introduced error because the class definition exercise could be completed with a variety of RGB color selections, which would each give a somewhat different end result for the plaque percentage calculations. For example, when choosing pixels to represent the Plaque category, if the investigator includes more ambiguous blue-green pixels towards the transition edge of a plaque deposit, the software analysis may result in more pixels being designated as Plaque (plaque-weighted output) compared to a class definition where those same blue-green pixels are chosen to represent the Teeth class instead (teeth-weighted output). To illustrate this aspect of potential subjectivity, the same photo, deliberately re-processed

with plaque-weighted or a teeth-weighted class definition, was compared to the investigator's initial best-representation output (Figure 6).

6A 6B 6C

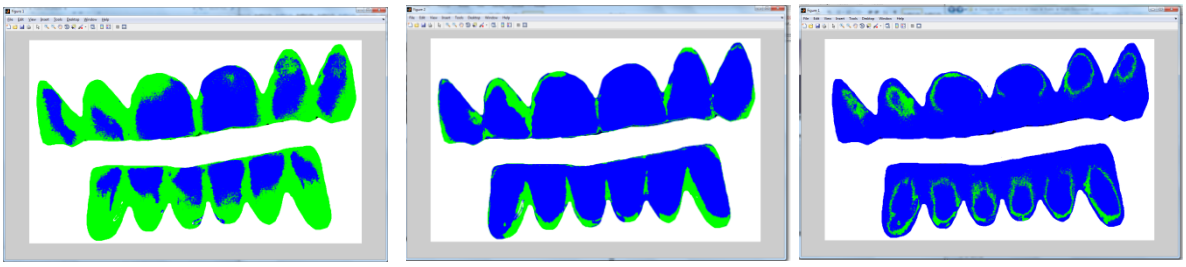


Figure 6. Comparison of differently-weighted outputs.
(A) Plaque-weighted. (B) Best-representation. (C) Teeth-weighted.

In this study, we were comparing changes in plaque percentage between groups and appointments. Therefore, any investigator biases in pixel class definitions would be applied to all photos equally, which we believe reduced the negative impact on the outcomes. Additionally, the visual outputs were compared to the original photos after DPIA analysis to make sure that the processed classification output appeared as close to real-life as possible. It would have been helpful to be able to identify a visually valid RGB color profile for each class and then have another input box in the software where those same RGB values could be entered again for consistency in pixel analysis.

5.9 Limitations of the Study and Future Research

There were several limitations to the present study. First, the sample size was very small which made the ability to show a statistical difference difficult. It is recommended that larger scale clinical studies be conducted to better analyze the clinical potential of the test toothpaste and increase the power of the study. Because the brushing instructions did not highlight the presence of the dye, subjects may not have clearly made the educational connection between the presence of the dye and any remaining plaque that they should brush away. In future studies, the brushing instructions should be modified to reflect the packaging instructions of the test toothpaste. In addition, more intervention should be made by the investigator to direct subjects' attention to the mirror for the entire duration of the brushing sequence.

Regarding the photographic setup and analysis, a power cord should be used for the ring flash in order to maintain lighting consistency. Or possibly investigators could switch to the more powerful and less glare-prone wired flash setup used by Procter & Gamble. More testing should also be done prior to data collection to optimize the photographic environment to ensure the best quality photos are obtained. The digital photo analysis software developed for this study was an excellent entry-level tool. However, time should be spent to increase the software sophistication allowing for more consistent pixel analysis and specific RGB value input for increased repeatability.

It would also be interesting to conduct a future study on orthodontic subjects in order to see if using an indicating dye-containing toothpaste can help patients reduce plaque accumulation around fixed appliances, which is a major problem during

orthodontic treatment. Future studies could also compare the test toothpaste's efficacy and patients' palatability and affinity to that of disclosing tablets or mouth rinses. Regardless of the inconclusive results of this study, the test toothpaste has valuable potential as an educational oral hygiene adjunct for dental patients of all ages.

6. CONCLUSIONS

- The null hypothesis was retained. There was no statistically significant difference in mean plaque reduction between an indicating dye-containing toothpaste and a traditional non dye-containing toothpaste when subjects are not given specific instructions to brush off the stained plaque.
- Digital plaque imaging analysis (DPIA) is a valuable method for analyzing subject plaque accumulation and brushing efficacy. However, more work needs to be done to improve the adapted version of DPIA employed in this study.
- Despite the lack of statistically significant differences between the test toothpaste and the placebo, the test toothpaste does represent a promising educational oral hygiene aid.

