2014

Chemical Dental Plaque Control: Chlorhexidine Tooth Staining and Efficacy of Common Whitening Procedures

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Chemical dental plaque control:

Chlorhexidine tooth staining and efficacy of common whitening procedures

A Thesis Presented

by

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To the Keck Science Department

Of Claremont McKenna, Pitzer, and Scripps Colleges

In partial fulfillment of

The degree of Bachelor of Arts

Senior Thesis in Biochemistry

9 December 2013

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Oral hygiene and its health implications

Oral hygiene has important implications for overall health and well–being. Dental infections are arguably the most common bacterial infections in humans.[1] Tooth decay caused by bacterial infections is one of the most prevalent chronic diseases worldwide. Targeting bacteria in the oral cavity is an effective way of maintaining oral health whether it be through the regular removal of dental plaque by toothbrushing with fluoride toothpaste or using chemical plaque preventative agents such as chlorhexidine mouthrinse.[2] In the absence of proper oral hygiene, oral bacteria counts increase dramatically, which can then lead to tooth decay or even to bacteremia, the introduction of bacteria into the blood stream.[3] It is therefore crucial to maintain healthy oral bacteria levels through good oral hygiene practices.

Dental plaque is the accumulation of bacteria present on the surface of teeth in the form of biofilms. Biofilms form when bacteria selectively bind to the acquired pellicle, the outermost layer of the tooth consisting of adsorbed proteins and other macromolecules found in the oral environment. The pellicle contains components from the diet, saliva, gingival crevicular fluid, blood, bacteria, and mucosa. The acquired pellicle serves many functions, including lubricating the tooth surface to facilitate chewing and speech. It also acts as a semi-permeable barrier between the enamel and the oral environment regulating remineralization and demineralization processes.[4]

Pellicle formation is a highly dynamic process due to adsorption–desorption events, modification of adsorbed molecules by microbial or host enzymes, and intermolecular complexing with other macromolecules. The first stage of pellicle formation occurs when salivary proteins are adsorbed at the enamel surface. Adsorption is attributed to electrostatic interactions between calcium and phosphate ions in the enamel and the charged side chains of proteins. Van der Waals forces and hydrophobic interactions also contribute to the adsorption process. The initial phase of pellicle formation is rapid, occurring immediately upon tooth eruption, and lasts only a few minutes. The second phase is much slower and involves the continuous adsorption of biopolymers from saliva onto the tooth surface. This process is characterized by protein–protein interactions. Both single proteins and protein aggregates participate in this secondary adsorption process.[4]

Plaque is a biofilm formed on the acquired pellicle and consists of a matrix of polysaccharides, proteins, and DNA secreted by cells.[2] Once plaque is established it is characterized by microbial homeostasis.[5] Environmental factors can break down this microbial homeostasis, such as salivary flow and composition, fluoride exposure, and sugars in the diet.[2,6] Disturbance of this microbial homeostasis can lead to dental decay as cariogenic, decay–causing, bacteria levels increase.

Dental decay can cause pain and discomfort, and ultimately can lead to tooth loss. Decay is the localized destruction of dental hard tissues by acidic metabolic by-products of cariogenic bacteria. If decay is advanced, cavitation

occurs.[2] Only a few species indigenous to dental plaque are cariogenic. *Streptococcus mutans* and *Lactobacilli* are two cariogenic bacteria found in dental plaque. These bacteria produce acidic by-products during the fermentation of dietary carbohydrates such as sucrose. This fermentation causes a local pH drop, which can cause demineralization beneath the tooth surface. Between meals, the pH levels return to normal and demineralization is repaired. Remineralization occurs when calcium and phosphate ions present in dental plaque diffuse into the lesion driven by supersaturation of these ions in saliva. When the balance between demineralization and remineralization is disturbed, cavitation occurs.[1]

Diet plays an important role in the prevention of decay. Increased consumption of fermentable carbohydrates leads to more drastic and prolonged drops in pH at the tooth surface. Cariogenic bacteria grow and metabolize best in an acidic environment, and therefore these species are selected for, causing a shift in the plaque population. As the population of cariogenic bacteria increases, more acidic by-products are produced, and the population continues to increase.[1,5] Regular removal of dental plaque prevents the proliferation of cariogenic bacteria on the tooth surface, thereby preventing cavitation and decay. Dental decay is reversible in its early stages presuming that enough of the biofilm can be removed by brushing or with chemical agents.[2]

