The relationship of hydrogen peroxide exposure protocol to bleaching efficacy

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THE RELATIONSHIP OF HYDROGEN PEROXIDE EXPOSURE PROTOCOL TO BLEACHING EFFICACY

by

So Ran Kwon

A thesis submitted in partial fulfillment of the requirements for the
Master of Science degree in Operative Dentistry
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To my dear parents, for their endless love and support.

To my husband Seok Hoon, for his devoted love and faithful prayers.

To my children Youngwon and Youngmin for their faith and love.

To all my family and friends, who are all my well wishers.

To my mentors for their love and guidance.

To our mighty God for his grace and everlasting love.
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CHAPTER 1
INTRODUCTION

Tooth bleaching is a noninvasive and highly effective method to whiten discolored teeth. It is a treatment option to enhance the esthetics of the teeth that has been practiced in dentistry for over 100 years. In modern society that places great emphasis on appearance. Discolored teeth, if left untreated, may cause a decrease in self esteem and self confidence leading to social and psychological problems.

The nature of the discoloration dictates the prognosis of bleaching and can be broadly classified into extrinsic and intrinsic discoloration. Extrinsic discoloration is a common problem that is associated with superficial accumulation of stains as a result from intake of coffee, tea, or other highly colored food or the use of tobacco. Bleaching is very effective in these cases. Whereas staining occurring deep within the tooth structure due to a medication given systemically, or excessive fluoride ingestion during the formation of teeth can be more challenging to treat. However, with careful diagnosis and proper treatment planning, bleaching can be the simplest, least invasive and most affordable approach to lighten teeth even in these challenging situations.

Bleaching techniques for vital teeth can be classified into three categories: in-office bleaching, home bleaching or over the counter bleaching. The procedure and treatment duration differ according to the category but the basic bleaching material is either hydrogen peroxide or carbamide peroxide. Hydrogen peroxide is very unstable and decomposes rapidly, remaining active only for 30 to 60 minutes. Due to this property, it is commonly used for in-office bleaching procedures where the highly concentrated
hydrogen peroxide is placed onto the tooth surface for a short period and activated with a light or heating device to facilitate the dissociation of hydrogen peroxide into oxygen and perhydroxyl radicals. Carbamide peroxide is hydrogen peroxide added to urea to make it more stable so that it releases its active ingredient gradually over 4 to 6 hours after application. This makes it ideal for the use in home bleaching which involves the fabrication of a custom fitted tray, which is usually worn overnight with the bleaching gel in place.

While bleaching is widely used, the mechanism of bleaching is not yet fully understood. However, it is known that when the low molecular weight hydrogen peroxide is applied to the tooth surface, it readily penetrates the enamel and dentin reaching the pulp within 5 to 15 minutes. During the course of penetration, the peroxide interacts with organic stain molecules breaking them down into smaller molecules. This conversion of stain molecules seems to change the light reflection of the tooth making it appear lighter.

Hydrogen peroxide moves according to a diffusion gradient; that is, from a point of origin with higher concentration to an end point of lower concentration. The tooth surface where the bleaching material is applied is the origin point, and the inside of the tooth, the pulp cavity is the final destination. Thus diffusion of hydrogen peroxide is dictated by Fick’s second law, which not only takes the diffusion gradient into consideration but also factors such as the distance, the area and the diffusion coefficient of the substrate. The penetration of hydrogen peroxide, \textit{in-vitro} is influenced by the concentration, application time, light and heat application and the age of the tooth. The bleaching efficacy, \textit{in-vivo} however, is not necessarily affected by the concentration and light or heat application.
Conventional in-office bleaching procedures advocate the use of highly concentrated hydrogen peroxide with activating light sources. The bleach material usually has to be changed 2 to 3 times during one in-office session, making the procedure laborious and costly.

The sealed bleaching technique uses a linear low density polyethylene wrap to cover the bleaching gel thus creating a sealed environment that prevents the dehydration of the gel. This removes the necessity to replenish the gel and also facilitates control of the diffusion gradient. By keeping the diffusion gradient low, the penetration of hydrogen peroxide into the pulp cavity might be reduced and increase retention in the tooth structure, so that more interaction with stain molecules might be expected.

While previous studies on the kinetics of hydrogen peroxide have shown factors such as concentration, exposure time, heat, and light affecting penetration, little is known about the relationship between the amount of hydrogen peroxide penetration and the resultant color change of the tooth.

1.1. Purpose of Study

This study will compare the relationship of the amount of hydrogen peroxide penetration into the pulp cavity between two different in-office application methods and correlate penetration levels with the color change of the tooth.
CHAPTER 2
LITERATURE REVIEW

2.1. History of Bleaching

Bleaching is a conservative and effective method to lighten discolored teeth and has been practiced in dentistry for many centuries. During the course of development, careful observations and research on various bleaching techniques and materials enabled the dental profession to introduce more effective, safer and more predictable methods of bleaching.

Dentistry emphasizing esthetics was a very popular topic in the late 1800s, however concerns were raised regarding the aggressive removal of tooth structure for the placement of crowns \(^1\). Bleaching was a promising alternative for crowns in the treatment of discolored teeth and from the middle 1800s until the early 1900s. Reputable journals published 40 to 60 articles a year on tooth bleaching \(^2\).

During this time, the chemistry of bleaching was well discussed, and the major issues addressed in operative dentistry textbooks were the efficacy, indications, longevity and safety of bleaching \(^3\). Despite the plea for conservative dentistry and preservation of tooth structure, practitioners who opposed bleaching argued that it was technique sensitive, treatment duration was too long and relapse of color to the original shade was too frequent \(^3\). However, scientific approaches to avoid failure and minimize relapses reported longevity averaging 6 to 25 years \(^3\).

In the late 1800s, numerous bleaching agents which were either direct or indirect oxidizers were employed for the bleaching of non-vital teeth \(^3\). All agents including
aluminum chloride\(^4\)\(^5\), oxalic acid\(^6\), pyrozone\(^7\), hydrogen dioxide\(^8\), sodium peroxide\(^8\), sodium hypophosphate\(^5\), chloride of lime\(^5\)\(^9\) and cyanide of potassium\(^10\) acted on the organic portion of the tooth\(^3\). The variety of bleaching agents used reflects the diverse nature of the discoloration. Oxalic acid was used for the removal of iron stains associated with pulp necrosis and hemorrhage\(^11\), chlorine was indicated for silver and copper stains encountered with amalgam restorations\(^3\), ammonia readily removed stains produced from iodine used for root canal therapy\(^12\). Cyanide of potassium removed the most resistant stain originating from metallic salts of metallic restorations, but, its use was not recommended due to the highly poisonous nature\(^9\).

Although most of the early dental literature focused on non-vital bleaching, oxalic acid was employed for bleaching of vital teeth as early as 1868\(^13\). In 1877, Chapple published the first dental report on tooth bleaching using oxalic acid\(^14\). In the following years, Taft and Atkinson suggested the use of chlorine for bleaching, Taft using calcium hypochlorite and Atkinson using Labarraque’s solution, a liquid chloride of soda\(^14\). In 1884, Harlan published what is believed to be the first report of hydrogen peroxide which he called hydrogen dioxide\(^14\). Since there were few chemical manufacturing companies in the 1800s, most dentists mixed a variety of solutions in their office\(^2\). Attempts to speed up the bleaching process in the office were performed by using an electric current\(^15\), ultra-violet rays\(^16\), and other heating instruments and lights\(^17\). Manufacturing companies started to introduce bleaching products early in the 1900s which led to the drawback of limiting the choices of materials to the dental profession\(^2\). Superoxol which was introduced by a manufacturing company, developed into the bleaching agent used by the majority of dentists because of its efficacy and safety\(^2\).
During World War I, the Depression, and World War II, very little was written about bleaching. However, articles began to appear in the 1940s and 1950s as the dental profession began recognizing and treating fluorosis, tetracycline-stained teeth, and discolored teeth saved by root canal therapy.

Non-vital tooth bleaching using pyrozone applied to the external surface of the tooth was superseded by internal bleaching within the pulp chamber using 35% hydrogen peroxide. A method known as the ‘walking bleach technique’ used a mixture of sodium perborate and water which was left in situ for one week. The patient would return to have the procedure repeated until the desired lightening effect was reached. A mixture of superoxol and sodium perborate was used to modify the walking bleach technique and achieve a synergistic effect. The synergistic effect of these materials might have increased the efficacy of bleaching but the technique suffered adverse effects, such as cervical resorption, even though it was recommended that the gutta percha be sealed prior to bleaching.

The innovative technique of home bleaching can be traced back to the late 1960s and an orthodontist, Bill Klusmier in Fort Smith, Arkansas. While treating a patient who had sustained trauma to the mouth during the orthodontic retention phase, he recommended the patient to use an oral antiseptic containing 10% carbamide peroxide, Gly-oxide (Marion Merrel Dow, Inc., Kansas City, MO, USA), by placing it into the orthodontic positioner at night to facilitate tissue healing. He noticed a significant improvement in tissue health and even more surprisingly, lightening of tooth color. Although the technique was not published by Dr. Klusmier, he presented his findings in several dental meetings from 1970 to 1975, so that the technique spread by word of
mouth to many practitioners including Dr. Freshwater, who presented the technique to the Coastal Dental Study Club in Jacksonville, North Carolina. While Dr. Haywood was presenting a lecture at the Coastal Dental Study Club whose members were familiar with the newly introduced technique, he was encouraged to do further research on that subject. This led to the first publication on ‘Nightguard Vital Bleaching’ in 1989, using a 10% carbamide peroxide in a custom made tray worn at night. The first product for nightguard vital bleaching was White & Brite (Omnii International, St Petersburg Florida, USA). It was a 10% carbamide peroxide solution and sold as a daytime-use-bleaching product. The main impact of this technique was a major shift from in-office bleaching advocating the use of highly concentrated hydrogen peroxide with activating lights to home bleaching using lower concentrations of carbamide peroxide. This technique offered the possibility of whiter vital teeth to a wider section of the general patient population at a lower cost, with much less danger and fewer side effects. It also influenced the treatment of non-vital teeth by filling an open chamber with 10% carbamide peroxide, together with external application with the aid of a custom made tray.

The initial 10% carbamide solutions were quite runny, and the trays had to be designed to better retain these gels. The addition of carbopol to the carbamide peroxide solution slowed down the release of oxygen and increased the viscosity of the gel. In order to reduce sensitivity and other adverse effects of home bleaching, fluoride, potassium nitrate and amorphous calcium phosphate were added to the bleaching gel.

As the public demand and awareness for white teeth increased, manufacturers realized that they could reach the general public directly by the introduction of over-the-
counter products (OTC). The early OTC products which were introduced in 1990, were three step systems including an acid pre-rinse, a lower strength peroxide material and a final tooth paste. Most often these systems were overused and misused, resulting in damage to the enamel. However, the strip technology using a 6.5 % hydrogen peroxide on a clear strip of tape, which was applied to the teeth for 30min twice a day for 14 to 21 days, brought about innovative advances in materials and delivery methods of OTC systems.

The changes and continuous progresses of the materials and techniques on bleaching reflect the efforts of the dental profession towards preservation of tooth structure, enhancing esthetics and restoration of smiles. The future will likely bring about even more innovative systems that are aimed to benefit the general public.