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APPENDICES

APPENDIX A

UNIVERSITY OF ILLINOIS
AT CHICAGO

Office for the Protection of Research Subjects (OPRS)
Office of the Vice Chancellor for Research (MC 672)
203 Administrative Office Building
1737 West Polk Street
Chicago, Illinois 60612-7227

Approval Notice
Initial Review – Expedited Review

February 14, 2013

Katharine Stevens, BS, DDS
Orthodontics
801 S. Paulina
M/C 841
Chicago, IL 60612
Phone: (858) 699-6741

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

Dear Dr. Stevens:

Members of Institutional Review Board (IRB) #1 reviewed and approved your research protocol under expedited review procedures [45 CFR 46.110(b)(1)] on February 12, 2013. You may now begin your research.

Please note the following information about your approved research protocol:

Protocol Approval Period: February 12, 2013 - February 12, 2014
Approved Subject Enrollment #: 100 Total
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: Department of Orthodontics
Research Protocol(s):

a) Protocol "Effectiveness of Plaque Removal," Version 1 - 2/1/13

Recruitment Material(s):

a) Flyer "Effectiveness of Plaque Removal," Version 2 - 2/11/13
b) UIC Event Calendar Ad "Effectiveness of Plaque Removal", version 1 - 2/1/13
c) Study Debrief Sheet 'Effectiveness of Plaque Removal" Version 1 - 2/1/13

Informed Consent(s):

APPENDIX A (continued)

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) Consent "Effectiveness of Plaque Removal," Version 2 - 2-11-13
d) Subject Information Sheet "Effectiveness of Plaque Removal," Version 2 - 2/11/13

Your research meets the criteria for expedited review as defined in 45 CFR 46.110(b)(1) under the following specific categories: 1, 6, 7
(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.,

(6) 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 :

Receipt Date	Submission Type	Review Process	Review Date	Review Action
02/05/2013	Initial Review	Expedited	02/12/2013	Approved

Please remember to:

→ Use only the IRB-approved and stamped consent document(s) enclosed with this letter when enrolling new subjects.

→ 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://tigger.uic.edu/depts/ovcr/research/protocolreview/irb/policies/0924.pdf)

Please note that the UIC IRB has the right to ask further questions, seek additional information, 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.

APPENDIX A (continued)

We wish you the best as you conduct your research. If you have any questions or need further help, please contact the OPRS office at (312) 996-1711 or me at (312) 355-1404. Please send any correspondence about this protocol to OPRS at 203 AOB, M/C 672.

Sincerely,

Sheilah R. Graham, BS
IRB Coordinator, IRB # 1
Office for the Protection of Research Subjects

Enclosure(s):

1. **Informed Consent Document(s):**
   a) Consent "Effectiveness of Plaque Removal," Version 2 - 2-11-13
   b) Subject Information Sheet "Effectiveness of Plaque Removal," Version 2 - 2/11/13
2. **Recruiting Material(s):**
   a) Flyer "Effectiveness of Plaque Removal," Version 2 - 2/11/13
   b) UIC Event Calendar Ad "Effectiveness of Plaque Removal", version 1 - 2/1/13
   c) Study Debrief Sheet 'Effectiveness of Plaque Removal" Version 1 - 2/1/13

cc: Carlotta A. Evans, Faculty Sponsor, Orthodontics, M/C 841
Bruce S. Graham, Dean, Dentistry, M/C 621

APPENDIX B

DRUG FACTS

Active ingredient(s) — *Purpose*

Use(s)
Aids in the prevention of dental cavities.

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

Stop use 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 2 years and over	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	Instruct children under 6 years of age in good brushing and rinsing habits (to minimize 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, DICALCIUM PHOSHATE, SORBITOL, GLYCERIN, SODIUM LAURYL SULFATE, ANNATTO (BIXA ORELLANA) SEED EXTRACT, SODIUM HYDROXIDE, NATURAL AND ARTIFICIAL FLAVORS, DISODIUMM EDTA, CELLULOSE GUM, SODIUM SACCHARIN, TETRASODIUM PYROPHOSPHATE, FD AND C BLUE NO.1

APPENDIX C

From: "Rights and Permissions" <permission@karger.com>
Subject: AW: Requested Karger Material
Date: Wed, September 10, 2014 1:30 am
To: "ksteve9@uic.edu" <ksteve9@uic.edu>
Cc: "Meier, Silvia" <s.meier@karger.com>,"Thommen, Moritz" <m.thommen@karger.com>

Dear Dr. Stevens

Thank you for your email. As to your request, I am pleased to inform you that permission is granted herewith to use figure 6 and 7 from the book

Faller RV (ed): Assessment of Oral Health. Monogr Oral Sci. Basel, Karger, 2000, vol 17, pp 130-143 (DOI:10.1159/000061638)

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Thank you for your understanding and cooperation.

Hopefully, I have been of assistance to you with the above.

Best regards,
David Schaub

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