Title
Reaction kinetics of hydrogen peroxide in teeth for teeth whitening applications

Author
Fang, Grace C.

Publication Date
2013

Peer reviewed|Thesis/dissertation

UNIVERSITY OF CALIFORNIA
Los Angeles

Reaction kinetics of hydrogen peroxide in teeth for teeth whitening applications

A thesis submitted in partial satisfaction

of the degree requirements for the degree Master of Science

in Biomedical Engineering

by

Grace Chia-Ann Fang

2013

ABSTRACT OF THE THESIS

Reaction kinetics of hydrogen peroxide in teeth for teeth whitening applications

by

Grace Fang

Master of Science in Bioengineering

University of California, Los Angeles, 2013

Professor Benjamin M. Wu, Chair

Clinical parameters for dental whitening such as peroxide concentration and treatment time have been empirically derived. However, limited quantitative analyses examine reactivity of hydrogen peroxide on *in vivo* tooth stains under various catalytic settings. The wide range of possible activators and stains are challenging in creating a standardized tooth model to isolate various effects for clinical applications. This study uses three model systems to determine the effects of heat, light, metal catalysts, and pH on peroxide bleaching. By first using a model chemical stain, alkaline pH levels (7.4), 35°C heat treatment, and 490nm light activation were optimal for increasing overall bleaching efficacy. When the same conditions were applied to bleaching stained bovine teeth using liquid peroxide, only 35°C heat treatment showed catalytic effect, increasing overall luminosity and decreasing yellowness. A final evaluation of the activation parameters on teeth treated with commercially used gel peroxides revealed positive catalytic activity for heat alone. These results, in conjunction with future clinical studies, can provide the basis for optimizing clinical whitening parameters and ultimately control peroxide reactivity to enhance bleaching efficacy while minimizing undesired side-effects.

The thesis of Grace Chia-Ann Fang is approved.

Daniel T. Kamei

Min Lee

Benjamin M. Wu, Committee Chair

University of California, Los Angeles

2013

Table of Contents

Part 1 – Background and Motivation

1.1 – Tooth Whitening

1.1.1 Overview

Teeth whitening, also known as dental bleaching, is a widespread cosmetic treatment for tooth discoloration. Causes for tooth discoloration range from internal aging, trauma, blood discoloration, and various metabolic diseases to extrinsic deposition of colored food stains [1]. Under traditional bleaching procedures, hydrogen peroxide or carbamide peroxide bleaching agents are applied to the external surface of the enamel [2][3]. The oxidative bleaching agents diffuse into the tooth and react with stain molecules within the enamel and dentin layer causing the overall whitening effect. Given the thin and relatively translucent nature of enamel, dentin is the primary target site for bleaching as it is largely responsible for the overall tooth color [4].

1.1.2 Bleaching mechanism

While the exact mechanism of bleaching is unknown, it is hypothesized that the exogenous stain molecules are degraded upon reacting with hydrogen peroxide dissociation products such as hydroxyl radicals, perhydroxyl radicals, and perhydroxyl anions [5][6]. These highly oxidative products are formed in a thermodynamically favorable manner and the process can be catalytically enhanced [7] . Stain molecules found in teeth are organic molecules that carry alternating single and double bonds that can be broken when upon reacting with unstable

radicals [6]. Interrupting the conjugation in the colored chromophores creates the overall bleaching effect.

1.1.3 Teeth whitening methods

Teeth whitening procedures can be classified into three major classes including in-office, at-home, or over-the-counter treatments. In-office treatments are often more aggressive using up to 25-35% hydrogen peroxide applied in multiple time increments for 30 to 60 minutes [6]. The advantage of using in-office treatment is the potential to use heat and light activation for accelerated bleaching. At-home bleaching, also known as nightguard vital bleaching, uses lower concentrations of hydrogen peroxide (3 – 7.5%) or carbamide peroxide (10 – 20%) under the supervision of a dental professional [8]. Under nightguard vital bleaching procedures, the custom tray is coated with the bleaching gel and the tray is impressed on the patient's teeth for 1-2 hours during the day or night for daytime and nighttime treatments respectively for 1-2 weeks. Over-the-counter bleaching procedures, with the least amount of peroxide, use thin strips of bleaching agents are wrapped around the surface of the teeth daily for approximately 30 minutes in treatment spans of 1-2 weeks.

A comparison of the three bleaching methods found at-home bleaching procedures have the highest whitening efficacy [9] while other studies suggest no differences in whitening efficacy between in-office and at-home treatments [10][11][12]. Another study found that the same whitening effects can be achieved using all three treatments but require different treatment times with 3.15 cycles, 7.15 cycles, and 31.85 cycles of in-office, at-home, and OTC treatments respectively [13].

For tooth whitening gels using lower peroxide concentrations, carbamide peroxide is often used in place of hydrogen peroxide. As an adduct of hydrogen peroxide and urea, carbamide peroxide dissolves in water to release free hydrogen peroxide, with the hydrogen peroxide concentration being approximately one-third of the original concentration [14]. Therefore, based on molecular weight, a 16% carbamide peroxide gel contains approximately 5.9% hydrogen peroxide formulation.

Other commercially used whitening products that treat extrinsic stains include chemicals such as sodium citrate in whitening toothpastes [15] and blue covarine in mouthwash [16]. The silica in blue covarine gives the appearance of whiter teeth because the blue hue adheres to and cancels out the yellow surface beneath. Physical abrasion methods using hydroxyapatite [17] and silicon carbide [18] have also shown to increase overall tooth whitening.

1.1.4 Clinical significance

Over $1.6 billion was spent on dental hygiene and oral care in 2010 and is expected to increase to $2 billion by 2017 with tooth whitening being a major component of the cost [19]. One survey demonstrates that 32% of the population is unsatisfied with their teeth color [20] and another study revealed that tooth whitening is the highest desired cosmetic treatment among women [21].

1.1.5 Risks of teeth whitening

The most common side effect associated with dental whitening is teeth sensitivity found in 4-50% of patients using home whitening systems [22][23] and 67-78% for in-office techniques that utilize heat catalysis [24]. The hydroxyl radicals move past the stains at the enamel and dentin level, penetrate the tooth pulp, and undesirably irritate sensitive tissue and surrounding nerve. Irritation and discomfort usually result in premature termination of treatment and detour patients from future procedures [25]. Sensitivity has shown to last from 4 days [26] [25] to 39 days[27].

While teeth hypersensitivity, caused by pupal damage, is normally reversible [1], cases of irreversible damage have been reported under clinical settings[27]. Several studies recommend using carbamide peroxide under 10% concentration for minimal risk [28] [29]. Risk of irreversible hypersensitivity increases significantly when heat is used in whitening procedures [30]. Other reported adverse side effects include root resorption and changes in surface topology and chemical composition of enamel [31].

1.2 – Stains found in teeth

The origins of tooth stains vary widely and can be categorized by both color and region of occupancy. A comprehensive overview of various stains can be found in Table 1 [32].

Table 1 Intrinsic and extrinsic teeth stains sources categorized based on color and region found.

	Extrinsic	Intrinsic (Localized)	Intrinsic (Regional)	Intrinsic (Generalized)
Brown stains	• Tobacco products • Dental plaque • Tea, coffee, wine, and other beverages • Certain foods • Metals • Iodine • Chlorhexidine rinse • Cetylpyridinium chloride rinse • Stannous fluoride • Khat leaf • Doxycycline	• Severe trauma during enamel formation (secondary teeth), • Periapical infection of primary tooth • Traumatic injury to primary tooth or teeth • Composite, glass ionomer, or acrylic restoration • Caries (active or remineralized) • Pulpal trauma with hemorrhage	• Infection during enamel formation • Moderate fluorosis (short-term exposure) • Trauma during enamel formation	• Congenital erthorpoietic porphyria • Tetracycline (long-term exposure) • Ochronosis • Alkaptonuria
Black stains	• Tobacco products • Betel nut • Dental plaque • Chromogenic bacteria • Tea, coffee, wine, and other beverages • Certain foods • Metals	• Amalgam restoration • Glass ionomer or acrylic restoration • Metal crown margin associated with porcelain fused to metal crown • Pulpal trauma with hemorrhage	• Tetracycline (short-term exposure)	• Tetracycline (long-term exposure) • Minocycline therapy
Green stains	• Chromogenic bacteria • Tea • Metals		• Diseases causing hyperbilirubinemia	• Diseases causing hyperbilirubinemia
Orange stains	• Chromogenic bacteria • Metals • Doxycycline			
Yellow stains		• Moderate trauma during enamel formation (secondary teeth) • Periapical infection of primary tooth • Traumatic injury to primary tooth or teeth • Trauma without hemorrhage • Composites or glass ionomer or acrylic restoration • Caries (active) • Focal tooth abrasion	• Diseases causing hyperbilirubinemia • Regional tooth abrasion or erosion • Epidermolysis bullosa • Nutritional deficiency • Trauma during enamel formation • Moderate fluorosis (short-term exposure) • Infection during enamel formation	• Fluorosis • Amelogenesis imperfecta • Dentinogenesis imperfecta • Dentinal dysplasia • Epidermolysis bullosa • Diseases causing hyperilirubinemia • Hemolytic diseases • General tooth abrasion or erosion • Ageing • Haemoglobin and haem moieties
White stains		• Mild trauma to teeth during enamel formation (secondary teeth) • Periapical infection of primary tooth • Traumatic injury to primary tooth or teeth • Incipient caries (primary or secondary teeth)	• Trauma during enamel formation • Moderate fluorosis (short-term exposure) • Infection during enamel formation • Nutrition deficiency	- Mild fluorosis - Amelogenesis imperfecta

1.2.1 Extrinsic

Extrinsic stains deposited on the pellicle include free microorganisms such as chromogenic bacteria, chlorohexidine deposition, food stains, and dental plaque [32][33]. Food stains composed of polyphenolic compounds, usually in the form of tannin, are classified into three groups: hydrolyzable, non-hydrolyzable, and phlorotannins [34]. Stain buildup is usually a result of ionic interactions exemplified in the carboxyl and phenolic group reactivity in coffee stains. Similar interactions can be seen in tea, wine, and other heavily pigmented food products. Metallic extrinsic stains from exposure to mouthwash, antiseptics, and chemicals are also sources of discoloration [35].

1.2.2 Intrinsic localized stains

Intrinsic stains generally result from changes in structural composition and thickness of the underlying tissue. Coloration is categorized based on blue, green, and pink tints of enamel and yellow to brown dentin beneath [36]. Developing dentition diseases such as ochronosis or alkaptonuria and congenital erthorpoietic porphyria cause build-up of acid and compounds leading to reddish-brown discoloration of deciduous dentin. Moderately widespread intrinsic stains arise from endemic fluorosis- hyponaturation of the enamel that causing spotting, tetracycline exposure- resulting in yellow-brown stains, and antibiotic use. Commonly found intrinsic stains stem from natural causes such as ageing and the accumulation of haemoglobin and haem moieties through the dentinal tubules embedded in the pulp [32][35][37].