2.2. Causes of Tooth Discoloration

The human tooth is composed of three dental hard tissues: enamel, dentin and the cementum. The basic tooth color is primarily determined by the reflectance of the dentin modified by the absorption, scattering, and the thickness of the enamel. The cementum however seems to have minimal influence on tooth color. Tooth color varies among people, among the teeth in the same person and within the same tooth. Tooth color is not constant over lifetime and changes for various reasons.

The etiology and type of discoloration is an essential component of proper diagnosis and treatment planning for bleaching and usually dictates the prognosis of the bleaching outcome. Thus a thorough clinical examination followed by a review of the patient’s oral hygiene practices, dietary habits, exposure to chemicals, trauma and intake
of medications are required to make a final diagnosis on the nature of the discoloration. Discoloration can be classified as extrinsic, intrinsic or a combination of both 31.

Extrinsic discoloration is caused by the accumulation of stains on the enamel surface and can be accentuated by pitting or irregularities of the enamel, salivary composition, salivary flow rates and poor oral hygiene 32. Affinity of the stains to the tooth surface plays a critical role in the deposition of extrinsic stains 33. The types of attractive forces include long-range interactions such as electrostatic and van der Waals forces and short-range interactions such as hydration forces, hydrophobic interactions, dipole-dipole forces, and hydrogen bonds 34. Extrinsic stains can usually be removed easily without altering the gross enamel structure and can be classified as follows.

Brown stain is a thin, bacteria free, pigmented pellicle found most commonly on the buccal surface of the maxillary molars and on the lingual surface of the mandibular incisors, with the lowest incidence on the labial surfaces of maxillary anterior teeth 35. It is commonly associated with poor oral hygiene and it is believed to result from the deposition of tannin found in tea, coffee, and other beverages 32.

Tobacco stain presents as a tenacious dark-brown discoloration mainly on the cervical one third to one half of the teeth. Staining results from the deposition of tobacco products on the tooth surface that might penetrate the enamel 36. The severity of the discoloration seems to be influenced by the characteristics of the tooth surface rather than the amount of tobacco consumption 37.

Black stain is a continuous narrow black line along the gingival margin of the enamel encircling the tooth 38. It is usually associated with a mucinous plaque that is infiltrated with chromogenic bacteria, primarily *Actinomyces* 39. The black stain is a ferric
sulfide formed by the reaction between hydrogen sulfide, a metabolic byproduct of bacteria and iron in the saliva and gingival exudate. It occurs more commonly in females and may occur in individuals with excellent oral hygiene.

Green stain is caused by the infiltration and growth of fluorescent bacteria and fungi such as *Penicillium* and *Aspergillus* on the labial surface of the maxillary anterior teeth at the gingival third. The growth of these bacteria was considered to be associated with the remnants of the primary dental cuticle or Nasmyth membrane, but this has not been substantiated. It is common in children, affecting boys more frequently than girls.

Orange stain appears as a yellow, orange, or reddish-orange line in the cervical third of the incisors. The stain is associated with chromogenic bacteria, such as *Serratia marcescens* and *Flavobacterium lutescens* and can be easily removed by dental prophylaxis.

Metallic stains are common in industrial workers exposed to metal containing dust or individuals who have received certain orally administered drugs or locally applied therapeutic agents. The color varies according to the metal that combines with the acquired pellicle at the tooth surface. Mercury and lead dust stains are grey. Copper and nickel stains are green to blue-green and chromic acid fumes produce a deep orange color in the enamel. The use of iodine solution and stannous fluoride may produce a brown discoloration and silver nitrate a black discoloration.

Antiseptic stain has been observed after prolonged use of chlorhexidine mouthrinses. The stain is characterized by a brown and diffuse discoloration of the teeth.
Unlike extrinsic discolorations that occur on teeth surfaces, intrinsic discolorations are attributable to the presence of stain molecules within the enamel and dentin, incorporated either during tooth formation or after eruption. Pre-eruptive stains include dental fluorosis, tetracycline stain, inherited developmental defects of enamel or dentin without systemic features, and hematologic disorders.

Dental fluorosis is the most common cause of intrinsic discoloration because of the wide range of availability of fluoride from multiple sources. It manifests as a subsurface hypomineralization or porosity of tooth enamel caused by chronic ingestion of fluoride during odontogenesis. The nature and severity depend on the dosage, duration of exposure, stage of ameloblast activity, and individual variation in susceptibility. The teeth may not be discolored on eruption but as the exposed porous surface gradually absorbs the chromogenic substances in the oral cavity, discoloration can become more evident. Clinically, mild fluorosis presents as delicate accentuation of the perikymata pattern to white opaque spots or lines. In severe cases, brown pitting patches, or localized loss of external parts of enamel may occur.

Tetracycline staining was first reported in the mid-1950s, after the introduction and widespread use of tetracycline which is a broad-spectrum bacteriostatic antibiotic. Exposure to tetracycline between the second trimester in-utero and approximately 8 years of age can affect the teeth, the skeleton and the fingernails. The tetracycline molecule appears to chelate with calcium in hydroxyapatite crystals, predominantly involving the dentin, forming tetracycline-calcium orthophosphate complex. The color of tetracycline stained teeth become more intense on chronic exposure to artificial light and sunlight owing to the photo-oxidation of this complex. The severity of stains depends
on the time, duration, and amount of drug intake, and the type of tetracycline. Proper diagnosis is very important since tetracycline stained teeth are considered one of the most difficult stains to remove. Diagnosis is established through history taking, clinical appearance and fluorescence under ultraviolet light.

Developmental defects of enamel or dentin can be associated with amelogenesis imperfecta, dentinogenesis imperfecta, and enamel hypoplasia. Amelogenesis imperfecta is a hereditary disorder of enamel formation involving both the primary and permanent dentition and can be classified into three main types: hypoplastic type exhibiting reduced enamel thickness, hypocalcified type characterized by deficient calcification and hypomaturation type showing impaired crystallite maturation. Discolorations associated with amelogenesis imperfecta tend to aggravate with time as the rough surfaces allow stains to accumulate more easily. Dentinogenesis imperfecta is a hereditary disorder affecting both dentitions exhibiting abnormal dentin formation. Affected teeth exhibit slender roots, small or obliterated pulp chambers and root canals with enamel that easily chips away from the dentin. Enamel hypoplasia is incomplete or defective formation of the enamel matrix induced by systemic or local factors. Hypoplasia can be mild showing pits and grooves or very severe with marked pitting or irregular horizontal banding of the crowns. There are numerous factors that can adversely affect the ameloblast and cause enamel hypoplasia including nutritional deficiencies, viral exanthematous diseases, trauma to the developing teeth, birth trauma, metabolic diseases, hemolytic diseases of the newborn, local infection, ingestion of chemicals and genetic causes.
Hematologic disorders cause a deposition of blood pigments in the dentin or enamel resulting in discoloration of the tooth structure. Erythroblastosis fetalis is characterized by destruction of red blood cells that may also induce severe jaundice in the newborn. The bluish-green staining is usually attributed to the accumulation of bilirubin in the developing dentition. Sickle cell anemia and thalassemia cause a deposition of blood pigments in the dentinal tubules, and congenital erythropoietic porphyria results in accumulation of porphyrin pigments making the teeth appear reddish-brown in appearance.

Intrinsic discoloration may also be acquired after eruption resulting in local discoloration. The severity of the discoloration varies according to the etiology and may vary from mild yellow orange to very dark brown and black staining.

Dental caries is a major cause of local discoloration that can start as an opaque, white halo and develop into an unsightly brown to black area due to the reaction of bacterial by-products with decalcified dentin.

Restorative materials that have leaking margins may allow debris or chemicals to enter and discolor the underlying dentin. Amalgam pigmentation is very common and can result in greenish-black pigmentation caused by the products of tin oxidation.

Pulp necrosis as a result of bacterial, mechanical, or chemical irritation to the pulp induces the release of noxious by-products that can discolor the affected dentin. Pulpal hemorrhage can be caused by pulp extirpation or severe trauma that subsequently results in the flow of blood components into the dentinal tubules. The hemolysis of red blood cells releases heme which then combines with the putrefying pulpal tissue to form iron. The iron reacts with hydrogen sulfide a bacterial by-product.
to form dark colored iron sulfides. These products penetrate deep into the dentinal tubules producing a dark discoloration of the whole tooth 41.

Endodontic materials and sealers have various staining potentials that cause intrinsic discoloration of the root canal filled tooth over time 56, 57.

Age related discoloration is a combination of extrinsic staining, thinned enamel, and darkened dentin due to the physiologic deposition of secondary dentin affecting the light-transmitting properties of the tooth 41.

The success of bleaching depends on the proper diagnosis of the etiologic factor of the discoloration. Although discolorations are categorized as extrinsic or intrinsic in most cases the etiology is multi-factorial, so that careful examination and history taking is required to properly determine all components that might have contributed to the discoloration.

2.3. Types of Bleaching Agents

There are a wide variety of bleaching products available but most are based on hydrogen peroxide (CAS No. 7722-84-1) as the active agent 44. Hydrogen peroxide may be applied directly, or produced in a chemical reaction from sodium perborate (CAS No. 7632-04-4) or carbamide peroxide (CAS No. 124-43-6) 58.

2.3.1. Hydrogen Peroxide

*Hydrogen peroxide* (*H₂O₂*) is a pale blue liquid, slightly more viscous than water with a molar mass of 34.0147g/mol 59. Because of its low molecular weight it can
penetrate dentin and release oxygen that breaks the double bonds of the organic and inorganic compounds inside the dentinal tubules \(^6^0\).

In dentistry, hydrogen peroxide is used as a bleaching material at different concentrations from 5\% to 35\% \(^3^3\). In order to improve its ease of use in bleaching teeth, it also comes in different delivery systems, most commonly in the form of gel. It acts as a strong oxidizing agent through the formation of reactive oxygen species and hydrogen peroxide anions. The rate of decomposition and the type of active oxygen formed is dependent on the temperature and concentration of the peroxide, as well as the pH and the presence of co-catalysts and metallic reaction partners \(^4^1\). Homolytic cleavage (the shared bonding electrons split apart leaving an unshared electron) is favored by light and heat: \(\text{HOOH} \rightarrow \text{H}^+ + \cdot\text{OOH} \) or \(\text{HO}^\cdot + \cdot\text{OH}\), and forms free radicals. Whereas, heterolytic cleavage (deprotonation leaving the electron pair) occurs at increased pH: \(\text{HOOH} \rightarrow \text{H}^+ + \cdot\text{OOH}^\cdot\), and generates perhydroxyl anions \(^6^1\). A third pathway is derived by a combination of both to generate active oxygen that is both an anion and a free radical. \(\text{HOO}^\cdot + \text{OH}^- \rightarrow \text{O}^\cdot_2 + \text{H}_2\text{O}\) in basic conditions and \(\text{HOO}^\cdot \rightarrow \text{O}^\cdot_2 + \text{H}^-\) in acidic conditions \(^6^1\). The active oxygen is attracted to the electron rich areas of stain molecules and by cleaving the double bond contribute to the decrease of color or the removal of the compound \(^6^2\).