There exist other health risks associated with poor oral hygiene. Bacteremia, the introduction of oral bacteria into the blood stream, can lead to a host of systemic diseases such as cardiovascular disease, infective carditis, bacterial

pneumonia, low birth weight, and diabetes mellitus. Bacteremia can occur following tooth extraction, endodontic treatment, periodontal surgery, and root scaling. Barriers exist in the oral cavity which prevent penetration of bacteria from dental plaque into tissues. The surface epithelium serves as a physical barrier, antibody-forming cells act as an immunological barrier, and defensins, small host-derived antibacterial peptides, are found in the oral mucosa epithelium. In the absence of proper oral hygiene, oral bacteria counts increase drastically and despite the barriers in place, more bacteria are introduced into oral tissues and eventually into the bloodstream.[3]

Managing bacterial levels in the oral cavity thus has important implications for overall health. Oral bacteria serve as an effective target to manage and prevent oral disease, tooth decay, and systemic infections. Good oral hygiene practices traditionally include regular toothbrushing, although chemical plaque control agents such as chlorhexidine mouthrinses may be available in the future as an alternative method of plaque control. Chlorhexidine has been proven to be highly effective in combatting plaque formation and therefore has potential to be used as a chemical plaque control agent when mechanical tooth cleaning may not be possible or inadequate, such as with children or those with disabilities, which makes brushing difficult.

What is chlorhexidine?

Chlorhexidine is a cationic antiseptic used for chemical plaque control and the prevention of gingivitis (see figure 1).

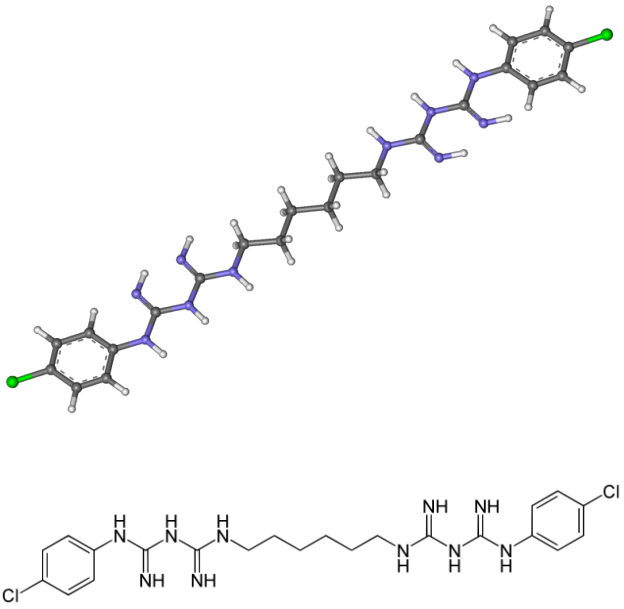

Figure 1. Chlorhexidine's structure.
http://en.wikipedia.org/wiki/File:Chlorhexidine_ball–and–stick.png
http://en.wikipedia.org/wiki/File:Chlorhexidin.svg

Chlorhexidine, which has been studied extensively since as early as the 1950s, is considered the gold standard of chemical plaque control agents.[7] One of the most important *in vivo* studies that highlighted chlorhexidine as a highly effective anti-plaque agent was published by Löe and Schiott in 1970. These authors found that two daily rinses with 10 mL of 0.2% chlorhexidine mouthrinse prevented plaque formation and gingivitis development in the absence of normal

mechanical tooth cleaning. Further, they found that chlorhexidine continued to prevent plaque and gingivitis in the oral cavity up to 24 hours after use[8].

The mode of action of chlorhexidine is purely topical. It does not penetrate the oral epithelium. Even when ingested, it is nontoxic as it is poorly absorbed through the gastrointestinal tract. The small amount that is absorbed is metabolized in the liver and kidney.[7]