1.2.3 Stain classification

The Nathoo classification system is the most prevalently used algorithm for classifying stains based on chromogen characteristics. Nathoo categorizes stains based on the color binding material and staining reactivity into three types: N1 chromogen binds to the tooth surface and is similar in color to tea, coffee, wine, chromogenic bacteria, and metal induced stains; N2 chromogen are N1-tye food stains that have darken with time; N3 type stains, caused by carbohydrate-rich foods, stannous fluoride, or chlorhexidine, are colorless or prechromogenic and only stain after undergoing a chemical reaction from binding to the tooth [36].

1.3 – Bleaching extrinsic stains

1.3.1 Factors affecting hydrogen peroxide bleaching

Various catalysts such as heat, pH, light irradiation, and the introduction of metal ions have been shown to potentially increase hydroxyl radical generation from hydrogen peroxide [38][39]. Hydrogen peroxide becomes more unstable under alkaline environments so increasing the physiological pH of the oral cavity of 6.2-7.4 would also yield a greater number of free radicals. Transition metals iron, manganese, copper, and vanadium are known to induce hydroxyl radical formation. However, they leave residual when not combined with a carrier. Iron has been shown to have significant catalytic effect due to the induction of Fenton reactions allowing for increased peroxide dissociation and radical formation [3].

Heat has been known to accelerate the bleaching process since 1918 when power bleaching, a process using high-intensity light to heat peroxide, was first introduced. While the use of heat (30-55°C) activation on vital teeth has shown to be effective in several clinical reports, other studies have shown irreversible side effects [6][40] [41]. Thermocatalysis occurs due to the following principle: H_2O_2 + 211kJ/mol→2HO• where a rise of 10°C theoretically corresponds to an increased rate of decomposition by a factor of 2.2. Since radical formation is the rate-limiting step for teeth whitening, increasing radical release theoretically increase efficacy [41]. However, the useful temperature range for thermocatalysis is limited by possible dental pulp damage. The useful range of heat increase should not exceed 5.5°C due to the possibility of pulpal damage [30].

Photolysis is thought to contribute to hydroxyl-radical release based on the equation $H_2O_2 + h\nu \rightarrow 2HO\bullet$ where the specific frequency ν breaks the peroxide into its radical components. Chemically, high frequency light that corresponds to wavelengths less than 248nm are preferred but potential adverse side effects of high-intensity light in the oral cavity demands a balance between efficacy and safety. Therefore, a variety of light sources have been examined and used. Light sources ranging from incandescence lamps such as quartz-tungsten-halogen lamps, plasma arc lamps, lasers, and LEDs have been used in bleaching procedures. Sources vary from lasers emitting narrow monochromatic light of a single wavelength to lamps that emit a wide range covering ultraviolet, visible, and infrared wavelengths. While these lamps often include UV and IR filters to reduce side effects, it is impossible to suppress all of the incoming radiation. Wavelengths around 3000nm bordering IR have high absorption coefficients in water and tooth surface minerals making them biocompatible whereas light in the red and near IR

range penetrates biological tissues potentially causing thermal damage to pulpal tissue. Violet, blue, and green light in the visible spectra are also known to absorb more readily [41].

1.3.2 Tooth bleaching models

While clinical surveys do provide insight for optimal and safe treatment conditions, controlled in vitro models allow for more control and precise color assessment. Few in vitro models have been generated to study the effects of various catalysts. The challenge is partly attributed to the wide variety of stains found in vivo, making it difficult to select a chemical dye for relevant modeling. Lee et al evaluated three dyes, comparable in structure, with hydrogen peroxide treatment to model reactivity of peroxide [3].

Other studies created an in vitro model using tea stained teeth samples. One investigated the effects of various peroxide concentrations using tea stained teeth samples with various peroxide concentrations using the Vita Shade Guide [42] while another compared the Vita Shade Standard to spectrophotometry readings also using a tea stain model [43]. Several studies have invested the individual effects of light or heat but no study has evaluated the effects of various parameters using a uniform in vitro model.

1.4 – Limitations in existing literature

While several bleaching trends for chemical model stains suggest the importance of ideal catalytic conditions, they fail to compare the significance of each factor using a controlled and isolated reaction chamber. Furthermore, these trends lack clinical significance as they may not

apply to tooth models. Extrinsically stained enamel may bleach differently than a suspended dye solution due to multiple factors including the degree of stain retention, diffusivity of peroxide, and the limited surface area contact. The use of liquid peroxide model systems rather than conventional gel peroxide systems presents another variable that may yield different results.

A translatable tooth model should ideally evaluate all parameters that affect bleaching efficacy including pH, heat, light, and the presence of metal catalysts. The optimal combination of conditions can then be applied clinically for a significant impact in patient outcome.

1.5 – Effects of diffusivity

Hydrogen peroxide based teeth whitening relies on both the diffusion of peroxide through the tooth as well as the reaction of peroxide with the surrounding stains. Therefore, while the primary focus of this study is to study the reaction kinetics of hydrogen peroxide in teeth, the diffusion component must also be considered during experimental setup and data analysis. Diffusion constants for hydrogen peroxide and teeth have been previously studied [24] and will be used to evaluate the rate limiting step in bleaching processes.

Part 2 – Experimental methods

2.1 – Goals

There are three sub-aims in this study to assess the potential clinical implications of catalysts.

2.1.1 Goal 1: Given the wide range of potential catalysts, which are the most influential in improving hydrogen peroxide reaction kinetics?

To assess reaction kinetics, an ideal experimental setup would only involve a reaction component with no diffusion parameter. Therefore, the simplest model is a simple liquid-liquid reaction of a well-characterized model dye and peroxide. Given the large variety of in vivo stains, correct dye selection is vital to constructing an accurate representation of in vivo conditions. The dye of choice should contain carbon-ring and double bond structure with similar connectivity, have a small molecular weight to easily diffuse into the tooth, and be decomposable upon reaction with hydrogen peroxide.

While model chemical stains are not found in clinical settings, the aim of this setup is to assess the catalytic potential of various parameters, specifically temperature, light, pH, and transition metals to narrow the range of ideal bleaching conditions. The experimental setup should be easily reproducible and only differ in one independent condition for meaningful cross-experiment comparisons of results.

2.1.2 Goal 2: Do the catalytic effects seen in a pure reaction setup translate to a stained tooth model with a diffusion parameter?

Once a range of bleaching conditions have been established using the chemical stain setup, an in vitro tooth model must be used to validate that clinical translatability of using various catalysts. To determine the optimal bleaching conditions for stain degradation in teeth, the experimental setup must utilize commonly found in vivo stains and minimize the effects of diffusivity. Furthermore, relevant in-office peroxide concentrations and comparable treatment times should be used when assessing bleaching efficacy.

The simplest in vitro tooth model would stain teeth samples with widely found extrinsic food stains such as tea and coffee. Using a liquid peroxide bleaching agent instead of conventional in-office peroxide gels will minimize the effects of diffusion to better isolate reactivity between peroxide and the stained substrate.

2.1.3 Goal 3: While catalysts influence the bleaching efficacy of hydrogen peroxide, do the ideal conditions also optimize commercially used peroxide gels on stained teeth?

The third aim of the experimental studies is to determine whether the optimal bleaching conditions of the liquid peroxide model apply to conventionally used in-office peroxide gels. Given the viscous nature of the gels and the presence of other substances, the catalytic effect of various parameters may differ from both the liquid peroxide and chemical stain model.

While the gel peroxide model introduces a diffusion component, a previous study conducted in the Wu lab at UCLA Bioengineering [24] suggests that reaction occurs quickly

relative to diffusion with a Thiele modulus $\phi_n{}^2 \gg 1$ (assuming a pseudo-first order process and a reaction constant $k = 10^{-4}s^{-1}$). Thus, at small time scales of relevant treatment times, the diffusion effect becomes minimal.

2.2 – Sample preparation

Brilliant blue FCF (Sigma, Saint Louis, MO) was diluted in deionized water to [5x10^{-5}]M and disodium phosphate buffer (Sigma, Saint Louis, MO) was buffered to alkaline pH levels ranging 7.3-7.5.

Bovine central incisors were obtained (Tri-state Beef Co, Cincinnati, OH) and screened for major defects including ridges and cracks. The teeth were cleaned and stored in 50% bleach solution and soaked in deionized water before use. Teeth samples are subsequently sand-blasted with 25 micron aluminum oxide to create homogenous surface roughness for optimal stain retention and the teeth are washed again in deionized water to remove residual debris.

To create the stain solution, Lipton tea bags (2.51±0.02g) are soaked in 100mL of boiling water for 5 minutes. Each tooth is then incubated in 10mL of cooled tea solution for 24 hours. Spectrophotometry color measurements of air dried tooth samples are taken before and after staining.

2.3 – Color change quantification

Brilliant blue FCF concentration was assessed quantitatively through UV spectroscopy, taking measurements at 620nm using a microplate reader (Tecan Group Ltd., Mannedorf,

Switzerland). Brilliant blue standards were prepared by forming serial dilutions from a [1x10]M solution and the Brilliant blue standard concentrations ranged from $1x10^{-5}$M to $5x10^{-5}$M (the upper limit of the linear portion of the Beer's law plot) (Supplemental Fig. 1). A standard curve for Beer's law analysis was constructed and used to calculate Brilliant blue sample concentrations based on measured absorbance.

Spectrophotometry was used to measure color on tooth samples using the LaB color meter [44] where L measures luminosity, a measures degree of red (positive) or green (negative), and b measures degree of yellow (positive) or blue (negative). Conventionally, ΔE represents the average overall color where $\Delta E = \sqrt{L^2 + a^2 + b^2}$ and is used for assessing color change.

Differences in surface roughness and microstructure of the bovine teeth samples, the samples retain varying amounts of stain. Darker stained teeth would need more extensive bleaching compared to lighter stained teeth to achieve a net positive effect; thus, a normalized bleaching parameter must be created. A novel bleaching parameter β was developed in this study and is defined as the degree of bleaching relative to the degree of staining or $\beta = \frac{E - E_{post\ stain}}{E_{prestain} - E_{post\ stain}}$ (β ranges from 0 to 1 with 1 denoting high bleaching efficiency).

2.4 – Parameter controls

The effects of various catalysts on Brilliant Blue bleaching were assessed individually with the initial focus on pH effects. Disodium phosphate solutions were buffered to pH 7.33, 7.40, 7.51, and 7.53 to evaluate bleaching at physiologically relevant alkaline environments and an optimal pH level was determined. All subsequent experiments were conducted using that standard. The effects of adding various metal catalysts were also examined using 0.005% wt/wt

ferrous gluconate and manganese(II)-nitrate tetrahydrate (Acros Organics, Geel, Belgium) in the presence and absence of the buffer solution. To study the temperature effects, Brilliant Blue samples were heated to 30°C, 35°C, and 40°C using an incubator. Finally, the effects of light were assessed using a multi-wavelength Polilight PL500 (Rofin, Australia) at 415nm, 450nm, 470nm, 490nm, and the Zoom™! Lamp™ (Discus Dental, Culver City, CA) on buffered and non-buffered samples. All light studies were calibrated to the same power of 72mW using 620nm light, characteristic of dental whitening lamps, and conducted in a water bath system to isolate light and temperature effects.