Hydrogen peroxide is naturally produced, controlled, used, and destroyed during normal body functioning. The human body uses the glutathione redox cycle, catalase, ascorbate, superoxide dismutase, prostaglandin E1, glutathione peroxidase, vitamin E, and plasma peroxidase to protect itself against oxidative stress \(^6^1\).
2.3.2. Sodium Perborate

*Sodium perborate* (*NaBO₃*) is a white, odorless, water-soluble chemical compound available as a powder ⁶³. It has been employed as an oxidizer and bleaching agent especially in washing powder and other detergents since 1907 ⁵⁵. It is stable when dry, however in the presence of acid, warm air, or water it breaks down to form sodium metaborate, hydrogen peroxide and nascent oxygen ³³. There are various types of sodium perborate: monohydrate, trihydrate, and tetrahydrate, which differ in oxygen content and thus determine their bleaching efficacy ⁶⁴. Sodium perborate is easy to control and widely used in intracoronal bleaching.

2.3.3. Carbamide Peroxide

*Carbamide peroxide* (*CH₆N₂O₃*) is a white crystalline solid that releases oxygen in contact with water ⁶³. Concentrations used for bleaching purposes range between 10% to 35%. A 10% carbamide peroxide solution breaks down into 3.35% hydrogen peroxide and 6.65% urea ⁶⁵. Urea further breaks down into ammonia and water, and may provide some beneficial side effects, because it tends to increase the pH of the solution ²². Carbamide peroxide products usually contain either a carbopol or gycerine base ⁴¹. The carbopol base slows the release of hydrogen peroxide, making it more effective over a longer period of time ⁶⁶.

Although most bleaching products are based on hydrogen peroxide, claims that any use of hydrogen peroxide will bleach teeth should be carefully considered. Effectiveness and safety of the bleaching technique must not only evaluate the product but also the delivery method and treatment time ².
2.4. Mechanism of Bleaching

Knowledge on the basic mechanism is essential to determine the most effective and safest method of bleaching. However, the mechanism of bleaching has not yet been fully understood and explained. To provide a better understanding, the mechanism can be divided into two separate entities. First, the interaction of the bleaching agent with the stain molecules in the tooth and second, the movement or diffusion of bleaching agents into the tooth structure to interact with the stain molecules. The outcome of this combination would result in the final color change of the tooth after bleaching.

According to Albers\textsuperscript{62}, who explained the interaction of the bleaching agent with the stain molecules, oxygen and hydroxyl radicals released from hydrogen peroxide attack the complex ring structures of stain molecules and oxidize and convert them into more simple chain structures thus altering the reflective index of the tooth so that it appears lighter. The heavily discolored stain molecules usually possess ring structures with conjugated double bonds that exhibit electron-rich areas, to which the active oxygen molecules are attracted. These oxygen molecules produced from hydrogen peroxide cleave the double bonds and form more soluble compounds which lead to their removal and the color change of the tooth\textsuperscript{61}. Although the interaction of the bleaching agent with the stain molecules still needs to be explored further in detail, it is mainly regarded to be a chemical oxidation process.

The second aspect of the mechanism of bleaching which considers the diffusion and movement of the bleaching agent has been explored in many studies. The dental hard tissues are significantly permeable to fluids, and the greatest fluid flow in the enamel is in the interprismatic spaces\textsuperscript{67}. When hydrogen peroxide is applied to the tooth surface
externally, it diffuses into the enamel, dentin and into the pulp cavity. Enamel and dentin act as a semi-permeable membrane allowing for the diffusion of molecules according to Fick’s second law of diffusion: \( J_s = A \cdot D \cdot \Delta C/\Delta x \), where ‘\( J_s \)’ represents Diffusive flux of solute(mol/s); ‘A’ the Surface area available for diffusion(m\(^2\)); ‘D’ the Diffusion coefficient(m\(^2\)/s); ‘C’ the Concentration(mol/m\(^3\)); and ‘\( \Delta x \)’ the Diffusion distance(m).\(^68\)

Studies have shown that higher concentrations of hydrogen peroxide, heat, light activation, altered surface due to restorations, and characteristics like large open dentinal tubules of young teeth facilitate the diffusion and penetration of hydrogen molecules from the outer tooth surface into the pulp cavity.

In 1987, Bowles and Ugwuneri\(^{69}\) were the first to show, that in extracted teeth exposed to 30% hydrogen peroxide for 15 minutes at 37\(^\circ\)C, hydrogen peroxide levels of 25.4±8.5\(\mu\)g were detected in the pulp. 58 extracted maxillary anterior teeth were sectioned 3mm apical to the CEJ and pulp tissue was removed with a #4 round bur. The uniformity of dentin-enamel walls was evaluated with radiographs. 100\(\mu\)l of 2M acetate buffer with a pH of 4.5 was added into the pulp cavity to act as stabilizing agent for hydrogen peroxide penetrating into the pulp. The teeth were mounted and exposed to different concentrations of hydrogen peroxide (1%, 10%, 30%), and different temperatures (37\(^\circ\)C, 50\(^\circ\)C). The exposure time was the same for all groups (15min). After exposure to the hydrogen peroxide solution the acetate buffer was removed from the pulp cavity and placed into a 5ml volumetric flask. The amount of hydrogen peroxide was estimated with the method of Mottola et al.\(^70\) using leucocrystal violet and horseradish peroxidase. A spectrophotometer was used to read the absorbance level. The authors used three different H\(_2\)O\(_2\) concentrations (1%, 10%, 30%) and found that all three
concentrations exhibited a statistically significant different amount of penetration compared to the control group which was exposed to distilled water (p=0.01, p<0.005, p<0.001). The amount of hydrogen peroxide penetration was significantly increased by temperature (p<0.001).

This *in-vitro* model served as a very useful design for further studies evaluating the kinetics of hydrogen peroxide penetration. However, detailed description of the extracted teeth employed were lacking and the statistical analysis using the student’s t-test only analyzed the significance of the experimental groups compared to the control. Multiple comparisons within the groups with adjustments could have shown the significance among the three different concentrations. The study addressed the research question very well and showed that hydrogen peroxide penetrates into the pulp chamber of extracted teeth and that increased temperature increases the amount of hydrogen peroxide penetration.

Many studies followed after Bowles and Ugwuneri’s study adopting the newly introduced *in-vitro* model to investigate various factors that might influence the amount of hydrogen peroxide penetration into the pulp cavity.

In 1992, Cooper et al. 71 tried to determine whether carbamide peroxide used for home bleaching would also penetrate into the pulp and compared it to the penetration of hydrogen peroxide. They used a total of forty extracted human anterior teeth and assigned them into one control group and four different experimental groups. A slight difference between Bowles and Ugwuneri’s and this study was the amount of acetate buffer that was placed into the pulp cavity. Cooper et al. were much more conservative with the pulp
cavity preparation and were able to use only 25µl instead of 100µl of acetate buffer as a stabilizing agent.

The experimental groups were exposed to 10% carbamide peroxide gel, 15% carbamide peroxide gel, 5% hydrogen peroxide gel, and 30% hydrogen peroxide gel for 15 minutes at 37°C. The amount of hydrogen peroxide penetration level was measured according to the method of Mottola et al. 70 and ranged from 3.3 ± 0.38µg for the 10% carbamide peroxide group to 40.4 ± 3.51µg for the 30% hydrogen peroxide group. Analysis of variance, followed by Scheffe’s test, showed statistically significant differences among all five groups. It was interesting to note that the 15% carbamide peroxide group which essentially yields 5.25% of hydrogen peroxide showed less penetration (4.8 ± 0.27µg) than the 5% hydrogen peroxide group (10.4 ± 0.24µg).

In 1991, Rotstein et al. 72 aimed to assess the effect of different bleaching times (5, 20, 40, 60min) and temperatures (24, 37, 47 ºC) on the radicular penetration of hydrogen peroxide in 24 extracted premolar teeth which were treated endodontically and bleached intracoronally. The penetration of hydrogen peroxide occurred from the inside of the tooth to the outer surface, thus from the dentin to the enamel. The amount of hydrogen peroxide penetration was measured by adding ferrous ammonium chloride and potassium thiocyanate to form a ferrithiocyanate complex which absorbs light at the wavelength of 480nm. They found a positive correlation for both bleaching time and temperature on the radicular penetration of hydrogen peroxide. Hydrogen peroxide could not be detected at 5 minutes at any of the temperatures tested. However, increasing the bleaching time from 5min to 20, 40, and 60 min increased the hydrogen peroxide penetration level at each of the temperatures tested (p<0.01).
A study by Benetti et al. in 2004 showed the effect of the buccal surface condition on the amount of hydrogen peroxide penetration into the pulp cavity. They used 60 extracted bovine teeth and submitted 30 teeth to Class V preparations (2mm deep and 4mm wide, 3mm coronal to the cemento-enamel junction) followed by composite resin restorations. Teeth were exposed to distilled water, 10% carbamide peroxide, 35% carbamide peroxide for 60min at 37ºC, in the un-restored group as well as the restored group. The amount of hydrogen peroxide penetration was measured according to the method of Mottola et al. with the use of leuco crystal violet and the enzyme, horseradish peroxidase. Significant differences were found in the amount of hydrogen peroxide penetration according to the presence or absence of Class V composite resin restorations on the buccal surface (p=0.001), the concentration of the carbamide peroxide gel (p=0.001), and the interaction of the surface condition and bleaching agent concentration (p=0.001). They concluded that hydrogen peroxide penetration levels were higher with 35% carbamide peroxide compared to 10% carbamide peroxide, especially in restored teeth.

In 2005, Gökay et al. tried to determine the effect of different delivery systems and bleaching agents on the penetration of hydrogen peroxide in 50 extracted human maxillary central incisor teeth. All teeth were sectioned 3mm apical to the cemento-enamel junction and divided into a control group exposed to distilled water and 4 experimental groups exposed to a whitening strip containing 5.3% hydrogen peroxide, a paint-on liquid whitener containing 19% sodium percarbonate peroxide-equivalent to 5.3% HP, a paint-on liquid whitener containing 18% carbamide peroxide-equivalent to 6.5% HP, and a paint-on liquid whitener containing 8.7% hydrogen peroxide. The total
exposure time was 30 min at 37°C. The amount of hydrogen peroxide penetration into the pulp cavity was measured as described by Mottola et al. \textsuperscript{70}. The hydrogen peroxide penetration levels ranged from 0.175±0.012µg (paint-on liquid whitener containing 19% sodium percarbonate peroxide) to 0.726±0.024µg (whitening strip containing 5.3% hydrogen peroxide). There were statistically significant differences in penetration levels between all of the groups with the Kruskal-Wallis Analysis of Variance and the Mann-Whitney U-tests. The whitening strips containing 5.3% hydrogen peroxide showed the highest penetration levels followed by paint-on whiteners containing 8.7% hydrogen peroxide, 18% carbamide peroxide, and 19% sodium percarbonate peroxide, respectively. This study showed that both the delivery method and the active ingredient of the bleaching agent affected the amount of penetration of hydrogen peroxide into the pulp chamber.