Chlorhexidine is effective against a wide spectrum of targets including both gram-positive and gram-negative bacteria. At lower concentrations it has a bacteriostatic effect whereas at higher concentrations it is bactericidal. Specific concentrations at which this occurs depends on the bacterial species. Chlorhexidine is a strong base and is bi-cationic at pH levels above 3.5. Bacterial cells generally have a net negative charge. Being bi-cationic, chlorhexidine is strongly attracted to bacterial cells, adsorbing to phosphate containing compounds in the bacterial cell membrane. Adsorption to the outer membrane increases the permeability of the bacterial cell membrane, and therefore, small molecules such as potassium ions can leak from the cell. At this stage, the effect is bacteriostatic and reversible. At higher concentrations, chlorhexidine causes more damage to the cell membrane. Eventually, the leakage of small molecules subsides as phosphate complexes form in the coagulation and precipitation of the cytoplasm, which is irreversible and lethal to the cell.[7]

Although chlorhexidine is a highly effective antimicrobial agent, the mechanism of action by which it inhibits the formation of plaque remains

unconfirmed as biofilms such as plaque display complex and dynamic structures. It has been posited that chlorhexidine prevents bacteria from colonizing on teeth by

1. inhibiting the formation of the acquired pellicle by binding to the acidic groups of salivary glycoproteins,
2. adsorbing to the extracellular polysaccharides of the tooth in the acquired pellicle, or
3. competing with calcium ion agglutination factors in plaque.[7]

The prevention of biofilm formation has important implications for the prevention of dental decay.

Substantivity, the ability of chlorhexidine to remain effective in inhibiting plaque for an extended period of time, contributes to chlorhexidine's efficacy.[10] Chlorhexidine is maintained in the oral cavity after having been adsorbed onto the tooth surface and oral mucosal surfaces. The dicationic nature of chlorhexidine contributes significantly to its substantivity. The tooth surface has a net negative charge; therefore, chlorhexidine binds strongly to the tooth surface and remains there for upwards of 24 hours after treatment. Saliva also has antimicrobial properties for a period after the use of chlorhexidine.[7]

Intrinsic and extrinsic factors play a role in the substantivity of chlorhexidine. These factors were investigated by Tomás et al. in 2010. Intrinsic factors such as concentration, time of application, and temperature can affect the retention of chlorhexidine in the oral cavity. Concentration, volume, and duration were varied to investigate these intrinsic factors *in vivo*. Results showed the volume of

mouthrinse did not affect substantivity although concentration and duration did. Substantivity was increased with higher concentrations and longer treatment time. Eating, drinking water, chewing sugar-free gum, and smoking a cigarette were extrinsic factors investigated. It was found that the substantivity of 0.2% chlorhexidine decreased significantly with these activities.[10] These findings highlight the importances of dietary etiological factors.

Chlorhexidine staining

Chlorhexidine mouthrinse could act as an alternative form of dental hygiene when toothbrushing is difficult or not possible. However, long term use is not recommended due to extrinsic tooth staining associated with regular chlorhexidine use.[7] Tooth discoloration can take a number of forms, having a variety of causes and etiological factors.

The visible part of the tooth is called the coronal portion and consists of the enamel, dentin, and pulp (see figure 2). Altering one or more of these structures can lead to discoloration. Tooth discoloration can be classified as intrinsic, extrinsic, or internalized, depending upon which structures are affected. Intrinsic staining results from a change in the tooth's structural composition or the thickness of the hard tissues. Intrinsic staining occurs during tooth development and is often caused by metabolic disorders. Extrinsic stains are caused by the discoloration of the tooth surface or the acquired pellicle. Internalized staining occurs when an extrinsic stain becomes incorporated into the substance of the tooth and is most commonly seen in tandem with defects in the enamel or in the porous surface of exposed dentin.[11]

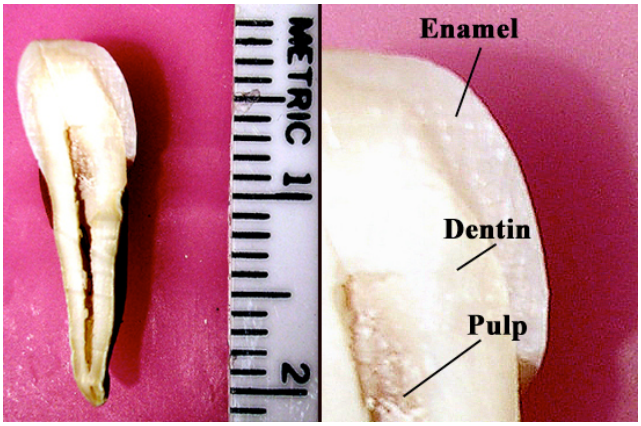

Figure 2. Detail of the coronal portion of the tooth.
http://en.wikipedia.org/wiki/File:Labeledandfulltooth.jpg