Based on the results from the Brilliant Blue model, the most influential factors will be evaluated in the liquid peroxide model. Temperature studies will be conducted in heated water baths under ambient light and light irradiation studies will be conducted at various wavelengths with the samples submerged in a water bath to minimize temperature effects. Similarly, the gel peroxide model samples will be heated using an incubator at the various temperatures and light studies will be conducted in the same manner as the liquid peroxide model.

2.5 – Reaction experiments

A summary of the experimental setups can be found in Supplemental Figure 1.

2.5.1 Brilliant blue model

The wells of a 24-well plate were filled with 0.5mL [$5x10^{-5}$]M Brilliant Blue, 0.5mL disodium phosphate buffer of various pH, and 0.5mL 50% hydrogen peroxide (Fisher, Houston,

TX). The effects of iron and manganese were measured using 0.5mL 0.005% wt/wt ferrous gluconate and manganese(II)-nitrate tetrahydrate in place of the buffer solution (pH 7.4) in the 24-well setup. The combinatory effects of transition metals and phosphate buffer were also evaluated using 0.25mL metal solution and 0.25mL phosphate buffer. Absorbance readings at 620nm were taken in 5 minute intervals for 60 minutes to back out dye concentrations as a function of time. All reactions proceeded under ambient light and room temperature conditions. Heat effects were assessed by placing samples containing 0.5mL [$5x10^{-5}$]M Brilliant Blue, 0.5mL phosphate buffer (pH 7.4), and 0.5mL 50% hydrogen peroxide in an incubator under ambient light and various temperatures while light effects were assessed under room temperature and various wavelengths. Again, absorbance readings were taken in 5 minute intervals for 60 minutes.

2.5.2 Liquid peroxide model

Sand-blasted, stained tooth samples are first air-dried and evaluated using a spectrophotometer (Konica Minolta, Ramsey, NJ). They are subsequently immersed in conical tubes containing 10mL phosphate buffer (pH 7.4) and 5mL 50% hydrogen peroxide under ambient light and room temperature. For temperature studies, the conical tubes were submerged in water baths of various temperatures. To assess light effect, the 4 tooth samples were placed on a petri dish containing 40mL phosphate buffer (pH 7.4) and 20mL 50% hydrogen peroxide so that the tooth samples were completely submerged with a minimal liquid layer above each tooth, limiting the effects of light scattering. The entire petri dish sat on a water bath to minimize the

temperature effects of light and the setup sat on a black backdrop to maximize absorption. Spectrophotometry readings were taken at the 30 minute and 60 minute time points.

2.5.3 Gel peroxide model

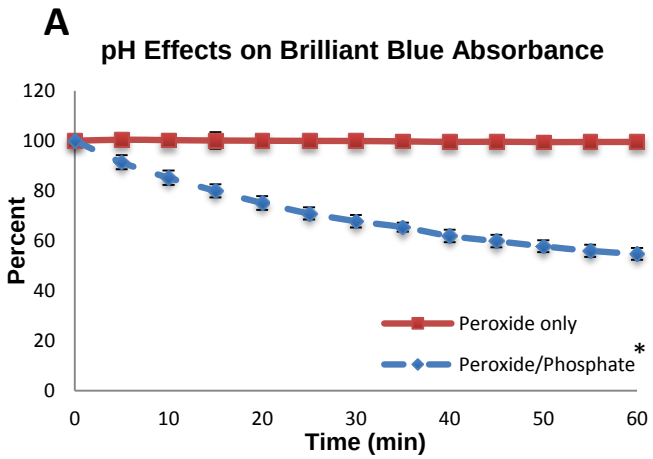
Similar to the liquid peroxide mode, the sand-blasted, stained tooth samples are air-dried and evaluated using spectrophotometry. Each bovine tooth was coated with 1mL 16% NiteWhite carbamide peroxide gel (Discus Dental, Culver City, CA) (pH 5.5) on the enamel surface and left to react under ambient light and room temperature conditions. Color measurements were taken at the 30 and 60 minute time points with the carbamide peroxide gel reapplied after the 30 minute reading. Samples were reacted at 35°C and 40°C in an incubator for temperature dependent experiments and light studies were setup in the same manner as the liquid peroxide model with 4 gel-coated tooth samples sitting on a petri dish, surrounded by water on a black backdrop. Again, spectrophotometry readings were taken at the 30 minute and 60 minute time point with gel reapplication.

Part 3 – Results

3.1 – Reaction rates of hydrogen peroxide with chemical model stain

3.1.1 Alkaline pH (7.4) optimal for Brilliant Blue degradation

Brilliant Blue experienced minimal degradation of 1% after 60 minutes of reaction with hydrogen peroxide. Since peroxide becomes unstable under alkaline pH levels, adding basic disodium phosphate buffer to the solution of dye and peroxide (pH 6.5) significantly increased bleaching efficiency. A pH range of 7.3-7.5 was tested to assess upper limit of physiologically relevant pH levels of the oral environment and the pH effects appeared to plateau around pH 7.4 (Fig. 1B), an acceptable alkaline level for normal oral environments. When comparing dye degradation between buffered and non-buffered solutions, buffered solutions experienced 45% greater bleaching efficiency (Fig. 1A) with a reaction time constant of $3x10^{-2}$ s^{-1} (Table 3). All subsequent experiments using disodium phosphate buffer were conducted at pH 7.4.



B

pH Level (t = 0h)	Percent Absorbance Decrease (t = 1h)
6.50 (without phosphate)	-0.18
7.33	39.17
7.40	45.07
7.51	48.62
7.52	**50**
7.53	49.82

Figure 1 pH effects on Brilliant Blue degradation with hydrogen peroxide A.) Comparing buffered and non-buffered samples using 0.5mL [$5x10^{-5}$]M Brilliant Blue and 0.5mL 50% H_2O_2 with 0.5mL water or 0.5mL disodium phosphate buffer (pH 7.4) (n=4 per group) B.) Overall bleaching effects under various pH conditions (n=4 per group). *Denotes statistical significance (student's t-test $p<0.05$)

3.1.2 Metal catalysts show minimal improvement of peroxide bleaching

Based on the Beer's law analysis, adding ferrous gluconate and manganese(II)-nitrate tetrahydrate in place of phosphate buffer yielded reaction time constants of $-2x10^{-4}$ s^{-1} and $4x10^{-4}$ s^{-1} respectively. In the presence of both buffer and transition metal, the reaction time constants were $1.5x10^{-2}$ s^{-1} for iron-phosphate samples and $1.4x10^{-2}$ s^{-1} for manganese-phosphate samples (Table 3). Phosphate buffered samples experienced 43.3±2.5% and 44.9±5.5% more bleaching after 60 minutes compared to non-buffered iron-treated samples (Fig. 2A) and non-buffered manganese-treated (Fig. 2B) samples respectively. In both cases, buffer alone showed greater bleaching effect than the combinatory effect of buffer and transition metals.

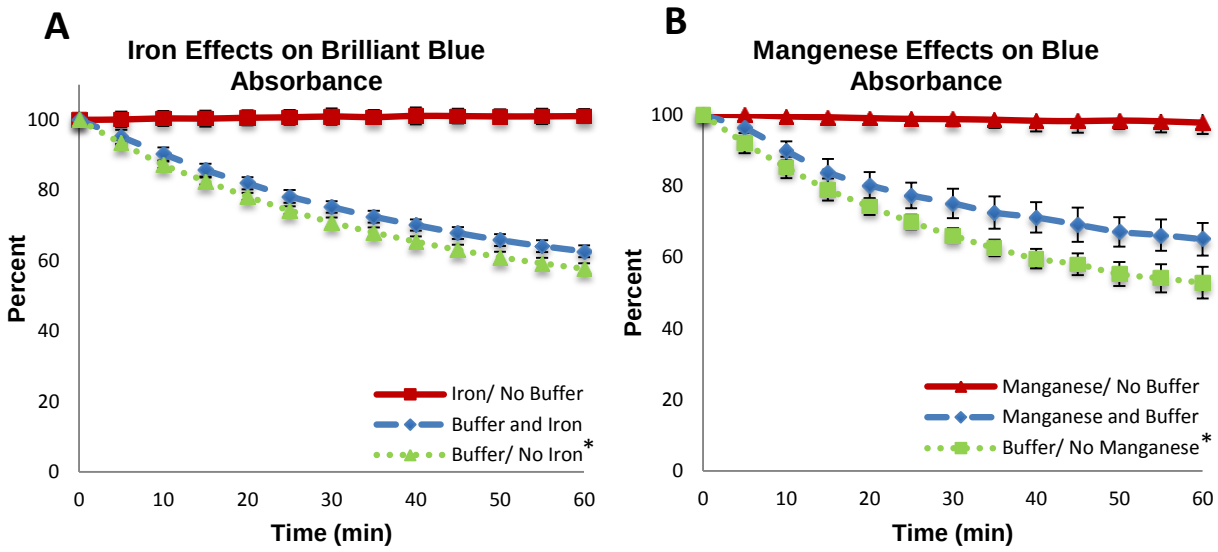


Figure 2 Transition metal effects on Brilliant blue degradation with hydrogen peroxide. Bleaching effects in the presence and absence of A.) iron and B.) manganese with and without buffer. Samples used 0.5mL [$5x10^{-5}$]M Brilliant Blue, 0.5mL 50% H_2O_2 with or without 0.5mL of 0.005%wt/wt ferrous gluconate or 10mM manganese tetrahydrate and 0.5mL disodium phosphate buffer (pH 7.4) (n=4 per group).*Denotes statistical significance (student's t-test $p<0.01$).

3.1.3 35°C temperature ideal for peroxide reaction

Using the disodium phosphate buffered system, the effects of temperature can be seen in Fig. 3 with the optimal temperature around 35°C. A one-way ANOVA test showed significant differences between the groups ($p<0.01$) with the 35°C treatment exhibiting 11.5±3.1% greater bleaching efficacy than room temperature conditions. Comparing the reaction time constants also shows a 10 fold increase in bleaching efficiency (Table 3). Surprisingly, samples heated to 40°C showed slightly less efficient bleaching than setups ran at 35°C and only slightly more efficient than room temperature treatments with overall bleaching percentages of 45.2±2.4%, 55.1±2.9%, 56.7±2.0%, and 50.8±8.8% after 60 minutes for room temperature, 30°C, 35°C, and 40°C treatments respectively (Fig. 3).