In all studies evaluating the kinetics of hydrogen peroxide penetration, the amount of hydrogen peroxide penetration had to be measured. There are several methods available to determine the amount of hydrogen peroxide including electroanalytical techniques, fluorimetry, luminescence, nanotechnology, high performance liquid chromatography, and mimetic peroxidase behavior of metal complexes \textsuperscript{75}. They provide very sensitive and selective quantification of hydrogen peroxide but the specialized equipment is very expensive and less versatile. Enzymatic assay by spectrophotometer is economical and easy to handle \textsuperscript{75}.

Mottola et al. \textsuperscript{70} showed that the use of leuco crystal violet and peroxidase as a catalyst is a very convenient and sensitive method for the determination of hydrogen peroxide in submicrogram amounts. Samples containing 0.6 to 5.0µg of hydrogen
peroxide in a 10ml volumetric flask received 1ml of LCV solution (0.5mg/ml), 0.5ml of HRP solution (1mg/ml), and an acetate buffer. The mixture was finally diluted to 10ml with water. Measurement was performed with a spectrophotometer at 596nm. Results showed that the amount of hydrogen peroxide could be determined at submicrogram amounts using a spectrophotometric calibration curve obtained through serial dilutions of 30% hydrogen peroxide solution with distilled water.

In 2007, Camps et al.\textsuperscript{68} aimed to determine the optimal renewal time for the walking bleach technique and investigated the difference of time course diffusion of carbamide peroxide in young human dentin versus old human dentin. They placed the hydrogen peroxide into the pulp cavity and measured the amount penetrating from the pulp cavity out of the dentin and concluded that young teeth exhibited a higher diffusive hydrogen peroxide flux than old teeth. 40 human premolar teeth were extracted and divided into young teeth group (12-20yrs) and old teeth group (41-62yrs). One operator performed the root canal treatment on all teeth with a profile device and lateral condensation with zinc oxide eugenol based sealers and gutta-percha points. Cavit\textsuperscript{TM} of 1mm thickness was placed as a base. The outer surface of the tooth was sealed with two layers of thick nail varnish. Rectangular class V cavities were prepared so that the remaining dentin thickness was 1mm. 20µl of 20% Opalescence PF (Ultradent Product Inc., South Jordan, UT) was placed in the pulp chamber, and the receiving medium was changed at 1hr, 24hrs, 48hrs and 120hrs. Hydrogen peroxide amount was quantified with a oxidation-reduction reaction which yields a red colored ferrithiocyanate complex. Readings of optical density were obtained at 480nm. The authors measured the concentration variation over time, estimated time for complete diffusion, maximal
hydrogen peroxide diffusion and the optimal renewal time. The concentration variation over time was analyzed, and a regression was fitted between hydrogen peroxide concentration and diffusion time: \( f(t) \). The estimated time necessary for complete diffusion (EDT) through young and old human dentin was calculated from the regression equation, \( f(t) \). The EDT was the time at which the diffusion was minimal and the value of \( 10^{-10}\text{mol/l} \) was retained as the diffusion end. The estimated maximal diffusion was also determined by the definite integral between the beginning of diffusion and EDT. The optimal renewal time was considered to be the time necessary to achieve 80% of the estimated maximal diffusion. They found that there was a rapid diffusion of hydrogen peroxide through dentin in the first hour for both young and old teeth. There was an inverse exponential relationship between the concentration of hydrogen peroxide and time (\( p<0.01 \)). The time necessary for complete diffusion was longer for young teeth (352hrs) than old teeth (291hrs). The maximal hydrogen peroxide diffusion was higher for young teeth (\( 2.31 \times 10^{-6}\text{mol} \)) than for old teeth (\( 1.14 \times 10^{-6}\text{mol} \)). The optimal renewal time was 33 hours and 18 hours for young teeth and old teeth, respectively. Overall, these results show that young teeth exhibit a higher diffusive hydrogen peroxide flux through dentin. However, this does not necessarily mean that young teeth respond better to the walking bleach technique as was suggested by the authors. Since the authors used only dentin as a substrate the results cannot be applied directly to optimal renewal time which would have to take diffusion through enamel into consideration as well. The authors also emphasized that in order to compare experimental study results, the differences of dentin permeability of the tooth has to be included for proper comparison which is a very important clue in standardizing the samples.
Camargo et al. \textsuperscript{76} evaluated the effect of LED and Nd:YAG laser on the amount of hydrogen peroxide penetration in bovine teeth. They concluded that activation of a 35\% hydrogen peroxide bleaching agent with LED or Nd:YAG laser increased the amount of peroxide penetration into the pulp chamber of extracted bovine teeth. Hydrogen peroxide was collected by using a modification of the experimental design by Bowles and Ugwuneri \textsuperscript{69} and the amount of hydrogen peroxide penetration was measured according to the protocol by Mottola et al. \textsuperscript{70}. 48 extracted bovine teeth were used and divided into three experimental groups and one control group with 12 samples in each group. Group A was exposed to 35\% hydrogen peroxide for 20min with four 1min exposures to LED light at an interval of 5min. Group B was exposed to 35\% hydrogen peroxide for 20min with four 1min exposures to Nd:YAG laser at an interval of 5min. Group C was exposed to 35\% hydrogen peroxide for 20min without light activation. The control group was immersed in distilled water for 20min. After the treatment, the acetate buffer solution was removed from the pulp cavity and added with Leuco crystal violet (100µl of 0.5mg/ml) and 50µl of 1mg/ml horseradish peroxidase. Distilled water was further added to have 3ml of solution in the flask. Optical density was compared at 596nm with a spectrophotometer to a standard curve with known hydrogen peroxide amounts. They found that activated groups showed statistically significant differences compared to the non-activated and the control group. (p<0.005). There was no significant difference between LED and Nd:YAG activation (p=0.170). The study used bovine teeth which are morphologically different from human teeth so that further interpretation and comparison of the results to other studies is difficult. Overall, it showed that light activation with LED and Nd:YAG laser affects the peroxide penetration into the pulp cavity.
Most of the studies on the kinetics of hydrogen peroxide evaluated the amount of hydrogen peroxide penetration into the pulp cavity and various factors that would affect the penetration levels. The penetration levels were considered to be very important in terms of possible adverse effects to the pulp. However, there have been no studies attempting to relate the penetration levels with the color change of the tooth.

In 2005, Sulieman et al. were the first to try to quantify the penetration of 35% hydrogen peroxide into enamel and dentin and relate this to the overall shade change of the teeth. The authors compared the shades of 24 extracted maxillary incisors at baseline, after tea staining and after bleaching using three different shade assessment methods (Standard dental shade guide, shade vision system, electronic chromameter). The teeth were sectioned horizontally at the cemento-enamel junction and immersed in a standard tea solution for 24 hours at room temperature. Teeth allocated to the bleaching group were exposed to 35% hydrogen peroxide three times for 10 minutes with plasma arc light activation for 6 seconds. Teeth in the control group were immersed in distilled water for 30 minutes. Final shades were taken using the three shade evaluation methods prior to sectioning the teeth longitudinally either in a mesio-distal or bucco-lingual direction. The cut specimens were positioned onto a flat-bed scanner and the remaining stain areas were evaluated with a stain penetration analysis software. They found significant differences in mean shade change with the shade guide system between bleached and water soaked control groups (p<0.01). Percentage stain area remaining was significantly higher in water soaked controls than in bleached groups (p<0.001). The study mainly looked at the bleaching effect on tea chromogens and compared the bleaching efficacy of 35% hydrogen peroxide to distilled water. Overall, the results showed that bleaching is more
effective in removing stains than water soaking but the authors failed to address their research question and properly relate depth of hydrogen peroxide penetration into enamel and dentin to the color change of the tooth.

Another approach to evaluate the kinetics of hydrogen peroxide was used by Al-Qunaian et al. 78 where the authors performed an in-vivo study measuring the degradation of 3% hydrogen peroxide in the tray according to a time gradient. The study design adopted and modified the method that was originally used by Matis et al.26 to evaluate the in vivo degradation of 10% carbamide peroxide used in whitening teeth. Ten subjects were enrolled according to the inclusion criteria. Dental prophylaxis, alginate impressions, stone pouring, and tray fabrication were performed. 3% hydrogen peroxide strips were placed in the tray and weighed on an analytical balance. Subjects had to wear the tray with the gel on separate days for 5, 10, 20, 30, 45 and 60 minutes. At the end of each treatment the bleaching agent was retrieved from three samples: the tray, the teeth, and the mouth. The amount of peroxide in each sample was measured according to a method in the United States Pharmacopeia. The following formula was used to calculate the concentration of hydrogen peroxide (wt %) for tray and teeth samples.

\[
\text{Concentration of hydrogen peroxide} = \frac{V(0.025)(1.7)}{W}
\]

\(V=\text{volume of sodium thiosulfate (ml)}\)

\(W=\text{weight of sample (mg of a recovered sample that incorporates gel and saliva into it)}\)

The following formula was used to calculate the concentration of the recovered gel.

\[
\text{Concentration of HP at time} = \frac{\text{total gel recovered} \times 100}{\text{initial gel delivered}}
\]

The kinetics of hydrogen peroxide penetration for each treatment time was the ratio of,
Concentration of hydrogen peroxide (t) \( \times \frac{100}{\text{initial concentration of hydrogen peroxide}} \)

Hydrogen peroxide concentration decreased significantly faster in the tray and teeth samples \( p=0.0001 \) and \( p=0.0002 \) respectively, from 5 to 10 minutes compared to 10 to 60 minutes. The amount of hydrogen peroxide in the rinse and tray samples decreased significantly faster \( p=0.0004 \) and \( p=0.0012 \) respectively, from 5 to 10 minutes compared to 10 to 60 minutes. The total amount of HP decreased significantly faster between 5 and 10 minutes compared to 10 to 60 minutes \( (p=0.0001) \). Overall, these results suggest that hydrogen peroxide has a faster degradation rate than carbamide peroxide and therefore is more suitable for daytime application. However, the relationship of the rate of degradation of the peroxide to the bleaching efficacy has not been determined, yet, and needs further studies.

Numerous studies have been performed to elucidate the mechanism of bleaching. The kinetics of hydrogen peroxide diffusion has been determined mainly by peroxide penetration and peroxide degradation studies. Although neither approach could relate the penetration levels or rate of degradation of peroxide to bleaching efficacy the results obtained were very consistent. The consistent results might be due to similar \textit{in-vitro} designs employed in all the studies. In summary, they showed that time, concentration of hydrogen peroxide, heat, activating lights and tooth substrate were positive factors influencing the amount of hydrogen peroxide penetration into the pulp cavity. In terms of degradation, hydrogen peroxide exhibited a similar kinetics curve as carbamide peroxide but with a faster degradation rate.
2.5. Factors Affecting Efficacy of Bleaching

Various factors influencing the efficacy of bleaching have been investigated similar to studies on peroxide penetration levels into the pulp cavity. However, compared to *in-vitro* studies employed in penetration studies, efficacy studies have been mainly performed clinically. The effect of time or frequency, concentration of the bleaching agent, characteristics of the tooth substrate, activation with light, heat, plasma, or a sealed environment have been evaluated.