Extrinsic staining are more common than intrinsic or internalized staining. Direct extrinsic staining is the result of the acquired pellicle taking up dietary chromagens. Indirect extrinsic staining occurs when a staining agent is adsorbed to the acquired pellicle, though the agent is initially either colorless or has a color other than the color of the resulting stain. Common staining agents include beverages such as red wine and coffee, tobacco, mouthrinses, and metal salts found in prescription medications and dietary supplements. Indirect extrinsic staining is often associated with cationic antiseptics, such as chlorhexidine, and metal salts.[11]

Chlorhexidine staining shows marked variation between individuals. The mechanism by which it stains teeth has yet to be confirmed, due in part to this large variation.[11] Many theories regarding the mechanism of extrinsic chlorhexidine staining have been suggested. It has been proposed for example that extrinsic tooth staining associated with chlorhexidine and metal salts is due to the formation of metal sulfides. It is hypothesized that chlorhexidine denatures proteins in the acquired pellicle by splitting disulfide bridges to

produce reactive sulfhydryl groups which can react with iron or tin ions to produce pigmented products (see figure 3).[7]

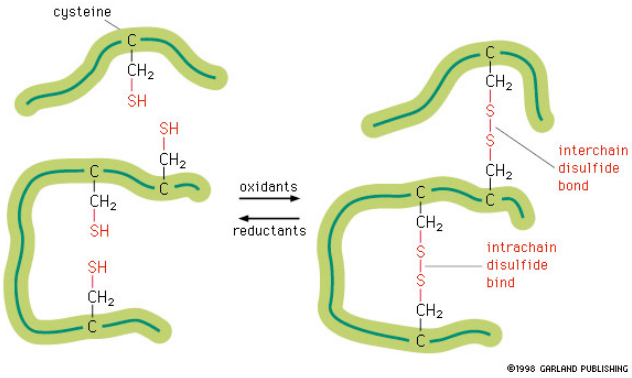

Figure 3. The oxidation of disulfide bridges present in pellicle proteins results in reactive sulfhydryl groups.
http://www.bio.miami.edu/tom/courses/protected/ECBCH05/5_22.jpg

Addy et al. investigated the hypotheses of chlorhexidine staining due to metallic sulfide formation or dietary precipitation in a 1995 *in vitro* study.[12] They sought to determine whether chlorhexidine staining is caused by surface precipitation of dietary chromagens by adsorbed chlorhexidine or if staining is the result of the denaturation of pellicle proteins. Denaturation of pellicle proteins forms reactive sulfhydryl groups, which then react with metallic salts causing brown staining.

To investigate the hypothesis that denaturation of pellicle proteins results in metallic staining, recently extracted human teeth and polymethyl methacrylate blocks were mechanically cleaned and polished removing soft tissue, plaque, and calculus (mineral deposits on the tooth surface), soaked in saliva, and washed in distilled water. Samples were then treated with chlorhexidine or the known denaturants gluteraldehyde or formaldehyde, which acted as positive

controls. All samples were then treated with either ferric or stannous chloride to induce staining. In both the control and chlorhexidine groups, the resulting ferric chloride stain was the same color as the original solution and stannous chloride did not result in staining. There was thus no evidence of a reaction having taken place, and it was concluded that the mechanism of chlorhexidine staining was not due to the initial denaturation of pellicle proteins followed by the formation of metallic sulfides.

The same procedure was used to investigate the role of denaturation in tea staining, treating samples with gluteraldehyde, formaldehyde, or chlorhexidine followed by tea. If chlorhexidine and known denaturants increased the degree of tea staining, the denaturation hypothesis would be supported. Gluteraldehyde and formaldehyde produced no increase in tea staining, though chlorhexidine did. Therefore, protein denaturation followed by dietary chromagen uptake is not likely to be the mechanism of chlorhexidine staining.

To investigate the role of dietary precipitation in staining, chlorhexidine and mono-, di-, and trivalent metal salts were mixed with different dietary solutions (tea, wine, juices, etc.) and the amount of precipitate formed was graded relative to that of water. Metal salts were expected to precipitate varying amounts of chromagens from dietary solutions, and therefore the degree of precipitation by chlorhexidine was graded relative to metal salts. Tea, coffee, curry sauce, red wine, and soy sauce resulted in the most precipitate. Stannous chloride resulted in the most precipitate followed by ferric chloride and silver nitrate which showed comparable levels of precipitation as chlorhexidine.