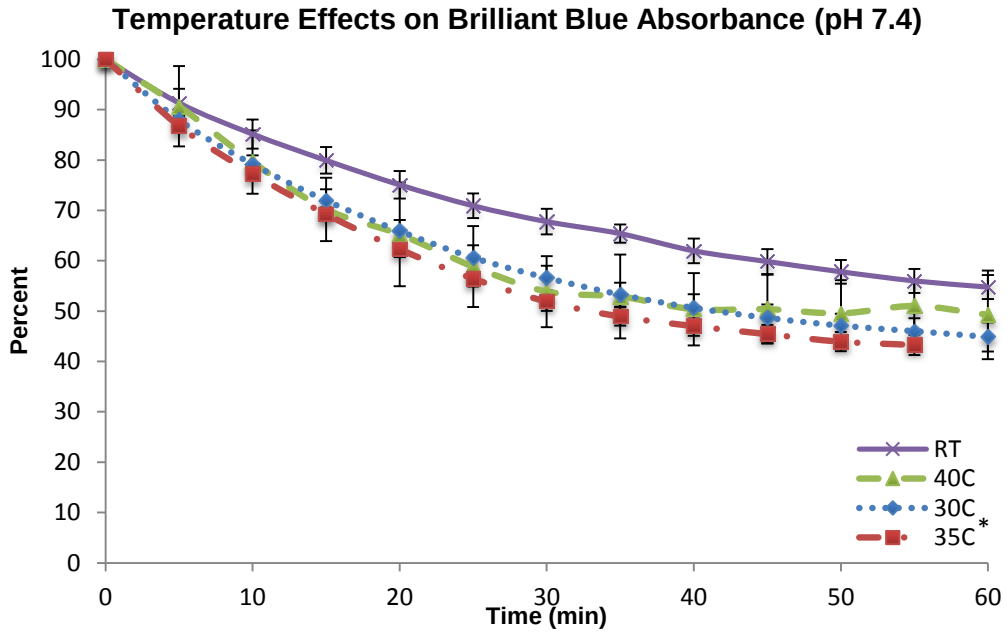


Figure 3 Temperature effects on Brilliant Blue degradation with hydrogen peroxide using a 1:1:1 solution of [$5x10^{-5}$]M Brilliant Blue, 50% H_2O_2 and disodium phosphate buffer (pH 7.4) in an incubator setup (n=4 per group). *Denotes statistical significance (student's t-test $p<0.05$)

3.1.4 490nm light irradiation enhances Brilliant Blue breakdown

The effects of light on phosphate buffered and non-buffered Brilliant Blue samples were assessed at 415nm, 450nm, 470nm, 490nm, and using the Zoom!™ dental whitening lamp (Table 3). Light irradiation effects were influenced by the presence and absence of phosphate in the system (Table 2). A one-way ANOVA showed significant difference between treatments ($p<0.01$ in the presence and absence of buffer). Without pH modification, 415nm irradiation had the greatest catalytic effect, increasing bleaching efficiency by 23.2±2.1% after 60 minutes. Under basic conditions, however, 490nm light showed the most catalytic potential, increasing bleaching efficiency by 14.0±3.1% after 60 minutes. Additionally, the Zoom™ dental lamp exhibited no enhancing effect in both buffered and non-buffered samples. The reaction rate constant of Brilliant Blue with 490nm treatment was twice that of samples under ambient light conditions and all other wavelengths including the Zoom™! show minimal differences in reaction rate (Table 3).

Table 2 Percent absorbance decrease after 60 minutes using various light-activation treatments. Samples used a 1:1:1 solution of [$5x10^{-5}$]M Brilliant Blue, 50% H_2O_2 and disodium phosphate buffer (pH 7.4) under various light wavelengths (72mW) (n=4 per group). *Denotes statistical significance (student's t-test $p<0.05$)

Condition	Ambient Light	415nm	450nm	470nm	490nm*	Zoom™!
Without Buffer	0.5±0.6	23.6±2.0	7.9±1.3	-15.6±5.1	5.1±0.4	-17.5±2.3
With Buffer	45.2±1.1	46.3±1.1	43.8±1.1	53.0±2.8	59.2±1.2	50.5±3.1

Table 3 Reaction time constants of Brilliant Blue with hydrogen peroxide using a first-order analysis for various treatment conditions. Rates derived from standard curve and Beer's law calculations. *Denotes statistical significance (student's t-test $p<0.05$)

Condition	With Buffer (s^{-1})	No Buffer (s^{-1})
No Treatment	$3.1x10^{-2}$	Near 0
Iron	$1.2x10^{-2}$	Near 0
Manganese	$1.4x10^{-2}$	$4x10^{-4}$
30°C	$8.5x10^{-2}$	---
35°C*	$1.4x10^{-1}$	---
40°C	$4.3x10^{-2}$	---
415nm	$3.6x10^{-2}$	---
450nm	$3.3x10^{-2}$	---
470nm	$5.2x10^{-2}$	---
490nm*	$6.9x10^{-2}$	---
Zoom™!	$3.7x10^{-2}$	---

3.2 – Bleaching efficacy of liquid hydrogen peroxide on bovine teeth

Based on the results from the Brilliant Blue model and the controversy in literature, the effects of light and temperature on teeth were the primary focus of the teeth models.

3.2.1 35°C heat treatment enhances teeth whitening under moderate peroxide concentrations

Initial temperature studies assessed the LaB measurements and overall color change, ΔE, for a 16% hydrogen peroxide treatment of tea-stained bovine teeth under room temperature and at 35°C (optimal bleaching temperature for the Brilliant Blue model). Heat appears to have catalytic effect at after both 30 minutes and 60 minutes of reactivity (Fig. 4). When the liquid peroxide model was extrapolated to various peroxide concentrations, heat still had a significant catalytic effect after 30 minutes of reactivity at both higher (25% H_2O_2) and lower concentrations

(5.9% H_2O_2). However, after 60 minutes of treatment, heat did not increasing bleaching when tooth samples were treated with lower peroxide concentrations.

Dissecting the overall color change, ΔE, into the LaB components further reveals that heat substantially enhances tooth luminosity (L) at all peroxide concentrations after 30 minutes of treatment and after 60 minutes at moderate to high peroxide levels. Heat only reduced yellowness (b) under moderate to high peroxide conditions and had no catalytic effect on red-green color change (a) in any treatment condition (Supplemental Table 1).

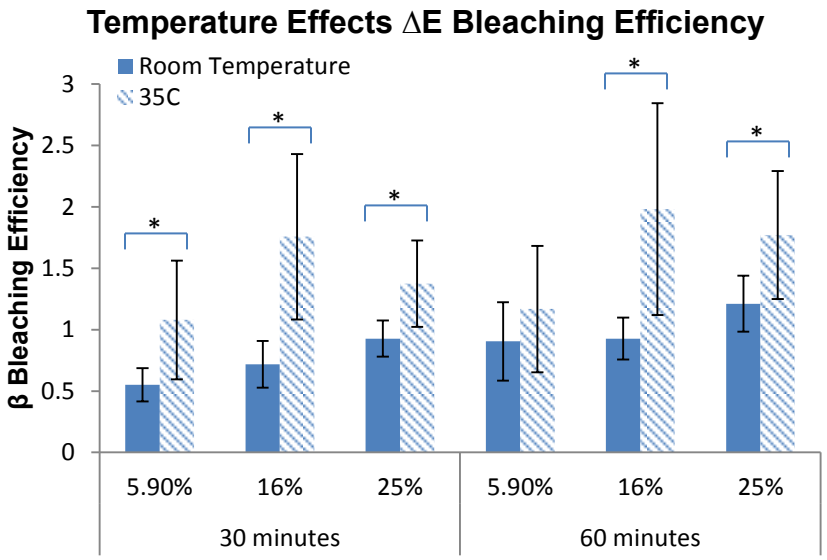


Figure 4 Temperature effects on the bleaching efficacy using the liquid peroxide tooth model under various peroxide concentrations. ΔE values are calculated based on LaB measurements at 30 and 60 minute time points. Tooth samples treated with 2:1 volume of phosphate buffer (7.4) and peroxide. (n=8 per treatment) . *Denotes statistical significance (student's t-test $p<0.05$)

3.2.2 490nm light irradiation has not catalytic effect on teeth bleaching

Light irradiation studies evaluated LaB measurements and overall color change, ΔE, for 16% hydrogen peroxide treated, tea-stained bovine teeth under ambient light and at 490nm (the optimal bleaching wavelength for Brilliant Blue) conditions. By placing the tooth samples in a

water bath setup, temperature effects were minimized with an overall surface enamel temperature change of 1.1°C after 60 minutes of 490nm treatment. The 490nm light irradiation showed no catalytic effect on bleaching at all time points (Fig. 5). The same results were seen when the liquid peroxide model was extrapolated to higher and lower peroxide concentrations.

When assessing individual LaB components of tooth samples, 490nm light treatment only significantly decreased yellowness after 60 minutes of reactivity with 25% hydrogen peroxide (student's t-test, $p<0.05$). Luminosity and red-green color were not affected by the wavelength specific light treatment (Supplemental Table 1).

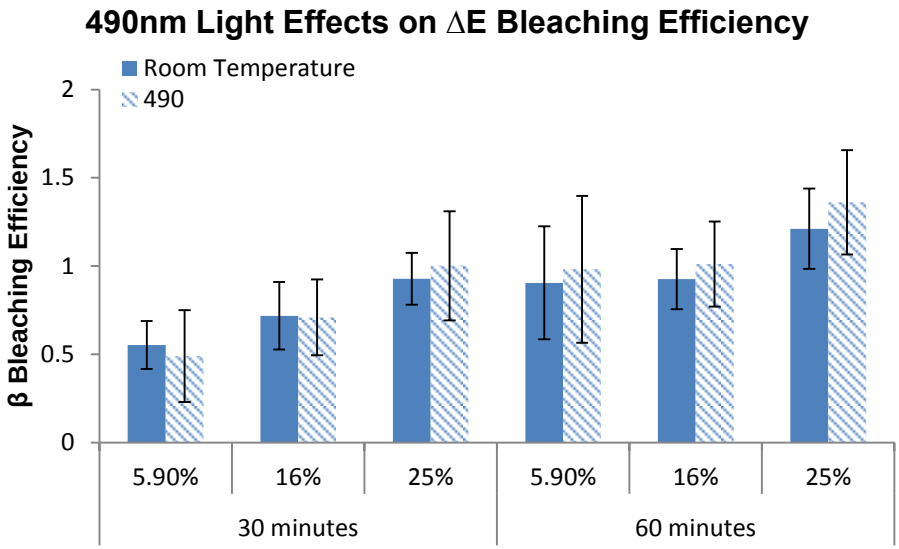


Figure 5 Light effects on the bleaching efficacy of the liquid peroxide tooth model using various peroxide concentrations. ΔE values are calculated based on LaB measurements at 30 and 60 minute time points. Tooth samples treated with 2:1 volume of phosphate buffer (7.4) and peroxide. (n=8 per treatment). No statistically significant differences are seen between control and light treatment groups.