2.5.1. Time and Frequency

In 1991, Rosenstiel et al.\textsuperscript{79} aimed to determine how many bleaching sessions were required to achieve a given degree of color change. The authors used 24 extracted teeth and subjected 8 teeth to 37% phosphoric acid for 20 seconds followed by 35% hydrogen peroxide bleaching activated with a bleaching light for 30 minutes. The remaining 16 teeth were assigned into two control groups. One control group was acid etched but mock-bleached with distilled water and the other control group was stored in water without receiving any treatment. They found that after one treatment, $\Delta E$ was 3.33 ± 1.2, 1.67 ± 0.8, and 0.48 ± 0.2 for the experimental group, mock-bleaching control and the water stored control group, respectively. After six treatments, the overall $\Delta E$ was 3.82 ± 1.0, 2.41 ± 0.8, 1.38 ± 0.6 for the three groups. Analysis of variance (ANOVA) with repeated measures showed significant differences between the groups ($p<0.01$). Tukey’s multiple range test for the comparison of color differences of each group at different time intervals showed minimum significant color differences with values ranging from 0.96 to 1.19 ($\alpha = 0.05$). They concluded that a single bleaching treatment resulted in a mean
color change of 3.33 and that subsequent bleaching produced much smaller changes than the initial bleaching treatment.

Overall, this in-vitro study showed what degree of color change can be expected from one office bleaching session and the effect of adding further bleaching sessions to the color change of the tooth. However, the six bleaching treatments were performed over 6 days consecutively which would usually not be recommended clinically. Keeping the bleaching interval longer might have resulted in a different result favoring the increase of frequency in terms of bleaching efficacy.

Contrary to Rosenstiel et al.’s study, Al Shetri et al. 80 concluded that tooth lightness could be improved by continuing with a second in-office bleaching treatment. 20 subjects received an in-office bleaching treatment with either 35% hydrogen peroxide gel (Starbrite, Interdent, Inc, Los Angeles, CA, USA) or a 38% hydrogen peroxide gel (Opalescence Xtra Boost, Ultradent Products, Inc, South Jordan, UT, USA) for 30 minutes. A half-mouth design was used so that each subject served as his or her control. The final color evaluation was postponed until one week after bleaching to exclude any dehydration effect. One week after the second bleaching treatment mean ΔE was 2.45 for Opalescence Xtra Boost and 2.31 for Starbrite. The study showed that a second office bleaching procedure could enhance the efficacy of bleaching but also raised a question regarding the appropriate time for color measurement after bleaching. The authors tried to avoid the effect of dehydration, however by postponing the measurement until one week, they also increased the possibility of including many other factors that might have contributed to a change of color.
A study by Gottardi et al. supported the use of multiple in-office bleaching treatments in terms of patient satisfaction. The upper and lower teeth of 73 patients were bleached with 35% hydrogen peroxide and LumaArch unit light activation. The total bleaching time was 24 minutes and bleaching was repeated every 2 weeks until the patient was satisfied. They found that the mean color change per bleaching appointment was 2.1 to 3.7 on a 16-scale Vita Classic shade guide and that the majority of patients were satisfied after 3 bleaching treatments.

An *in-vivo* pilot study by Matis et al. comparing eight different in-office bleaching products (15~35%) showed that contact time (15~60min) was important while concentration was not. 32 subjects who met the inclusion criteria were randomly assigned to groups of four. Color measurements were made with a colorimeter at baseline, immediately after treatment and one, two, four and six weeks after bleaching. They found that all eight products were effective in bleaching teeth in the office. The mean $\Delta E$ measured immediately after bleaching was 6.77. And at one and six weeks after bleaching the mean $\Delta E$ regressed to 3.31 and 2.34, respectively. Statistical analysis on the efficacy of various products was not possible, due to the small number in each group. However, the in-office products with the lower concentrations (15~25%) had the highest $\Delta E$ values immediately after bleaching, and the three products with the shortest exposure time (15~45min) had the three lowest $\Delta E$ values. The authors suggested that contact time is more important than the concentration and speculated that other agents added to the product must have catalyzed the peroxide.

Kihn et al. aimed to evaluate the influence of increasing bleaching time on the color change for the tooth. They compared the color differences of 15 subjects after using
15% hydrogen peroxide gel used as a touch-up bleaching agent. They found significant differences of color change with 60min compared to 30 and 45minutes.

According to the above studies on the effect of time and frequency on the efficacy of bleaching it can be concluded that increasing the exposure time to the bleaching agent and the frequency of the bleaching session will improve the whitening efficacy in terms of color change. The number of bleaching sessions required and the optimal concentration will probably differ according to the type of the discoloration and the characteristics of the tooth substrate. Further studies are needed to address these issues.

2.5.2. Concentration of Bleaching Agent

Zekonis et al. \(^8^4\) aimed to evaluate the color change, color relapse and sensitivity associated with a highly concentrated in-office bleaching product and a low concentration at-home bleaching product. Twenty subjects who met the inclusion criteria were given a sensitivity sheet to self-evaluate the level of tooth and gum sensitivity they experienced during and after the bleaching treatment. Bleaching was performed with a 10% carbamide peroxide product for 14days and a two session in-office bleaching with 35% hydrogen peroxide product for 30min. The type of bleaching treatment was randomly assigned to the right or left side of the maxillary anterior teeth by flipping a coin. Color was measured with a colorimeter, shade guide, and clinical slide photographs at baseline and three, six, twelve weeks from baseline. Repeated measures ANOVA for differences in mean \(\Delta L^*\), \(\Delta a^*\), \(\Delta b^*\), \(\Delta E\) showed that the two different bleaching treatments did not have significantly different baseline L* (p=0.56), a* (p=0.76), and b* (p=0.52). Compared to the in-office bleaching treatment home bleaching had significantly more
color change in $\Delta L^*, \Delta a^*, \Delta b^*$, and $\Delta E$ overall ($p=0.0001$) and at each follow-up evaluation ($p=0.0001$). Wilcoxon Sign Rank tests were used to determine differences in tooth and gum sensitivity. At-home bleaching showed significantly higher gum sensitivity than in-office bleaching in the overall test combining all days ($p=0.037$). There was no significant difference in tooth sensitivity between the two bleaching regimen ($p>0.15$). Color stabilization was observed at six weeks for both at home ($\Delta E=6.64$) and in-office bleaching treatment ($\Delta E=3.63$) at a level significantly different from baseline. However, the rate of color relapse was lower for the in-office bleaching compared to the at-home bleaching treatment.

Overall, this study showed that fourteen days of at-home bleaching was more effective than two sessions of in-office bleaching. It would be interesting to determine how many more in-office sessions might be required to obtain similar results to home bleaching and then correlate concentration versus application time for a specific whitening outcome.

Sulieman et al. determined the effect of various concentrations of hydrogen peroxide (5~35%) on the outcome of tooth whitening. They used extracted third molars and removed the root portion and further sectioned the crown portion vertically to expose them to a standardized tea solution for staining. Staining was performed to a uniform shade of C4. The stained specimens were then bleached with 5, 10, 15, 25, and 35% hydrogen peroxide with a total exposure time of 30 minutes. The number of applications required to reach the shade of B1 was evaluated. Results showed that 1, 2, 4, 7, 12 applications were required for 35%, 25%, 15%, 10%, 5% respectively to reach a uniform shade of B1. The authors concluded that the relationship of the number of applications
and the concentration of the bleaching gel showed an exponential relationship. This study demonstrated that with higher concentrations of hydrogen peroxide fewer applications are required to obtain a specific whitening outcome. However, there was no statistical data analysis and no descriptions on how many specimens were used for each group and descriptions on the baseline color prior to the standardized staining protocol were lacking.

Auschill et al. 86 used the same principle as Sulieman et al.’s study 85 in terms of reaching a defined level of whitening. They performed a clinical study comparing the efficacy of White strips (one cycle=30min, 5.3% hydrogen peroxide), Opalescence PF (one cycle=8hours, 10% carbamide peroxide) and Opalescence Xtra boost (one cycle=15min, 38% hydrogen peroxide) in 39 subjects that were randomly allocated to one of the three bleaching groups. Bleaching was continued until the defined level of six grades on the Vita shade guide was achieved. Mann-Whitney-U test for independent samples showed that all three groups differed significantly from each other in terms of treatment cycles and required treatment time (p<0.001). The mean treatment time required to achieve six grades of whitening was 31.85±6.63 cycles for Whitestrips, 7.15±1.86 cycles for Opalescence PF and 3.15±0.55 cycles for Opalescence Xtra Boost. They concluded that the higher the concentration of the bleaching agent the faster the whitening occurs. Overall, this study showed that all techniques were effective in lightening teeth with special consideration to the time required to achieve the defined level.

Several studies compared the efficacy of bleaching with different carbamide peroxide concentrations 87-90. Kihn et al. 87 aimed to determine the difference between
10% and 15% carbamide peroxide whitening agent in terms of whitening efficacy and development of tooth sensitivity. Fifty-six human subjects matched into 28 pairs received either 10% or 15% carbamide peroxide gel for 2 weeks of active home bleaching. The color was assessed with a value oriented shade guide at baseline, after 1 and 2 weeks after treatment and 2 weeks following the end of the treatment. Sensitivity was self reported on a 20-mm visual analog scale. They found that there was a significant efficacy difference between the two concentrations at the end of active treatment and at the 2-week post-bleaching evaluation. The mean color change for 15% carbamide peroxide was 9.4 ± 2.3 and 7.7 ± 3.0 for 10% carbamide peroxide. There was no significant difference in sensitivity between the two concentrations. This study evaluated the color at 2-weeks post-bleaching, a time where the color might have not yet stabilized. Incorporating longer post-bleaching evaluation periods might have given different information on the efficacy of the two concentrations.

Contrary to Kihn et al’s study several other studies\textsuperscript{88-90} found no significant differences in the efficacy of bleaching between different concentrations of carbamide peroxide.

Matis et al.\textsuperscript{91} aimed to evaluate the color change, rebound effect, and sensitivities associated with using 10% and 15% carbamide peroxide for 14 days. Twenty-five subjects used 10% and 15% carbamide peroxide in a split-mouth design on either the right or left side of their maxillary arch. Shade matching, intraoral photographs and colorimeter measurements were performed at 3 days and 1, 2, 3, and 6 weeks. Gingival and tooth sensitivity was self reported on a scale of 1 to 5. The $\Delta E$ values immediately after bleaching were 8.79 and 11.03 for 10% and 15% carbamide peroxide, respectively.
At 4 weeks post-bleaching the ΔE values regressed to 5.13 and 5.58 for 10% and 15% carbamide peroxide, respectively. Overall, there was a greater color change with 15% carbamide peroxide, but the difference was not statistically significant at 4 weeks post-bleaching. The self reported gingival and tooth sensitivity scores showed no significant differences between the two concentrations. Although there was more color change with 15% carbamide peroxide bleaching as was found in Kihn et al.’s study, increasing the post-bleaching evaluation time showed that the ultimate color change was similar for both concentrations.

However, there is still a need for studies to determine whether the use of 15% carbamide peroxide for a shorter period would result in the same ultimate bleaching change as 10% carbamide peroxide for a longer period of time.

A double blind randomized clinical trial of 92 subjects by Meireles et al. comparing the efficacy and safety of 10% and 16%CP showed no significant difference in color between the two groups immediately after bleaching (p=0.5).

Krause et al. compared the efficacy between 10% and 17% carbamide peroxide bleaching gel in a double blind study design of thirty patients and showed similar contentment in terms of color change but more pain with 17% CP.