To examine the role of dietary precipitation reactions in tea staining, recently extracted human teeth and polymethyl methacrylate blocks were treated with ferric chloride, stannous chloride, or chlorhexidine followed by a tea bath. Ferric and stannous chloride acted as a positive control as it was previously determined that both precipitate chromagens from dietary solutions. Ferric chloride produced a black coating on samples and stannous chloride produced a yellow-brown stain. Chlorhexidine resulted in the greatest degree of staining again supporting the hypothesis of chlorhexidine staining due to a precipitation reaction between dietary chromagens and adsorbed chlorhexidine.[12]

The findings of this study further the findings of a 1971 study by Nordbo et al. Nordbo performed an *in vitro* experiment investigating the interactions between chlorhexidine and aldehydes or ketones. Ketones and aldehydes are common intermediates in the metabolisms of oral bacteria. It was found that chlorhexidine reacts with aldehydes and ketones to form chromagens, the color being dependent on the aldehyde or ketone.[13]

In addition to the diet consumed, chlorhexidine concentration and dosage affects the degree of staining. Najafi et al. performed an *in vivo* study investigating the role of concentration on the severity of chlorhexidine staining. Subjects rinsed with either 0.12% or 0.2% chlorhexidine mouthrinse, or with a placebo rinse twice per day for 14 days. Both concentrations of chlorhexidine resulted in a significantly lower plaque and gingival index. The difference between the two concentrations of chlorhexidine in plaque and gingival index

was not significant, although the staining area and intensity was significantly higher in subjects using the 0.2% chlorhexidine mouthrinse compared with 0.12%. It was concluded that chlorhexidine should be prescribed in lower concentrations, as lower concentrations are just as effective in preventing plaque and gingivitis while reducing the severity of staining.[14]

These results support the findings of Segreto et al. This *in vivo* study followed 600 adults over a period of three months rinsing with 0.12% or 0.2% chlorhexidine mouthrinse. Subjects using chlorhexidine showed significantly less gingivitis and plaque though again there was no significant difference between 0.12% and 0.2%.[15] In order to reduce the staining effects of chlorhexidine mouthrinses without compromising plaque control efficacy, again it was concluded that 0.12% concentration should be used.

Despite the capacity of chlorhexidine to effectively treat and prevent plaque accumulation, its potential for long term use is compromised due to tooth staining. If staining could be safely and effectively prevented, chlorhexidine mouthrinses suitable for long term use may become available. Such a development could positively affect the oral health of populations in which traditional tooth cleaning procedures are either difficult or impossible. A chemical plaque control agent that does not cause staining and is approved for use in the long term could have important implications for the oral health industry, dental professionals, and public health sectors.

Tooth bleaching

Chlorhexidine has a myriad of benefits for its users though due to staining side effects, long–term use is discouraged. Due to the fact that the mechanism of chlorhexidine staining is poorly understood, developing a method by which to prevent or treat chlorhexidine staining is a challenge to researchers. Tooth whitening procedures have been formulated and studied for over 100 years. Since the development of the 'nightguard' bleaching system in the late 1980s, the popularity of tooth whitening has increased dramatically. Tooth bleaching procedures are simple and noninvasive, and the results are immediate, making them highly appealing to patients.[16]

There are two forms of bleaching procedures, vital and non-vital. In non-vital bleaching, a bleaching agent is applied internally within the pulp chamber of the tooth. The procedure is more invasive than a topical bleaching method as the bleaching agent is injected with a syringe into the pulp of the tooth. This method is most effective for treating intrinsic stains, those caused by alterations to the dental hard tissues. Vital bleaching involves applying a bleaching agent externally to the teeth. This method is used for extrinsic staining.[16]