3.3 – Bleaching efficacy of gel peroxide on bovine teeth

3.3.1 Both 35°C and 40°C heating increases overall whitening effect of peroxide gels

Heat effects on overall color change, ΔE, of tea-stained bovine teeth under gel peroxide treatment were studied at room temperature and at 35°C. Heat appears to have catalytic effect only after the full 60 minute treatment (Fig. 6). Increasing the temperature to 40°C yielded the same results with no significant bleaching enhancement at the 30 minute time point but noticeable catalytic effect after 60 minutes. Performing a one-way ANOVA shows statistically significant differences in overall bleaching across the three temperature groups ($p<0.05$).

Upon evaluating the individual LaB components of color, heat does not appear to enhance luminosity or red-green color changes at higher temperatures. However, at 40°C, heat significantly improves yellowness after 60 minutes of 16% hydrogen peroxide treatment (Supplemental Table 2).

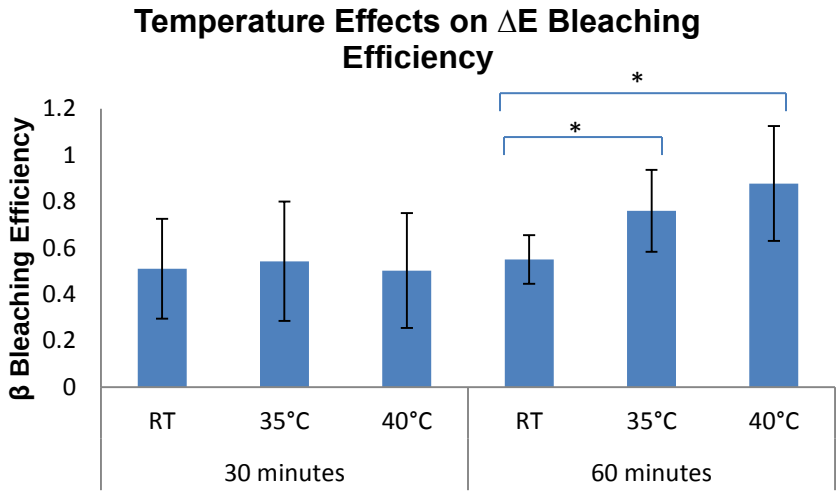


Figure 6 Temperature effects on the bleaching efficacy of the gel peroxide tooth model at 35°C and 40°C. ΔE values are calculated based on LaB measurements at 30 and 60 minute time points. Tooth samples treated with 1mL – 16% carbamide peroxide NiteWhite Discus Dental gel in an incubator setting. (n=8 per treatment). *Denotes statistical significance (student's t-test $p<0.05$)

3.3.2 490nm light irradiation does not improve gel peroxide whitening

Initial light treatment studies assessed overall color change, ΔE, for tea-stained bovine teeth under gel peroxide treatment in ambient light and 490nm light conditions (Fig. 7). No enhanced bleaching was seen after 60 minutes of 490nm treatment compared to ambient light conditions, verified with the student's t-test ($p>0.05$). When the gel peroxide model was extrapolated to higher and lower wavelength treatments ranging from 415nm to 530nm, no catalytic effect was seen at either time point.

Luminosity was also only enhanced after 60 minutes of 490nm treatment, showing parallel color trends with the overall color change. 490nm wavelength treatment significantly reduced tooth yellowness after 30 minutes and 60 minutes of reaction while no improvement was seen using other light wavelengths (Supplemental Table 2).

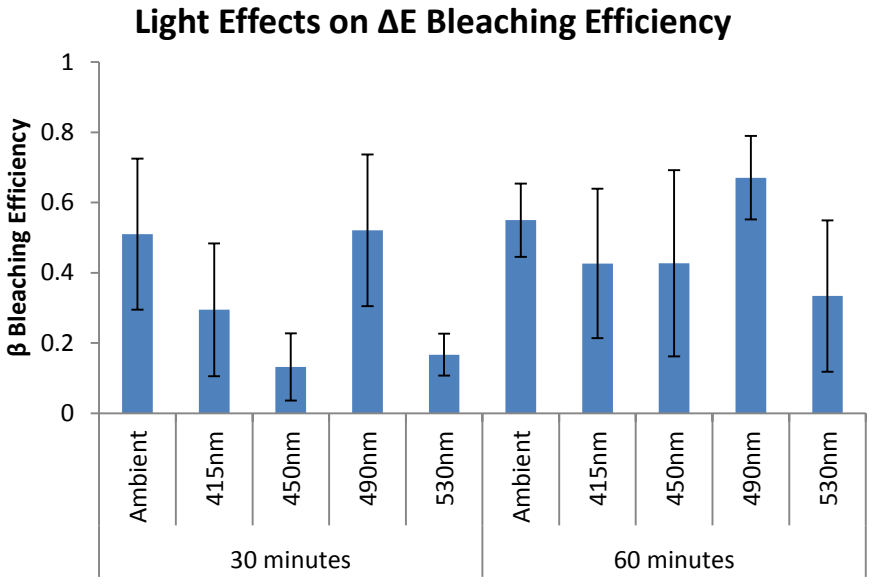


Figure 7 Light effects on the bleaching efficacy of the gel peroxide tooth model at various wavelengths (72mW). ΔE values are calculated based on LaB measurements at 30 and 60 minute time points. Tooth samples treated with 1mL – 16% carbamide peroxide NiteWhite Discus Dental gel in an incubator setting. (n=4 for 415nm, 450nm, 530nm; n=8 for ambient and 490nm).

Part 4 – Discussion

4.1 – Validity of chemical model stain

4.1.1 Validity of assumptions and simplifications

While Brilliant Blue is not a clinically found in vivo stain, it proves to be a strong candidate for modeling the reaction kinetics of hydrogen peroxide bleaching under various parameters. Previous studies have researched its bleaching kinetics for textile purposes [45] [46][47]and more recently, Brilliant Blue has served as a potential for enhancing photoelectrocatayltic activity in nanotubules [48]. For this study, its highly pigmented nature allows for sensitive and precise detection in a simple reaction setup. The conjugated ring structure provides comparable chemical reactivity with polyphenol extrinsic stains [3] [49] and the molecular weight of 792.85g/mol allows for diffusion of the compound through the enamel pores (10-300Å in radius) [50].

A liquid chemical stain reaction chamber yields relevant degradation profiles, comparable to stain degradation on tooth enamel surfaces because the diffusion rate of peroxide is much slower than the reaction rate of bleaching. Thus, for the time scales of interest, diffusion can be considered minimal.

4.1.2 Validity of reaction time constants derived

Although previous studies have reported the reaction rate of hydrogen peroxide to be 10^{-4} s^{-1} [51], the experimental reaction time constants ranging from $4x10^{-4}$ s^{-1} to $1.4x10^{-1}$ s^{-1} are realistic based on the conditions used in the reaction. The conditions used for the reported reaction rates are not stated and as shown in this study, the reaction time constant of hydrogen peroxide can fluctuate significantly with changes with pH, temperature, light conditions, and presence of catalytic metals (Table). pH changes showed the most influential effect as seen in the conversion of a non-reacting system to a first-order reaction with a reaction time constant of $3x10^{-2}$ s^{-1} when the acidic environment became alkaline in nature. Furthermore, adding heat showed the potential to increase reaction rate by another order of magnitude.

4.1.3 Comparison of experimental data to existing experimental data

The reaction kinetics of Brilliant Blue with hydrogen peroxide under specific parameter adjustments has not been studied. However, several have investigated the role of titanium oxide (TiO_2), UV radiation, and hydrogen peroxide on blue dyes. Muneer et al found reaction rates on the order of 10^{-3} s^{-1} for photocatalytic degradation of disperse blue 1 with optimal conditions using a combination of UV, TiO_2, and H_2O_2 [52]. Similar reaction time constants were found by the Sikarwar group where the influence of pH appeared to play the most dominant role in influencing reactivity [53]. These reaction time constants are relatively comparable to those obtained in this study given the range of parameter influences.

Farajzadeh et al found that the addition of iron nitrate (above a given threshold) to a solution of peroxide and Brilliant Blue significantly enhances the reaction rate due to the presence of Fenton reactions, catalyzing the formation of radicals [54]. While they observed an order of magnitude change in reaction rate, another study presents conflicting results suggesting that the introduction of iron alone does not have any enhancing effect [3]. No effect was seen in this study, potentially due to the differences in oxidation state and the presence of an alkaline buffer can potentially mask the effect of iron.

4.2 – Validity of the bovine teeth model

4.2.1 Justification of sand blast preparation

Variation in surface roughness of tooth samples creates discrepancies in degree of stain retention, making it difficult to assess bleaching efficiency across samples. Previous studies have shown that increased surface roughness increases plaque formation [55]; thus, an ideal sample set would contain homogenous, high surface roughness. Clinically, enhanced surface roughness is needed for binding resin composite materials [56] [57].

Two studies have shown the advantages of using an acid-etching method over a laser ablation method to achieve the desired surface topology for enhanced restorative material/enamel bond strength [58][59]. Furthermore, Retief et al has shown that etching increases the wettability of the enamel surface [60]. However, after acid etching the enamel surface with phosphoric acid gel, the tooth surface contained heterogeneous indentations and occasional micro-cracks as seen in SEM images (Fig. 8B).

Sandblasting, an alternative method to enhancing surface roughness [61], yielded more homogenous tooth samples with minimal grooves and micro-cracks (Figure). Treated teeth retained tea stain in comparable degrees, providing a standardized method for sample preparation (Fig. 8C).