It can be concluded that the higher the concentration of the bleaching agent the faster the bleaching rate will be. The ultimate color change and the time required for stabilization with different concentrations of hydrogen peroxide and carbamide peroxide needs to be examined in further studies.
2.5.3. Tooth Substrate

The tooth is composed of three different hard tissues, the enamel, dentin and the cementum. These tissues vary in their inorganic and organic composition and also demonstrate different structural components which undergo changes with age and internal and external stimuli\textsuperscript{92}. There are only a few studies that determined the effect of the characteristics of the tooth substrate on the efficacy of bleaching.

Contrary to the general belief that bleaching was only effective in enamel and not in dentin, McCaslin et al.\textsuperscript{93} showed that dentin can be bleached and that the change occurred through the dentin at a uniform rate rather than greater change from the outside and less inward. The authors used 10 extracted adult human anterior teeth and sectioned the teeth in half longitudinally. The cut specimens were positioned onto a glass microscope slide and the peripheries sealed with cyanoacrylate adhesive. The root portions were painted with nail varnish so that only the crowns were exposed to 10\% carbamide peroxide for 10 days. Bleaching changes were measured by taking photographs at baseline and after bleaching and by converting the images into an image processing software. A two-way ANOVA to test for differences in the mean gray-scale level between the outer dentin, inner dentin, and control showed significant differences in mean gray-scale level changes between the control and the outer dentin (p=0.01), and the control and the inner dentin (p=0.008). The difference in mean gray-scale level changes was not significant between the outer and inner dentin (p=0.89). Overall, the study showed that there are lightness changes in the dentin after bleaching and that the changes occurred at a uniform rate throughout the dentin, indicating that carbamide peroxide
easily penetrates the tooth and that the type of stain may be the determining factor for bleaching success.

Kugel et al. \textsuperscript{94} aimed to investigate the color changes in enamel and dentin separately and relate the differences to the overall color change of the tooth. 26 extracted human molar teeth were sectioned at the cemento-enamel junction and the color of each specimen was measured at three locations. On buccal surfaces of the crown, 1mm occlusal to the cemento-enamel junction to evaluate the overall color change of the tooth. At the mid-point of enamel on the cut surface and 1mm into dentin on the cut surface to measure the color change of enamel and dentin, respectively. The cut surfaces of the specimens were then mounted on a glass slab and peripheries were sealed with cyanoacrylate adhesive. The specimens were divided into an experimental group exposed to 15\% carbamide peroxide for 2 hours per day over a period of 14 days and a control group receiving a placebo gel without an active ingredient for 2 hours per day, 14 days consecutively. The authors measured post-bleaching changes at day 15 at the same predetermined locations. Treatments were compared using ANCOVA with baseline color as the covariate. Relative to the control group the buccal surfaces showed the greatest color change in terms of $\Delta b^*$ and $\Delta L^*$ values and dentin the least color improvement. They concluded that the overall color change of the tooth was caused by the color change in enamel rather than the color change of the dentin.

Lin et al. \textsuperscript{95} enrolled 91 subjects to determine whether gender, age, and initial tooth hue would influence the effect of laser tooth whitening. They concluded that baseline hue was an important determinant for color change, with hue A teeth exhibiting greater shade improvement than hue C and hue D teeth. The whitening response was better in younger
individuals (<30 years) than in older individuals (≥30 years), and gender was not a factor influencing the effect of bleaching.

Overall, these studies suggest that the characteristics of tooth substrate have an important influence on the effect of tooth whitening. Further studies are needed to determine which structures or processes within the enamel and dentin are most influenced by the active bleaching agent.

2.5.4. Light Activation

Quartz-tungsten-halogen (QTH) lamps, plasma arc lamps, laser systems with a variety of wavelengths, and light emitting diodes (LED) have been used for activation of bleaching agents used in power bleaching. The basic mechanism of action of these lights has not been well documented. The rationale of using activating lights is based on the release of hydroxyl-radicals from hydrogen peroxide through direct excitation by light. Following the equation: $\text{H}_2\text{O}_2 + \text{hv} \rightarrow 2 \text{HO} \cdot$ (h=Planck’s constant, ν=frequency of light), the absorption of light induces the photolysis of hydrogen peroxide into two hydroxyl-radicals. The required wavelength to induce such an energy corresponds to 248nm and lower which cannot be used in the oral cavity for safety reasons. However, indirect excitation of hydrogen peroxide via photo-activators that absorb the light energy and transfer it to the hydrogen peroxide molecules have been suggested with the use of lights in the range of the visible spectrum ranging from 380 to 750 nm.

There are a few studies comparing the effectiveness of bleaching with the use of light and without using the light. However, the results have been equivocal due to the variability of study designs and the use of different bleaching materials as well as
different bleaching lights. In order to understand the lack of agreement in the dental literature regarding the use of activating lights, several articles addressing the issues of light activation have been summarized below.

Papathanasiou et al. \(^9^7\) compared the effectiveness of light activation and no light activation of a 35% hydrogen peroxide bleaching agent in a randomized clinical evaluation using the split arch design. Twenty patients who had six maxillary anterior teeth with a shade darker than A3 were selected. The baseline color was evaluated by three independent evaluators with a classic Vita shade guide. The six maxillary teeth were exposed to 35% hydrogen peroxide for 20 minutes with teeth Nos. 6 to 8 receiving the halogen light activation. Post-bleaching color measurements were taken after 24 hours. Results showed that a 20 minute application of hydrogen peroxide was effective in lightening the teeth compared to the non-bleached mandibular teeth. The median degree of lightening was 7.21 and 6.78 in the light-activated group and in the non-light-activated group, respectively. 7 out of 20 patients showed greater degree of lightening in the light activated group, but there was no statistical significance using the Mann-Whitney U test (\(p>0.05\)). The authors concluded that there was no benefit of using light activation with 35% hydrogen peroxide. The split arch design used in this study is commonly used to compare two different treatment modalities. However, no effort was made to block the light from reaching the non-light-activated side and exposure to 35% hydrogen peroxide was limited to 20 minutes only, which might have influenced the overall result of the study.

Hein et al. \(^9^8\) in a clinical study of 15 patients comparing bleaching effects in a split arch design also showed that light nor the heat produced by the light activating unit
enhanced the efficacy of bleaching. The authors used three different in-office whitening systems differing in hydrogen peroxide concentration, chemical composition of activators, pH, light design, contact time recommended for gels and lights, and the distance of light source from teeth during the bleaching procedure. Color measurements were taken by three evaluators with a Vitapan 3D-Master Shade Guide at baseline, immediately after bleaching and 1 week post-bleaching. Additional laboratory tests were performed on chemical characterization of hydrogen peroxide, optical characterization of test lights, in-vivo gel temperature, bleach light decomposition of hydrogen peroxide, and thermal decomposition of hydrogen peroxide. Color changes at 1 week post-bleaching showed that there were no differences between the non-light-activated and the light activated teeth for any of the three in-office bleaching systems. However, one system required less time and one system used a lower concentration to achieve the same bleaching result. The authors concluded that the use of proprietary chemicals in the bleaching gel had a positive effect on the bleaching process rather than the activating light unit. This study was a comprehensive study including laboratory tests. However, no descriptive statistical data were given related to the clinical outcomes.

Marson et al. 99 evaluated the effect of different light activation units on the efficacy of a 35% hydrogen peroxide gel. 40 patients were selected and randomly assigned into four groups of ten subjects. Group 1 served as the control and was exposed to 35% hydrogen peroxide without light activation. Groups 2, 3, and 4 were bleached with 35% hydrogen peroxide in combination with a halogen curing light, LED light, and a LED/LASER light, respectively. All groups received two sessions of 45 minutes of bleaching, with a one week break between sessions. The color measurements were
performed with a spectrophotometer and a Vita Classic Shade Guide at baseline, first
week, second week, first month and six months after the bleaching treatment. Statistical
analysis using ANOVA showed no significant differences among the groups
(p=0.999993). Color stability was observed up to the sixth month after treatment.
Overall, the authors showed that the use of different light units did not affect the outcome
of in-office bleaching with 35% hydrogen peroxide. The strengths of this study compared
to others were that two separate sessions were used and that the color stability was
assessed up to six month. Weaknesses included small sample size and that the baseline
color was not considered as a covariate of the data analysis.

Tavares et al. \(^{100}\) were the first to show that light activation enhanced the efficacy
of a 15% hydrogen peroxide gel in a six-month parallel–design, blinded clinical
evaluation conducted in accordance with ADA guidelines. 87 patients were randomly
assigned to peroxide and light, peroxide gel only, and placebo gel and light group. The
total bleaching time was 60 minutes for all groups. Tooth shade was evaluated at baseline
and post-bleaching and at three and six months post-treatment with a Classic Vita Shade
guide and a chromameter. Kruskal-Wallis nonparametric analysis was performed to
evaluate the differences between the treatments. Results showed that the initial shade
change was greatest in the light and peroxide treatment group compared to the peroxide
alone and light alone groups. Approximately 88% of these effects persisted for six
months. The study contradicted to other studies claiming no benefit of the use of light
activating units for bleaching. Hein et al. \(^{98}\) especially noted that this study failed to
control for differences in subject’s teeth by not using a split arch design, and the use of
only one color judge throughout the study provided no confirmation of tooth colors
initially or at any of the recalls. The fact that the study was funded by the company whose product was used was criticized as well.

Luk et al.\textsuperscript{101} examined the effect of light energy on peroxide tooth bleaching in 250 extracted human teeth halves and showed that a combination of specific bleach and light can enhance bleaching efficacy.

Dostolova et al.\textsuperscript{102} showed on extracted human maxillary central incisors that selective diode laser irradiation with 38\% hydrogen peroxide can decrease the time of bleaching without surface modification of the enamel.

Ontiveros and Paravina\textsuperscript{103} aimed to visually and instrumentally evaluate the color changes associated with the use of a 25\% hydrogen peroxide system with and without the use of supplementary light. Twenty patients were treated with two separate 45-minute exposures of 25\% hydrogen peroxide in an opposing-arch design. The order of the arch (maxillary or mandibular) and the type of treatment (light or no light) was randomly sequenced into two appointments. Color measurements were taken at baseline and seven days post-bleaching with a Vitapan Classical (VC), Vita Bleachedguide 3D-Master (BG), and a contact-type intraoral spectrophotometer. Data analysis was performed with t-test and Wilcoxon Signed Ranks Tests. Results showed that there was a significant difference in color between the two groups ($\Delta E=6.0$, for the light-activated group, $\Delta E=4.7$ for the no light group) after seven days ($p<0.05$). No significant difference between the two groups was detected with the Vitapan Classic ($p=0.56$), whereas significant difference was obtained with the Vita Bleachedguide 3D-Master ($p<0.01$). This study was one of the few to show that supplementary light enhances the efficacy of in-office whitening. The
authors emphasized that the opposing arch design was used over a split arch design to eliminate the potential of radiation to the no light segment.