Hydrogen peroxide and carbamide peroxide are the two most popular bleaching agents. Carbamide peroxide, a white crystalline solid, is a loosely associated adduct of hydrogen peroxide and urea. When exposed to water

carbamide peroxide breaks down into urea and hydrogen peroxide, although carbamide peroxide produces a lower concentration of hydrogen peroxide than a standard hydrogen peroxide bleaching agent.[16] Evidence indicates that there is no significant difference in the efficacy of hydrogen peroxide over carbamide peroxide when the hydrogen peroxide concentrations and the mode of delivery are the same.[17] Other bleaching agents including sodium chlorite, sodium perborate, peroxymonosulphate, peroxide plus metal catalysts, and oxidoreductase enzymes although results have yet to be conclusive as to their respective efficacies.[16]

It is believed that these oxidizers enter the dentine of the tooth via enamel micropores in the form of reactive oxygen species. Chromaphores are generally organic compounds with extended conjugated pi–systems with variable side chains. Bleaching of these chromophores occurs when one or more of the double bonds is reduced, a conjugate chain is cleaved, or a chemical moiety is oxidized (see figure 4).[16] When chromaphore molecular bonds are cleaved the resulting fragments absorb less light or may even diffuse out of the tooth.[16]

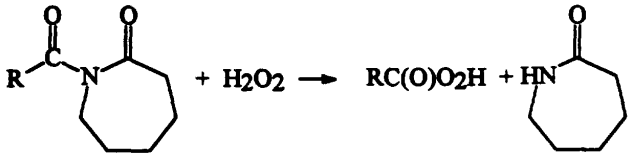

Figure 4. Oxidation of a chromophore by hydrogen peroxide.
http://www.google.com/patents/WO1996022350A1

A challenge facing researchers is the standardization of tooth whitening measurement. The most common method of judging the efficacy of a bleaching treatment is the use of a shade guide (see figure 5).

Figure 5. A shade guide used to determine tooth shade before and after bleaching procedures.
http://en.wikipedia.org/wiki/File:Zahnfarbe_Frabring_20100202_034.JPG

Although shade guides are standardized, their use is still subjective. Lighting, experience, age, eye fatigue, make up, room decor, and color blindness can affect how a tooth is rated. Colorimeters are another useful experimental method although this method is difficult to implement *in vivo*. More commonly used today is non-contact camera-based digital imaging. A photo is taken of the teeth under controlled lighting along with calibration tiles or standards. These photos are then analyzed using computer software.[16]

Many factors affect the efficacy of bleaching treatments, including concentration and time the tooth is exposed to the bleaching agent.[16] Increasing the exposure time and concentration increases the expediency of the bleaching process though not the overall efficacy.[17] It has been confirmed that 10% carbamide peroxide gel in a tray worn overnight, over-the-counter

hydrogen peroxide strips, and power bleaching using 35% hydrogen peroxide with or without light and/or heat activation are all effective and have predictable results.[18]

Ferrari et al. investigated the whitening response of hydrogen peroxide whitening strips at varying concentrations. Three different concentrations were tested: 1.8%, 3.3%, and 5.2%. Subjects used the strips twice daily for 30 minutes. Efficacy and safety were evaluated at day 7, 14, and 28. Whitening effects were evaluated using digital image processing, and the safety of the agents by clinical examination of oral irritation and sensitivity. All groups showed significant whitening as early as day 7. As predicted, concentration was directly proportional to the response time. The highest concentration had the quickest response time, followed by the intermediate concentration, and so forth. By day 28, the concentration response plateaued and no significant difference in efficacy between the high and intermediate concentrations was seen. Therefore, treatment duration has an effect only to a certain extent. Additionally, the treatments were well-tolerated and no subjects reduced or discontinued treatment due to sensitivity or oral irritation.[19]

Modern bleaching techniques are highly effective although upwards of two-thirds of patients using at-home bleaching systems experience increased sensitivity to temperature.[16] This increased sensitivity subsides within a few days though some studies have shown that sensitivity can last for up to 39 days. In addition, the hydrogen peroxide mouthrinses used in many studies investigating the efficacy of oxidizing agents in treating and preventing

chlorhexidine staining have been found to cause mouth irritation and discomfort, dryness, loss of taste, diffuse mucosal whitening, and elongation of filiform papillae (small prominences on the tongue surface).[18]

Gingival irritation is the second most common side effect of bleaching procedures. It has been reported in a number of studies that gingival irritation and sensitivity increased when higher concentrations of hydrogen peroxide were used in tray-based systems. The same has not been found for strip-based systems.[20]