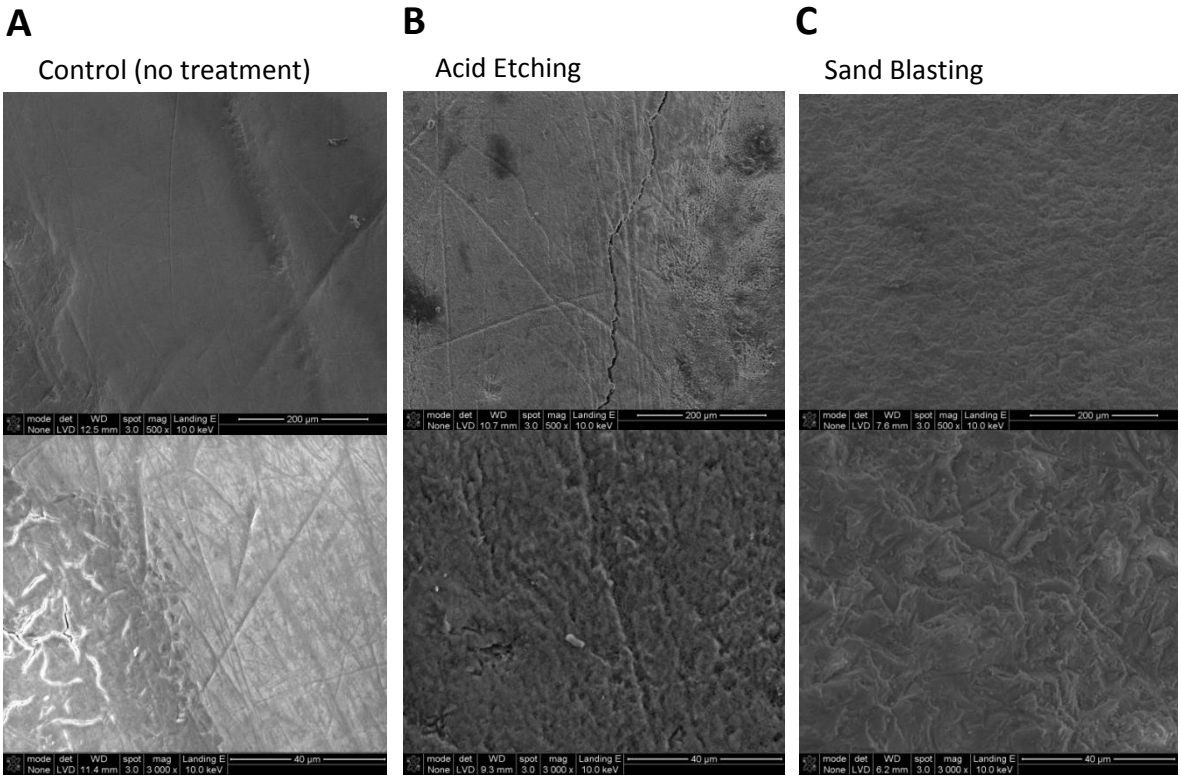


Figure 8 Scanning electron microscope images of bovine teeth enamel at 3000x and 500x magnification A.) without treatment B.) treated with phosphoric acid C.) treated with sandblasting. (n=2 per treatment)

4.2.2 Significance of LaB measurements

Color determination in teeth is often highly subjective due to difficulty in precise quantitative measurements. Unlike chromagen solutions such as Brilliant Blue, tooth color cannot be evaluated using single wavelength UV spectroscopy and must cover a wide range of possible color values. Thus, clinical standards utilize the quantitative CIE-Lab parameters,

obtained using spectrophotometry, and qualitative Vita Shade Guide [44]. By using a diffused illuminating integrating sphere system, the spectrophotometer can illuminate and sense both the wavelength, intensity, and angle of the reflected light providing a readout of sample luminosity, red-green color, and yellow-blue color (Konica Minolta site). While the spectrophotometer provides insight into individual parameters, deriving a reaction rate constant from the color measurements is difficult and potentially inaccurate. Subtle changes in sample positioning and ambient lighting can create noise between measurements. Furthermore, the overall color value, ΔE, is a function of three changing individual changing parameters. Thus, current standards report individual LaB values when measuring color [62][63].

4.3 – Inability to ignore diffusion

While the reaction rate obtained in this study is significantly larger than previously reported diffusion rate constants of peroxide through enamel [24] or water through enamel [64], the diffusion component cannot be completely ignored. Due to the viscous nature of in-office whitening gels, there is the presence of a lag time for the peroxide to diffuse out of the gel and into the enamel. Furthermore, reaction and diffusion of peroxide occur in an interdependent manner as the peroxide must approach the vicinity of the stain while the degradation occurs along the diffusion path [42]. Although the primary focus of this study is to quicken the reaction rate of bleaching, a more comprehensive approach would catalyze the rate of diffusion as well. Increasing temperature [65] and adding light activation [66] both appear to quicken diffusion of peroxide.

4.4 – Clinical significance

Based on the three models, there are several implications that may be suitable for improving clinical teeth whitening.

4.4.1 pH influence

pH plays a major role in determining the degree of reactivity but it must be carefully balanced to allow for sustained peroxide reactivity. Although alkaline conditions may appear to be favorable for bleaching, shelf-life of bleaching products and burst release of radicals must also be considered. pH levels of teeth whitening products have been found to range from 3-11 but surprisingly, most in-office bleaching products and dentist-supervised home-bleaching products have acidic pH levels ranging from 5-6 while over-the-counter bleaching products have alkaline pH levels [67].

Based on the results of this study, an ideal system would utilize slightly basic gel peroxides (around 7.4) for enhanced radical formation with maximal compatibility to oral environments.

4.4.2 Temperature influence

While temperature behaviors do not appear to directly follow the theoretical Arrhenius equation of reactivity, it does have a catalytic effect in improving bleaching. Increasing treatment temperature shows favorable color change and while the effect plateaus around 35°C for Brilliant

Blue, the same trends are not seen in gel peroxide model. This discrepancy is likely due to the differences between liquid peroxide and gel peroxide, where the gel bleaching is further influenced by viscosity and diffusion. Furthermore, the temperature heating effects appear to plateau after peroxide concentrations hit a threshold, as seen in the liquid peroxide model. Presumably, this is due to the fact that the high concentration peroxide system has already reached a saturated concentration of free radicals and the generation of more reactive species would not produce a significant effect.

Results from the gel peroxide model point to a 40°C treatment as the optimal condition. However, due to the sensitive nature of soft tissue in the oral environment and the potential for thermally induced damage, clinical whitening treatments should not exceed 5.6°C [68] above normal oral temperatures of 36°C [69].

4.4.3 Light influence

Several literature studies have reported catalytic effects light activated bleaching [40][70][71], while others report no advantages of light activated systems [72][71] and inconclusive results [41]. The heated controversy regarding the effects of light may lie in the close relationship between light activation and heat since the use of light has reportedly increased pulpal temperatures by 6°C – 11.7°C [73]. Buchalla et al proposes that light-activated systems actually rely on heat effects as the main mechanism of action for bleaching procedures as a small fraction of the light projected onto the bleaching agent is absorbed and converted to heat [41].

The results of this study after decoupling the effect of heat and light through the use of a water bath setup shows that light alone lacks catalytic effect in the 415-530nm (72mW) range.

Part 5 – Conclusions and future directions

5.1 – Conclusions

Based on the findings of the three models, the following conclusions can be made:

1. Brilliant Blue serves as a suitable chemical stain model, comparable to in vivo tooth stains, for studying the reaction kinetics of hydrogen peroxide.
2. The reaction rate of hydrogen peroxide is most heavily influenced by pH effects with optimal bleaching under alkaline environments.
3. Heat activation, rather than light irradiation, plays a catalytic role in improving bleaching efficacy with ideal temperatures at the upper threshold of the oral cavity.

5.2 – Optimizing tooth whitening parameters

5.2.1 Controlling reaction

Now that the optimal parameters for reaction have been experimentally derived, an ideal bleaching system would allow for increased and sustained stain degradation on the enamel and dentin level with minimal free radical activity in the pulp chamber. A potential platform to better control reactivity across the layers would utilize a temperature gradient—heating the external side of the tooth while simultaneously cooling the back side of the tooth during in-office procedures. By doping gels with colorants and matching specific wavelengths, light adsorption, and therefore heat conversion, would increase [41]. Lasers can also be used for high intensity

heat and pulpal cooling can be achieved by flowing cold water across the back side of the teeth. Together, the simultaneous heating and cooling could potentially enhance whitening while reducing the risk of side-effects, thereby leading to shorter treatment times. Further clinical studies may be needed to optimize bleaching temperatures without compromising pain and safety thresholds.

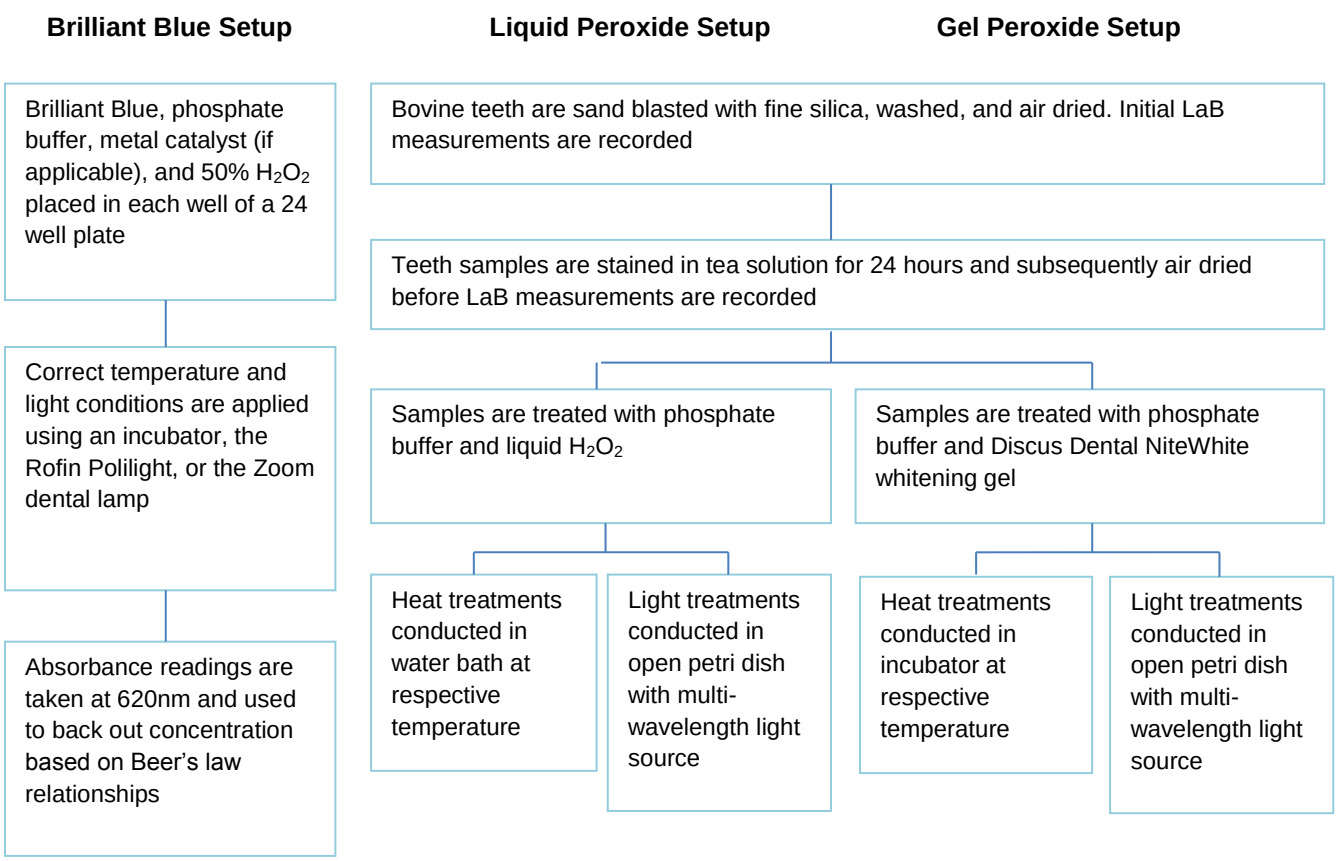
5.2.2 Controlling diffusion

Since hydrogen peroxide reaction occurs quickly relative to diffusion, it is sensible to target diffusion for controlling mass transport. With the diffusion portion of hydrogen bleaching as the rate limiting step, increasing diffusivity of peroxide through porous enamel and dentin would significantly enhance bleaching rates. The application of a heating and cooling system would theoretically also increase diffusion on the enamel side and slow, stop, or reverse diffusion on the pulpal surface, as diffusion has shown to be enhanced under thermal heating and discouraged under cooling. Altogether, the setup would prevent peroxide from reaching the pulp chamber and further prevent risk of pulpal damage and tooth sensitivity. Mathematical models using heat transfer theory and mass transport laws can be created to predict the temperature of the tooth at various layers within the teeth. Combining the heat map with a peroxide concentration profile can help determine clinically relevant and safe parameters for optimal bleaching.