2.5.5. Heat

Davidi et al.\textsuperscript{104} showed in extracted human molars and premolars, stained with tea solution that a mild increase of temperature from 37ºC to 45ºC enhanced the bleaching efficacy. However Zach and Cohen\textsuperscript{105} reported irreversible pulpal damage in 15% of monkeys for temperature elevations of 5.6 ºC. Baik et al.\textsuperscript{106} showed significant increase in temperature with the use of intense lights in a study on an extracted human upper central incisor fitted with thermocouples and simulating intrapulpal fluid flow. Eldeniz et al.\textsuperscript{107} reported a great increase of pulpal temperature associated with the use of a diode laser. Overall, these studies showed that careful consideration should be given to potential risks of heating teeth when using heat activating devices.

2.5.6. Ozone

The concomitant use of ozone to increase the oxidative capability of the bleaching agent has been suggested as a method of improving bleaching effectiveness\textsuperscript{108}. In order to substantiate this suggestion by dental research, the efficacy of ozone and Tooth Mousse\textsuperscript{TM} on the efficacy of peroxide bleaching was evaluated \textit{in vitro}. Manton et al.\textsuperscript{109} prepared 60 enamel specimens and incubated them in tea solution. The specimens were then randomly divided into six treatment groups receiving, ozone followed by carbamide peroxide bleaching; ozone concurrently with carbamide carbamide peroxide bleaching; carbamide peroxide bleaching alone; ozone followed by a mixture of carbamide peroxide
bleaching agent and Tooth Mousse; ozone concurrently with a mixture of carbamide peroxide bleaching agent and Tooth Mousse; and a mixture of carbamide peroxide bleaching agent and Tooth Mousse alone. Images were taken at baseline and after the different exposure protocols with a high resolution digital camera and analyzed with image analysis software. The authors found that the addition of Tooth Mousse into the bleaching gel or the application of ozone with carbamide peroxide did not affect the bleaching effectiveness compared to carbamide peroxide alone.

2.5.7. Sealed Environment

In 2007, Kwon\textsuperscript{110} introduced a new protocol of power whitening called ‘Sealed Bleaching Technique’ which prevents the evaporation and desiccation of active agents by placing a linear low density polyethylene (LLDPE) wrap onto the power whitening gel. The mechanism of sealed bleaching is that by creating a sealed environment, the activated whitening material remains concentrated near the tooth surface so that it is directed into the tooth rather than evaporating into the air. This technique not only improves the efficacy of whitening but also makes the procedure safer. Sealing prevents the whitening material from evaporating into the air and from unintentional exposure. The activated whitening material is also utilized more effectively so that lower concentrations can be used, and replenishment is not necessary making the procedure simple and cost effective. Lee et al.\textsuperscript{111} showed in a clinical study of ten patients using a split arch design that a greater shade difference was observed after whitening in the sealed bleaching group compared to the conventional bleaching group.
2.6. Color Related to Bleaching

2.6.1. Color Measurement

The concept of color is difficult to understand, not easy to define, and is often related more to art than science \(^{112}\). In order to measure and specify color using letters and numbers, the Munsell Color Order System was introduced by Albert H. Munsell as early as 1905 \(^{113}\).

The Munsell system is comprised of three attributes: hue (H), value (V), and, chroma (C). Munsell value (V) runs from 0 (black) to 10 (white) and is a visual perception according to the amount of reflection of incident light from the object. Munsell chroma (C) is an open-ended scale operating from 0 (achromatic colors) to maximum depending on the hues. Munsell hue (H) is the attribute according to which a sample appears to be one of the perceived colors \(^{29}\). The Munsell color order system is intuitive and easy to apply for visual assessment. However, it is not suited for computational or instrumental methods and for color difference measurements.

In 1976 and 1978 the Commission International de l’Eclairage developed a new system, called CIEL*a*b*, in which for the first time it was possible to express color by numbers and calculate the differences between two colors in a way that corresponded to visual perception \(^{114}\). In this system, color is expressed by three coordinates: L* value is the degree of lightness of an object, a* value is the degree of redness/greenness (positive value indicates red; negative indicates green), and b* value is the degree of yellowness/blueness (a positive value indicates yellow; negative value indicates blue) \(^{114}\).

The CIEL*a*b* system is regarded as a benchmark for scientific purposes, conceived for computational and instrumental methods and well suited for color
difference measurements. In the CIEL\*a\*b\* system a formula is used to calculate color differences:

\[ \Delta E_{ab} = [(L_1-L_2)^2+(a_1-a_2)^2+(b_1-b_2)^2]^{1/2} \]

This “\( \Delta E \)” value became pivotal in color science, for both industry and dentistry\textsuperscript{115}. Following this new fundamental approach to dental color science, instruments like colorimeters and spectrophotometers were quickly developed and improved to measure color and color differences\textsuperscript{112}.

2.6.2. Threshold

Recording an accurate baseline shade is extremely difficult due to the complex optical characteristics of tooth color, such as gloss, opacity, transparency, translucency, and optical phenomena such as metamerism, opalescence and fluorescence\textsuperscript{116}. Since spectrophotometers can detect small differences in color at a level that is not appreciable by the human eye, an important issue of color science in dentistry is to establish a reference value for evaluation of study results in terms of \( \Delta E \)\textsuperscript{112}. In other words, it is necessary to understand the clinical relevance of \( \Delta E \) and the ability of the human eye to perceive the color difference. However this value is subjective and difficult to establish. It varies between different groups of dental personnel\textsuperscript{117}. It also varies according to the material type that is compared.

In a study by Kuehni et al.\textsuperscript{118} that is frequently cited, industrial color matchers evaluated paint and textile specimens and “\( \Delta E = 1 \)” was discernible 50% of the time. Seghi et al.\textsuperscript{119} used translucent porcelain specimens and dental professionals were able to discern a difference 100% of the time when the \( \Delta E \) value was greater than 2.
Ragain et al. \textsuperscript{120} assessed the minimum color difference for discriminating mismatch between composite resin specimens and reported an average acceptability threshold of 2.72 \(\Delta E\) units.

Johnston and Kao \textsuperscript{121} in their clinical study showed that the average color difference (\(\Delta E\)) between compared teeth and composite restorations rated as a match in the oral environment was 3.7 units. Thus with different threshold values reported in different studies it was stressed that one single value was probably not the key solution to the problem, and that a differentiation between perceptibility (the difference that can be identified by the human eye) and acceptability (the difference that is considered tolerable) was proposed\textsuperscript{112}.

Douglas et al. \textsuperscript{122} determined the valid acceptability and perceptibility tolerances for shade mismatch in a clinical study using spectroradiometric instrumentation and reported that the predicted color difference at which 50\% of the dentist observers could perceive a color difference was 2.6 \(\Delta E\) units. Whereas the predicted color difference at which 50\% of the subjects would remake the restoration due to color mismatch was 5.5 \(\Delta E\) units.

In order to provide a range for the identification of the perceivable \(\Delta E\) value, Vichi et al. \textsuperscript{123} proposed three different intervals for distinguishing color differences. \(\Delta E\) values of less than 1 unit were regarded as not appreciable by the human eye; \(\Delta E\) values greater than 1 and less than 3.3 units were considered appreciable by skilled operators, but clinically acceptable; \(\Delta E\) values greater than 3.3 were considered perceivable by untrained observers.

Despite much effort on the identification of a \(\Delta E\) value in terms of perceptibility and acceptability the values are still controversial and require further investigation.
2.6.3. Shade Guides

Tooth shade matching is performed mainly by dental shade guides. The Vitapan Classical shade guide (VITA, Bad Sackingen, Germany) was introduced onto the market in 1956, and with some minor modifications made, it is still the gold standard for shade matching. It consists of 16 tabs arranged into four groups of different hues. According to the manufacturer, the hue representing group A is reddish-brown, group B is reddish-yellow, group C is grey, and group D is reddish-grey. Within each group, lightness and chroma are identified by a number. As the number decreases, lightness increases and chroma decreases. This shade guide used routinely by many dental practitioners has the advantage of ease of use, but it lacks a logical and adequate distribution in the color space as defined by the CIELab* specifications. In order to overcome the limits of the Vitapan Classical shade guide the Vitapan 3D Master shade guide (VITA, Bad Sackingen, Germany) consisting of 26 tabs divided into five groups according to lightness was developed. Within the groups, tabs are arranged according to chroma (vertically) and hue (horizontally). Compared to the Vitapan Classical shade guide the Vitapan 3D Master show a wider range of lightness; more chromatic tabs; extension of hue range toward the reddish spectra part; more uniformly spaced shade tabs; improved group division; better overall tab arrangement.

Other popular shade guides include the Chromascop shade guide (Ivoclar-Vivadent, Amherst, NY), that is divided into groups according to five hue criteria, and the Vintage Halo shade guide (Shofu Dental, Menlo Park, Calif) that is basically keyed to the Vitapan Classical shade guide.
It is always beneficial to use a shade guide made of the corresponding restorative material. To make color matching for composite resin restoration more feasible, manufacturers provide resin-made dental shade guides such as Esthet-X (Dentsply/Caulk, Milford, Del), Miris (Coltene/Whaledent, Cuyahoga Falls, Ohio), Tetric Ceram (Ivoclar Vivadent, Armherst, NY), Venus (Heraeus Kulzer, South Bend, Ind), Vit-l-escence (Ultradent, South Jordan, Utah). However, factors such as disinfecting solutions, heat, and age can cause color changes of the resin made ones \(^{29}\).

2.6.4. Shade-Taking Devices

Advancements in technology have enabled the use of specialized devices for shade determination. The main advantages of using technology-based shade determination is that the measurement is not influenced by the human eye, the environment and light source and the results are reproducible \(^{124}\). Clinical studies have shown that technology based shade determination is more accurate and more consistent than human shade assessment \(^{125}\).

Shade systems can be broadly classified into three types according to the underlying mechanism. RGB devices acquire red, green and blue information to create a color image such as most video or digital cameras. Spectrophotometers measure and record the amount of visible radiant energy reflected or transmitted by an object one wavelength at a time for each value, chroma, and hue present in the entire visible spectrum giving accurate and extensive data on color. Colorimeters measure color stimulus more directly and operate using a three broad-band filter \(^{126}\). All of these devices can be further classified according to the area of measurement. Spot measurement (SM)
devices measure a small area on the tooth surface, while complete-tooth measurement (CTM) devices cover the entire tooth.

In terms of tooth whitening, spectrophotometers and complete-tooth measurement devices provide reliable data about the color map of the entire tooth. With the use of a spectrophotometer, a smile analysis permits observation of a patient’s entire set of teeth in order to obtain an overall view. This is useful in recording an objective and accurate baseline shade and serves as a useful tool to motivate the patient to initiate tooth whitening. However, the use of technology based instruments is more time consuming and expensive so that overall cost-benefit ratio should be carefully considered.
CHAPTER 3
MATERIALS AND METHODS

3.1. Introduction

Hydrogen peroxide readily penetrates into the pulp chamber when applied to the external surface of the tooth indicating that dental hard tissues exhibit a substantial degree of permeability\textsuperscript{128}. While previous studies on the kinetics of hydrogen peroxide penetration have shown factors such as concentration, exposure time, heat, and light affecting the penetration, little is known about the relationship between the amount of hydrogen peroxide penetration and the resultant color change of the tooth.

This \textit{in-vitro} study compared the relationship of the amount of hydrogen peroxide penetration into the pulp cavity between two different in-office application methods and correlated penetration levels with the color change of the tooth.

Recently extracted human canines without any identifiers were obtained from the University of Iowa, and confirmation was received of the exempt status from IRB submission and review prior to initiating the study.