Many factors can effect the tolerability of whitening procedures. Gerlach et al. investigated the effects of concentration and pre-treatment brushing on the efficacy and tolerability of strip-based bleaching procedures. Three groups of adult volunteers used whitening strips twice daily for 2 weeks. All participants used the same type of toothbrush and toothpaste. One group was instructed to brush immediately prior to using 5.3% hydrogen peroxide strips. Another group was instructed to brush immediately prior to using 6.5% hydrogen peroxide strips. The last group used 6.5% hydrogen peroxide strips without brushing prior to treatment. The efficacy was measured using digital imaging analysis. All three groups showed significant whitening although the 6.5% hydrogen peroxide strips were more effective than the 5.3% hydrogen peroxide strips. Pre-treatment brushing was found to have a modest impact on the efficacy of the whitening strips, although it was concluded that pre-treatment brushing reduced overall tolerability of the process, due to increased

sensitivity and gingival irritation. Therefore, to minimize the increase in sensitivity and oral irritation, pre-treatment brushing should be avoided.[20]

Treatment and prevention of chlorhexidine staining

Gentler bleaching agents such as peroxyborate have garnered much attention since the early 1980s as a potential agent for the prevention and treatment of chlorhexidine discoloration. Ellingsen et al. were the first to suggest the use of an oxidizing agent to treat chlorhexidine stained teeth. Subsequent research has supported this hypothesis beginning with work by Eriksen et al. in 1983. A solution of 1% peroxymonosulfate was used in addition to 0.2% chlorhexidine rinses twice daily for two weeks. The results showed a significant difference between the severity of staining between the chlorhexidine alone and the chlorhexidine–peroxymonosulfate mixture.[21]

These results were later confirmed by Addy et al. in an *in vitro* study. They also investigated the efficacy of peroxyborate in both preventing developing stains as well as its efficacy in treating established chlorhexidine stains. To test established stains, human teeth and acrylic samples were bathed in black tea and twice daily were washed with human saliva followed by a two-minute wash in 0.2% chlorhexidine solution; the samples were then returned to the tea bath. On the sixth day samples were treated twice with peroxyborate or water. To test the efficacy of peroxyborate on developing stains, the same process was used in addition to a once daily peroxyborate treatment. Results showed that peroxyborate was effective in reducing developing chlorhexidine/tea stains and nearly eliminated tea-only staining. As with established stains,

water washing somewhat reduced staining, although not nearly as well as a peroxyborate wash.[22]

Addy et al. also performed an *in vivo* experiment in the same study. For three days four adults rinsed with 0.2% chlorhexidine for one-minute then rinsed with warm black tea for two minutes, followed by a one minute water rinse. On the fourth day, individuals rinsed with peroxyborate five times over the course of the day. Resulting staining varied greatly among the participants although all subjects displayed some degree of brown staining. It was concluded that peroxyborate was more effective than water in reducing both the area and severity of staining *in vivo*.[22]

Grundemann et al. furthered these findings in 2000, discovering that peroxyborate actually enhanced the efficacy of chlorhexidine in regard to plaque inhibition. It was postulated that chlorhexidine and peroxyborate have an additive effect due to differing mechanisms of plaque control. Chlorhexidine is purely topical, whereas peroxides release oxygen, killing obligate anaerobes implicated in oral infections.[23]

Despite the evidence supporting oxidizing agents as an effective agent in combatting staining by chlorhexidine, the mechanism is poorly understood, which is expected considering the mechanism by which chlorhexidine causes staining is also poorly understood. Regardless of the mechanism of action, the research shows that using an oxidizing agent in conjunction with chlorhexidine mouthrinses can prevent and treat staining.

These findings are key to the development of a chlorhexidine mouthrinse that does not cause staining and is suitable for long term use.

Trial Design

Goal

If Chlorhexidine staining is due to precipitation reactions with chromagens onto the acquired pellicle, standard bleaching vis-á-vis oxidizing procedures should be effective in chlorhexidine stain removal. Variations of the treatment duration, concentration, and type of bleaching agent should be investigated in order to establish protocols that reduce the negative side effects associated with these bleaching procedures. The ultimate goal is to formulate a chlorhexidine mouthrinse which is suitable for long term use through the development of effective methods by which to treat and prevent staining.

Methods

The proposed trial will last four weeks with adult volunteers in overall good oral health. All subjects will receive prophylaxis prior to the trial and their baseline tooth shade will be determined using digital imaging. Normal tooth brushing will be discontinued for the duration of the trial. Chlorhexidine mouthrinse will act as the only form of plaque control.