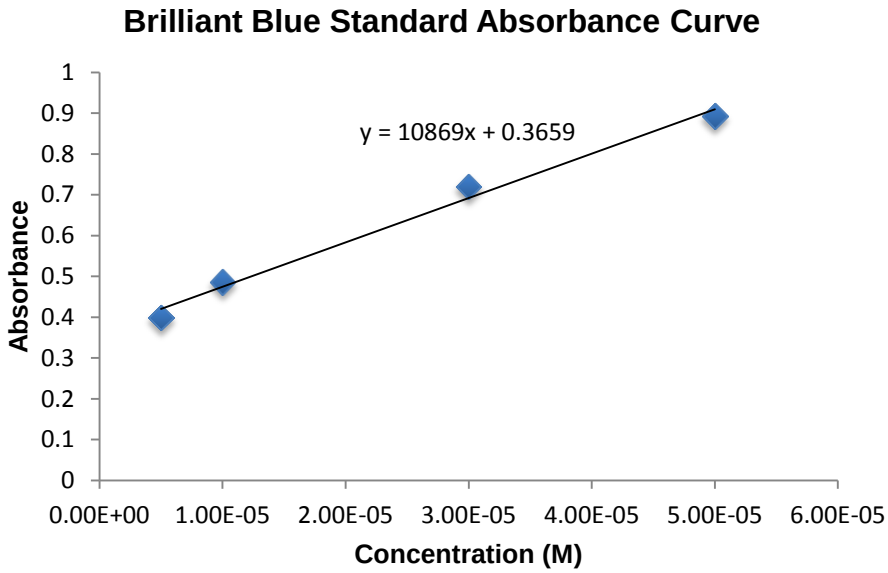
5.2.3 Iontophoresis

A potential alternative to controlling diffusion and reaction is the use of iontophoresis. Although it is traditionally used for transdermal drug delivery [74], the application can be used for active transport of peroxide for dental whitening procedures. Iontophoresis utilizes the application of an electric potential to a drug-filled transdermal patch, driving the charged drug molecules across the skin. If charged catalytic ions such as iron were introduced to peroxide gels, the ions could serve as both a pulling agent, adding the diffusion of peroxide, as well as a reaction catalyst, increasing in the rate of bleaching. Furthermore, the system can be tuned based on the ion charge, size, and applied potential (positive or negative) to selectively enhance bleaching on the external surface of the tooth while minimizing peroxide effects near the pulp chamber.

Supplemental Figures



Supplemental Figure 1 Basic schematic for the experimental setup of all conditions



Supplemental Figure 2 Standard curve for Brilliant Blue relating concentration and absorption at 620nm.

Time Point	[H_2O_2]	Treatment	L	a	B
30 minutes	5.9%	RT & Ambient	0.6±0.1	0.8±0.2	0.6±0.2
		35°C	1.0±0.3	1.0±0.3	0.9±0.2
		490nm	0.5±0.2	0.6±0.8	0.5±0.1
	16%	RT & Ambient	0.8±0.1	1.0±0.2	0.8±0.2
		35°C	1.4±0.3	1.0±0.1	1.1±0.3
		490nm	0.8±0.2	0.8±0.3	0.8±0.3
	25%	RT & Ambient	0.9±0.1	-1.0±3.0	0.9±0.5
		35°C	1.3±0.3	1.9±0.6	1.2±0.4
		490nm	0.9±0.3	-1.9±6.7	0.9±0.3
60 minutes	5.9%	RT & Ambient	0.9±0.2	0.9±0.3	0.8±0.1
		35°C	1.1±0.4	1.0±0.3	1.0±0.3
		490nm	0.9±0.3	0.8±1.4	0.8±0.1
	16%	RT & Ambient	1.0±0.1	1.1±0.2	1.1±0.2
		35°C	1.5±0.4	0.9±0.0	1.2±0.3
		490nm	1.0±0.1	1.1±0.3	1.2±0.3
	25%	RT & Ambient	1.1±0.1	-1.0±3.2	0.7±0.3
		35°C	1.6±0.4	1.8±0.8	1.2±0.4
		490nm	1.3±0.2	-3.5±8.8	1.1±0.3

Supplemental Table 1 LaB measurements of liquid peroxide model tooth samples under given conditions. Measurements were taken on air dried samples at respective time points.

Time Point	Condition	Treatment	L	a	B
30 minutes	Control	RT & Ambient	0.5±0.2	0.9±0.1	0.5±0.1
	Temperature	35C	0.5±0.3	0.9±0.1	0.6±0.1
		40C	0.5±0.3	1.3±4.0	0.2±0.5
	Light	415nm	0.4±0.3	-1.8±2.9	0.8±0.4
		450nm	0.2±0.0	0.8±0.2	0.2±0.1
		490nm	0.5±0.2	1.0±0.1	0.6±0.0
		530nm	0.2±0.0	1.5±1.0	0.2±0.1
60 minutes	Control	RT & Ambient	0.6±0.1	0.9±0.1	0.6±0.1
	Temperature	35C	0.8±0.2	0.9±0.0	0.7±0.1
		40C	0.9±0.3	0.8±3.5	0.1±0.4
	Light	415nm	0.5±0.3	-2.7±3.7	0.8±0.3
		450nm	0.5±0.2	1.1±0.1	0.5±0.1
		490nm	0.7±0.1	1.0±0.1	0.9±0.0
		530nm	0.3±0.3	-2.0±2.8	0.2±0.2

Supplemental Table 2 LaB measurements of liquid peroxide model tooth samples under given conditions. Measurements were taken on air dried samples at respective time points.

References

[1] S. Camargo, M. Valera, C. Camargo, M. Mancini, M. Menezes, Penetration of 38% hydrogen peroxide into the pulp chamber in bovine and human teeth submitted to office bleach technique. *Journal of endodontics* **33**(9) 1074–7 (2007).

[2] C. Coldebella, A. Ribeiro, N. Sacono, F. Trindade, J. Hebling, C. Costa, Indirect cytotoxicity of a 35% hydrogen peroxide bleaching gel on cultured odontoblast-like cells, *Brazilian Dental Journal*. **20**(4) 267–274 (2009).

[3] B. Lee, S. Huang, Y. Chiang, Y. Chien, C. Mou, C. Lin, Development of in vitro tooth staining model and usage of catalysts to elevate the effectiveness of tooth bleaching, *Dental materials : official publication of the Academy of Dental Materials*. **24**(1) 57–66 (2008).

[4] J. Bosch and J. Coops, Tooth Color and Reflectance as Related to Light Scattering and Enamel Hardness. *Journal of Dental Research* **74**(1) 374–380 (1995).

[5] P. Sun, J. Pan, Y. Tian, N. Bai, H. Wu, L. Wang, C. Yu, J. Zhang, Tooth Whitening with Hydrogen Peroxide Assisted by a Direct-Current Cold Atmospheric-Pressure Air Plasma Microjet. *IEEE Transactions on Plasma Science* **38**(8) 1892–1896 (2010).

[6] A. Joiner, The bleaching of teeth: a review of the literature. *Journal of dentistry* **34**(7) 412–9 (2006).

[7] K. Kawamoto and Y. Tsujimoto, Effects of the Hydroxyl Radical and Hydrogen. *Journal of Endodontics* **300**(9) 45-50 (2004).

[8] C. F. Pinto, V. Cavalli, M. Giannini, Peroxide bleaching agent effects on enamel surface microhardness , roughness and morphology. *Braz Oral Res* **18**(4) 306–311 (2004).

[9] B. a Matis, M. a Cochran, and G. Eckert, Review of the effectiveness of various tooth whitening systems. *Operative dentistry* **34**(2) 230–235 (2009).

[10] L. Giachetti, F. Bertini, C. Bambi, M. Nieri, D. S. Russo, A Randomized Clinical Trail Comparing At-Home and In-Office Tooth Whitening Techniques: A Nine-Month Follow-up. *Journal of the American Dnetla Association* **141**(11) 1357-1364 (2010).

[11] J. B. da Costa, R. McPharlin, R. D. Paravina, J. L. Ferracane, Comparison of at-home and in-office tooth whitening using a novel shade guide. *Operative dentistry* **35**(4) 381–8 (2010).

[12] R. Zekonis, B.A. Matis, M. A. Cochran, S. E. Al Shetri, G. J. Eckert, T. J. Carlson, Clinical Evaluation of In-Office and At-Home Bleaching Treatments. *Operative Dentistry* **28**(2) 114–121 (2003).

[13] T. M. Auschill, E. Hellwig, S. Schmidale, A. Sculean, N. B. Arweiler, Efficacy, side-effects and patients' acceptance of different bleaching techniques (OTC, in-office, at-home). *Operative dentistry* **30**(2) 156–639 (2005).

[14] G. R. Mokhlis, B. a Matis, M. a Cochran, G. J. Eckert, A clinical evaluation of carbamide peroxide and hydrogen peroxide whitening agents during daytime use. *Journal of the American Dental Association* 31(9) 1269–77 (2000).

[15] A. Joiner, Whitening toothpastes: a review of the literature. *Journal of dentistry* **38**(2) 17–24 (2010).

[16] A. Joiner, C. J. Philpotts, S. R. Ricketts, L. Z. Collins, Tooth Whitening Effects Delivered from a Mouthwash. IADR Abstract. July 2010.

[17] M. Niwa and T. Sato, Polishing and whitening properties of toothpaste containing hydroxyapatite. *Journal of Materials Science: Materials in Medicine* **12** 277–281 (2001).

[18] D. C. Sarrett, Tooth Whitening Today. *JADA* **133** 1535–1538 (2002).

[19] iData Research. (2011), U.S. Market for Dental Hygiene and Oral Care 2011. www.idataresearch.net. 2013.

[20] British Academy of Cosmetic Dentists. (2009), BACD - Statistics. www.bacd.com. 2013.

[21] V. Swami, A. Arteche, T. Chamorro-Premuzic, A. Furnham, S. Stieger, T. Haubner, and M. Voracek, "Looking good: factors affecting the likelihood of having cosmetic surgery," *European Journal of Plastic Surgery*, vol. 30, no. 5, pp. 211–218, Dec. 2007.