All groups will use 0.12% chlorhexidine mouthrinse twice a day for one minute. To test the efficacy of bleaching agents in preventing chlorhexidine staining, whitening agents will be used once a day in addition to chlorhexidine. Bleaching agents, carbamide peroxide and hydrogen peroxide, and an oxidizing

agent, peroxyborate, in the form of mouthrinses will be used. The concentration and duration of treatment will be varied. The control group will use 0.12% chlorhexidine mouthrinse twice per day for one minute with no whitening agent.

Digital imaging analysis will be performed everyday as well as weekly clinical oral exams to determine level of tissue irritation, if any. Tooth sensitivity will be rated daily by subjects to be averaged at the end of the four week period to investigate severity of side effects.

Experimental Groups

Group	Whitening Agent	Concentration	Duration
1	Carbamide Peroxide	2%	1 minute/day
2	Carbamide Peroxide	2%	2 minutes/day
3	Carbamide Peroxide	4%	1 minute/day
4	Carbamide Peroxide	4%	2 minutes/day
5	Hydrogen Peroxide	2%	1 minute/day
6	Hydrogen Peroxide	2%	2 minutes/day
7	Hydrogen Peroxide	4%	1 minute/day
8	Hydrogen Peroxide	4%	2 minutes/day
9	Peroxyborate	2%	1 minute/day
10	Peroxyborate	2%	2 minutes/day

Group	Whitening Agent	Concentration	Duration
11	Peroxyborate	4%	1 minute/day
12	Peroxyborate	4%	2 minutes/day
13/Control	--	--	--

Expected Results

Carbamide peroxide, hydrogen peroxide, and peroxyborate have all been shown to be effective in treating extrinsic tooth staining, though the concentration and treatment duration should have an effect on both the rate and the overall efficacy. Higher concentration of the bleaching agent with greater treatment time should produce results more quickly than lower concentrations and shorter treatment times. As carbamide peroxide contains lower concentrations of hydrogen peroxide, it would be expected that hydrogen peroxide would be more effective overall. Hydrogen peroxide at 4% with a 2-minute treatment time should show the greatest whitening with the smallest response time. As few studies have compared peroxyborate to carbamide peroxide and hydrogen peroxide; it is unknown what the expected results would be.

Hydrogen peroxide at 4% with a 2-minute treatment time is expected to show the greatest degree of whitening, although it would also be expected that tooth sensitivity, gingival irritation, and other negative side effects would also increase. Lower concentrations and shorter treatment times would reduce the negative side effects.

The results of the study will give insight to the efficacy of whitening treatments on chlorhexidine staining specifically. Combining chlorhexidine treatments with whitening agents could eventually lead to the development of a formulation of chlorhexidine mouthrinse that does not cause staining and would therefore be appropriate for long term use. Determining appropriate concentrations and duration of use of the treatments so as to minimize any possible negative side effects would be the first step in the formulation of a purely chemical oral hygiene routine.

Future Work

In the proposed trial, the whitening treatment is used separately from the chlorhexidine mouthrinse. In future studies, a single mouthrinse may be investigated which contains both chlorhexidine and a whitening agent. Again, concentration and duration of treatment should be investigated in order to maximize the plaque inhibition and stain prevention, while minimizing negative side effects. The results will also provide insight into the stability and efficacy of chlorhexidine in an oxidizing environment.

The development of an oral hygiene routine which does not require mechanical tooth brushing could have important implications for public health, the oral health of children and those with disabilities, as well as the oral health industry. Mouthrinses are user friendly and could potentially be more effective in combatting plaque and dental decay than mechanical toothbrushing. Chlorhexidine is an excellent candidate for such a development for its plaque

inhibiting properties. If staining side effects can be minimized, chlorhexidine mouthrinses could be an integral part of the future of oral healthcare.

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Acknowledgements

I would like to sincerely thank Dr. David Hansen for his insight, guidance, and critical eye. I would also like to thank Dr. Nora Sullivan whose Microbewiki project inspired my topic. My thanks goes out to all of the teachers and professors I have had the opportunity to work with in my scientific education. I also thank my family, David, and my brilliant friends whose experiences writing theses I had the fortune of learning from.