[22] M. G. Jorgensen, W. B. Carroll, Incidence of tooth sensitivity after home whitening treatment. *JADA* **133** 1076-1081 (2002).

[23] R.M. Pohjola, W. D. Browning, S. T. Hackman, M. L. Myers, M. C. Downey, Sensitivity and tooth whitening agents. *J Esthet Restor Dent* **14**(2) 85–91 (2002).

[24] B. K. Petersen, Quantitative anaylsis of the diffusion of hydrogen peroxide through teeth.. UCLA Dissertations. 2012.

[25] R. H. Leonard, V. B. Haywood, C. Phillips, Risk factors for developing tooth sensitivity and gingival irritation associated with nightguard vital bleaching. *Quintessence international (Berlin, Germany)* **28**(8) 527–34 (1997).

[26] J. R. Schulte, D. B. Morrissette, E. J. Gasior, M. V Czajewski, The effects of bleaching application time on the dental pulp. *Journal of the American Dental Association* **125**(10) 1330–5 (1994).

[27] L. Tam, Clinical Trial of Three 10% Carbamide Peroxide Bleaching Products. *J Can Dent Assoc* **65** 201-205 (1999).

[28] J. O. Fugaro, I. Nordahl, O. J. Fugaro, B. a Matis, I. a Mjör, Pulp reaction to vital bleaching. *Operative dentistry* **29**(4) 363–8 (2004).

[29] J. E. Dahl and U. Pallesen, Tooth Bleaching--a Critical Review of the Biological Aspects. *Critical Reviews in Oral Biology & Medicine* **14**(4) 292–304 (2003).

[30] L. Zach and G. Cohen, Pulp response to externally applied heat. *Oral Surgery, Oral Medicine, Oral Pathology* **19**(4) 515–530 (1965).

[31] C. J. Tredwin, S. Naik, N. J. Lewis, C. Scully, Hydrogen peroxide tooth-whitening (bleaching) products: review of adverse effects and safety issues. *British dental journal*, **200**(7) 371–6 (2006).

[32] R. I. Vogel, Intrinsic and extrinsic discoloration of the dentition (a literature review). *Journal of oral medicine* **30**(4) 99–104 (1975).

[33] H. M. Eriksen and H. Nordbø, "Extrinsic discoloration of teeth. *Journal of clinical periodontology* **5**(4) 229–36 (1978).

[34] A. Kadman, M. Ganachari, B. Mahendra Kumar, S. Gurunath, Drug Induced Tooth Discolouration. *Internet Journal of Dental Science* **7**(2) (2009).

[35] A. Watts and M. Addy, Tooth discolouration and staining: a review of the literature. *British dental journal* **190**(6) 309–16 (2001).

[36] S. Nathoo, The chemistry and mechanisms of extrinsic and intrinsic discoloration. *J Am Dent Assoc* **128** 6S–10 (1997).

[37] F. N. Hattab, M. a Qudeimat, H. S. al-Rimawi, Dental discoloration: an overview. *Journal of esthetic dentistry* **11**(6) 291–310 (1999).

[38] M. Sulieman, An overview of tooth-bleaching techniques: chemistry, safety and efficacy. *Periodontology 2000* **48** 148–69 (2008).

[39] T. Attin, F. Paqué, F. Ajam, M. Lennon, Review of the current status of tooth whitening with the walking bleach technique. *International endodontic journal* **36**(5) 313–29 (2003).

[40] M. Tavares, J. Stultz, M. Newman, V. Smith, R. Kent, E. Carpino, J. M. Goodson, Light augments tooth whitening with peroxide. *JADA* **134** 167-175 (2003).

[41] W. Buchalla and T. Attin, External bleaching therapy with activation by heat, light or laser--a systematic review. *Dental materials : official publication of the Academy of Dental Materials* **23**(5) 586–96 (2007).

[42] M. Sulieman, M. Addy, E. MacDonald, J. S. Rees, The effect of hydrogen peroxide concentration on the outcome of tooth whitening: an in vitro study. *Journal of dentistry* **32**(4) 295–9 (2004).

[43] M. Sulieman, M. Addy, J. S. Rees, Development and evaluation of a method in vitro to study the effectiveness of tooth bleaching. *Journal of Dentistry* **31**(6) 415–422 (2003).

[44] S. Paul, a. Peter, N. Pietrobon, C. H. F. Hammerle, Visual and Spectrophotometric Shade Analysis of Human Teeth. *Journal of Dental Research* **81**(8) 578–582 (2002).

[45] S. Sadhasivam, S. Savitha, K. Swaminathan, Redox-mediated decolorization of recalcitrant textile dyes. *World Journal of Microbiology and Biotechnology* **25**(10) 1733–1741 (2009).

[46] E. Kissa, J. M. Dohner, W. R. Gibson, D. Strickman, Kinetics of staining and bleaching. *JAOCS* **68**(7) 532-538 (1991).

[47] D. R. Hunt and O. F. Makinson, Removal of plaque disclosing stains from clothing. *Australian dental journal* **29**(1) 5–9 (1984).

[48] Y. Li, C. Yang, S. Chen, Photoelectrocatalysis of Hydrogen Peroxide at Functionalized Multi-Walled Carbon Nanotubes (f-MWCNT) with Brilliant Blue Modified Electrode. *Int. J. Electrochem Sci.***6** 4829–4842 (2011).

[49] Y. Tanizawa, T. Abe, K. Yamada, Black tea stain formed on the surface of teacups and pots. Part 1 – Study on the chemical composition and structure. *Food Chemistry* **103**(1) 1–7 (2007).

[50] G. H. Dibdin, The Internal Surface and Pore Structure of Enamel. *Journal of Dental Research* **48**(5) 771–776 (1969).

[51] S. Satish, H. Ching, R. Dedhia, N. K. Voulgarakis, T. D. Johnson, C. J. Radke, Modeling Peroxide Release Dynamics and Pulp Penetration during Tooth-Bleaching Procedures. AADR Abstract. March 2010.

[52] M. Saquib, M. Abu Tariq, M. M. Haque, M. Muneer, Photocatalytic degradation of disperse blue 1 using UV/TiO2/H2O2 process. *Journal of environmental management* **88**(2) 300–6 (2008).

[53] R. Jain and S. Sikarwar, Photodestruction and COD removal of toxic dye erioglaucine by TiO 2 -UV process : influence of operational parameters. *Int. J. Phy. Sci.* **3**(12) 299–305 (2008).

[54] S. Y. Own and N. Me, Remediation of the Textile Dye Brilliant Blue FCF from Contaminated Water by Fenton-Like Process : Influence of Aromatic Additives. *Turkish J. Eng. Env. Sci.* **32** 367-376 (2008).

[55] M. Quirynen, M. Marechal, H. J. Busscher, A. H. Weerkamp, P. L. Darius, D. van Steenberghe, The influence of surface free energy and surface roughness on early plaque formation. *Journal of clinical periodontology* **17**(3) 138–44 (1990).

[56] G. Willems, P. Lambrechts, M. Braem, and G. Vanherle, Composite resins in the 21st century. *Quintessence international (Berlin, Germany : 1985)* **24**(9) 641–58 (1993).

[57] Y. H. Hong and K. K. Lew, Quantitative and qualitative assessment of enamel surface following five composite removal methods after bracket debonding. *European journal of orthodontics* **17**(2) 121–8 (1995).

[58] C. J. Arcoria, M. G. Lippas, B. A. Vitasek, Enamel surface roughness analysis after laser ablation and acid-etching. *Journal of oral rehabilitation* **20**(2) 213–24 (1993).

[59] M. T. Ariyaratnam, M. A. Wilson, I. C. Mackie, A.S. Blinkhorn, A comparison of surface roughness and composite/enamel bond strength of human enamel following the application of the Nd:YAG laser and etching with phosphoric acid. *Dental materials* **13**(1) 51–5 (1997).

[60] D. H. Retief, Effect of Conditioning the Enamel Surface with Phosphoric Acid. *Journal of Dental Research* **52**(2) 333–341 (1973).

[61] Y. Zhang, B. R. Lawn, E. D. Rekow, V. P. Thompson, Effect of sandblasting on the long-term performance of dental ceramics. *Journal of biomedical materials research* **71**(2) 381–6 (2004).

[62] W. J. O'Brien, H. Hemmendinger, K. M. Boenke, J. B. Linger, C. L. Groh, Color distribution of three regions of extracted human teeth. *Dental materials* **13**(3) 179–85 (1997).

[63] K. Dancy and M. E. Razzoog, Color measurements as quality criteria for clinical shade matching of porcelain crowns. *J Esthet Restor Dent* **15** 114–121 (2003).

[64] E. J. Burke and E. C. Moreno, Diffusion fluxes of tritiated water across human enamel membranes. *Archives of oral biology* **20**(5-6) 327–32 (1974).

[65] I. Rotstein, Y. Torek, I. Lewinstein, Effect of bleaching time and temperature on the radicular penetration of hydrogen peroxide. *Endodontics & dental traumatology* **7**(5) 196-198 (1991).

[66] L. D. Carrasco, D. M. Zanello Guerisoli, J. D. Pécora, I. C. Fröner, Evaluation of dentin permeability after light activated internal dental bleaching. *Dental traumatology* **23**(1) 30–34 (2007).

[67] R. Price, M. Sedarous, G. Hiltz, The pH of Tooth-Whitening Products. *J Can Dent Assoc* **66** 421-426 (2000).

[68] D. C. Attrill, R. M. Davies, T. A. King, M. R. Dickinson, A. S. Blinkhorn, Thermal effects of the Er:YAG laser on a simulated dental pulp: a quantitative evaluation of the effects of a water spray. *Journal of Dentistry* **32**(1) 35–40 (2004).

[69] M. Sund-Levander, C. Forsberg, L. K. Wahren, Normal oral, rectal, tympanic and axillary body temperature in adult men and women: a systematic literature review. *Scandinavian journal of caring sciences* **16**(2) 122–128 (2002).

[70] K. Luk, L. Tam, M. Hubert, Effect of light energy on peroxide tooth bleaching. **135** 194-201 (2004).

[71] Y. Li, S. S. Lee, M. Zheng, C. A. Forde, C. M. Carino, Effect of Light Treatment on in vitro Tooth Bleaching Efficacy. ADEA/AADR/CADR Abstract. March 2006.

[72] D. K. Hein, B. J. Ploeger, J. K. Hartup, R. S. Wagstaff, T. M. Palmer, L. D. Hansen, In-office vital tooth bleaching--what do lights add? *Compendium of continuing education in dentistry (Jamesburg, N.J. : 1995)* **24**(4) 340–352 (2003).

[73] A. U. Eldeniz, A. Usumez, S. Usumez, N. Ozturk, Pulpal temperature rise during light-activated bleaching. *Journal of biomedical materials research.* **72**(2) 254–9 (2005).

[74] P. Singh and H. I. Maibach, Iontophoresis in drug delivery: basic principles and applications. *Critical reviews in therapeutic drug carrier systems* **11**(2–3) 161–213 (1994).