The study aimed to address the following research questions.

3.2. Research Questions

1. Are hydrogen peroxide penetration levels affected by the type of application method (Conventional versus sealed bleaching technique)?

2. Is the amount of color change affected by the application method?
3. Does the labial tooth thickness affect the hydrogen peroxide penetration levels and the color change of the tooth?

4. What is the relationship among the labial tooth thickness, hydrogen peroxide penetration levels and the color change of the tooth?

### 3.3. Hypotheses

Based on the research questions the following hypotheses were presented for the study.

1. The color changes ($\Delta E$, $\Delta L$, $\Delta a$, $\Delta b$) will not differ according to the bleaching protocol employed.

2. The hydrogen peroxide penetration levels will not differ according to the bleaching protocol used.

3. There will be no correlation between color change parameters ($\Delta E$, $\Delta L$, $\Delta a$, $\Delta b$) and hydrogen peroxide penetration levels.

4. There will be no correlation between color change parameters ($\Delta E$, $\Delta L$, $\Delta a$, $\Delta b$) and tooth thickness.

5. There will be no correlation between color change parameters ($\Delta E$, $\Delta L$, $\Delta a$, $\Delta b$) and initial baseline color measurements ($L^*$, $a^*$, $b^*$).

6. There will be no predictors for color change parameters ($\Delta E$, $\Delta L$, $\Delta a$, $\Delta b$).

### 3.4. Operational Definitions

1. Conventional bleaching method: the bleaching gel is applied onto the tooth surface and replenished every 20 minutes for three times.
2. Sealed bleaching method: the bleaching gel is applied onto the tooth surface and covered with a linear low density polyethylene wrap without replenishment of the gel during any of the bleaching procedure.

3. Color change (ΔE): The color difference of the tooth before and after bleaching is measured as ΔE from the Commission Internationale de l’Eclairage which is the shortest distance in the CIEL*a*b* color space between the colors being compared. It is calculated from the following equation: 
   \[ \Delta E = (\Delta L^*{}^2 + \Delta a^*{}^2 + \Delta b^*{}^2)^{1/2}. \]

4. Remaining tooth thickness: The tooth thickness measured from the outer labial surface to the outer boundary of the pulp cavity at the cross-sectioned root 3mm below the cemento-enamel junction as measured using an electronic digital caliper.

5. Peroxide penetration level: The amount of peroxide that is recovered in the pulp cavity with the use of an acetate buffer after application of the bleaching material onto the tooth surface. The optical density of the retrieved acetate buffer as measured by a UV spectrophotometer is converted into µg levels of hydrogen peroxide.

3.5. Study Design

3.5.1. Sample Selection and Preparation

Eighty recently extracted human maxillary and mandibular canines were collected 3 months prior to the study and stored in 0.2% Thymol (Sigma-Aldrich, USA) and distilled water at 4°C. Thymol was used only to retard bacterial growth and minimize any
effect on the tooth surface that might alter the response with \textit{in vivo} conditions. Calculus was removed with a sickle scaler (Hu-Friedy, USA), and any surface debris was cleaned with plain pumice (Preppies\textsuperscript{TM}, Whipmix, USA) and purple prophy cups (Young, USA).

All teeth were observed under the microscope (Zeiss, Germany) for the absence of developmental anomalies, caries, existing restorations, deep crack lines or severe attrition. The roots were sectioned 3mm apical to the cemento-enamel junction and the pulpal tissue was removed with #25 to #40 H-files (Mailleffer files, Dentsply, USA) and a #4 round bur in a low speed contra angle. The pulp chamber was slightly enlarged towards the lingual in order to maintain intact labial tooth structure and still be able to encompass 30µl of acetate buffer.

3.5.2. Random Assignment of Teeth and Bleaching

Fifty maxillary and thirty mandibular canines were randomly assigned into the sealed bleaching group and the conventional bleaching group by arch of origin, in permuted blocks of size ten to achieve balance in treatment numbers. The tooth thickness was measured from the outer labial surface to the outer boundary of the pulp cavity at the cross-sectioned root 3mm below the cemento-enamel junction using an electronic digital caliper (Harbor Freight Tools, USA). The teeth were then stored individually in sealed glass vials with the numbers attached to the side of the vial. A jig was fabricated for each tooth by gently placing the lingual surface of the tooth into a polyvinylsiloxane putty impression material (Exaflex, GC America Inc., USA) at a 30 degree angle from the bottom, to stabilize the tooth during the shade measurement, and the bleaching procedure.
Photographs were taken with a digital camera (Nikon D200, Sigma two point flash) and the baseline color was measured with a spectrophotometer (Spectroshade Micro, MHT, Switzerland) to provide a topographical color map of the entire tooth in one image. The 45/0 option (illumination at 45 degrees and observation at 0 degrees) was used in a dark room to avoid any interference from surrounding light.

A resin barrier (OpalDam, Ultradent, Jordan, Utah) was placed to cover 0.5mm of tooth coronal as well as 2mm of root apical to the cemento-enamel junction and light cured for 20 seconds (Elipar, 3M). The resin barrier was used in the same manner as would be used in the clinic to protect soft tissue irritation from the highly concentrated bleaching material. In this study it also served to further stabilize the tooth on the polyvinylsiloxane jig during the bleaching procedure.

The pulp cavities were rinsed twice with 30µl of distilled water and dried with coarse paper points (Hygenic, USA) prior to the placement of freshly mixed 30µl, 2M acetate buffer (pH 4.5) with a 22 gauge blunt needle on a Hamilton syringe (Hamilton Company, Reno, Nevada). The acetate buffer acted as a stabilizing agent for peroxide that might have diffused into the pulp cavity. 38% hydrogen peroxide gel (Opalescence Boost, Ultradent, Jordan, Utah) was then placed onto the labial surface of the canines to 1.5mm thickness and subjected to the following groups.

*Conventional bleaching group (CBG)*: the bleaching gel (80 µl) was applied onto the tooth surface in a 1.5mm layer and replenished every 20 minutes for three times according to manufacturer’s directions. A microbrush (Kerr applicators, Kerr, USA) was used for the removal of the bleaching gel but no irrigation was performed to avoid any contamination with the acetate buffer in the cavity.
*Sealed bleaching group (SBG):* the bleaching gel (80 µl) was applied onto the tooth surface in a 1.5mm thick layer and covered with a linear low density polyethylene wrap (Professional Plastic Food Wrap Film, Bakers & Chefs, USA) without replenishment of the gel during the bleaching procedure (60 minutes).

All samples were kept in a closed humid chamber (General Glassblowing Co. Lab Apparatus, USA) with 100% relative humidity during the bleaching procedure to mimic the oral environment and to avoid contamination from the surrounding environment (Figure 1). At the end of the bleaching procedure, the acetate buffer was retrieved from the pulp cavities by Hamilton syringe and placed in 10ml volumetric flasks. The pulp cavities were thoroughly rinsed twice with 30µl of distilled water and the washes were added to the flasks. After removal of the acetate buffer the bleaching gel was removed with microbrushes and the teeth were rinsed with distilled water and stored in the individual boxes for two hours prior to measuring postoperative shades with the spectrophotometer.

3.5.3. Measurement of Hydrogen Peroxide Penetration Levels

Hydrogen peroxide penetration levels were estimated according to the method of Mottola \(^70\) which is a convenient and sensitive method for the determination of hydrogen peroxide in submicrogram amounts. Leuco crystal violet (LCV, Sigma Chemical Company, USA), 50mg was dissolved in 80ml of 0.5%(v/v) hydrochloric acid and diluted to 100ml with acid of the same strength to obtain a LCV solution of 0.5mg/ml. A solution of horseradish peroxidase (HRP type II, Sigma Chemical Company, USA) was freshly prepared as needed by mixing 10mg of the enzyme preparation with 10ml of
distilled water (1mg/ml). The acetate buffer was made by dissolving equal volumes of
2M sodium acetate and 2M acetic acid then adjusting the pH to 4.5 with glacial acetic
acid.  

1ml of LCV solution (0.5mg/ml), 0.5 ml of HRP solution (1mg/ml) was added to
the volumetric flasks containing the acetate buffer retrieved from the cavity. A blue color
developed immediately upon addition of the HRP solution. Further addition of 4ml
acetate buffer turned the mixture to violet. The mixture was then diluted with distilled
water to a total volume of 10ml. The intensity of the color was proportional to the
hydrogen peroxide concentration and was measured in a UV/Visible Spectrophotometer
(Perkin Elmer, Model Lambda 20, USA) at a wavelength of 596nm. The evaluator taking
the spectrophotometer reading was blinded regarding the treatment group. A standard
calibration curve with known amounts of hydrogen peroxide was used to determine the
amount of hydrogen peroxide in microgram equivalents in the samples.

3.5.4. Standard Calibration Curve

A hydrogen peroxide working solution of 33.3μg/ml was prepared by diluting the
commercial reagent (30% v/v, Sigma Chemical Company, USA) with distilled water. In
order to obtain samples with 0, 0.33, 0.83, 1.66, 3.3, and 6.66μg of hydrogen peroxide, 0,
10, 25, 50, 100, and 200μl of the working solution was placed into 10ml volumetric
flasks respectively. 1ml of LCV solution (0.5mg/ml), 0.5 ml of HRP solution (1mg/ml),
and 4ml of acetate buffer (0.2M, pH 4.5) were added to the flasks and the total volume
adjusted to 10ml with distilled water. The optical density was measured with a UV
Spectrophotometer and the standard calibration curve was plotted from 0 to 6.66µg of hydrogen peroxide.

3.5.5. Determination of Color Change

The color difference of the tooth before and after bleaching was measured as $\Delta E$ from the Commission Internationale de l’Eclairage which is the shortest distance in the CIEL*a*b* color space between the colors being compared. It was calculated from the following equation: $\Delta E = (\Delta L^*^2 + \Delta a^*^2 + \Delta b^*^2)^{1/2}$, with the use of a software analysis program (MHT Software Analysis version 2.43). Synchronization of before and after bleaching images is a powerful function of the SpectroShade system software that allows measurement of color difference of two images. Synchronous measurement was performed at the center of the tooth (Figure 2).

3.5.6. Data Entry and Storage

Data including the measured labial tooth thickness, optical density, and the color change were initially written down on a sheet of paper. The data were then entered into Excel followed by calculation of penetration levels. The data entry was confirmed twice by another evaluator.

3.6. Data Analysis

3.6.1. Preliminary Studies

In order to secure information on which to base sample size estimation, a pilot study was conducted, and recordings of overall color change ($\Delta E$) and level of $\text{H}_2\text{O}_2$
penetration were obtained for six experimental units bleached using the conventional protocol and six using the sealed protocol. Descriptive statistics for these data are provided in Table 1 and 2.

Examination of the data suggest considerable positive skewness, and evaluation of conformance to normality using the Shapiro-Wilk test were significant for ΔE (p=0.042) and suggestive for penetration level (p=0.08). Measures of variation also appeared to differ. Normalizing transformations were therefore considered, and reasonably satisfactory results were obtained using the natural log (ln) transformation. Shapiro-Wilk tests of normality were not significant (p=0.54 for ΔE and p=0.14 for penetration level). Box plots and descriptors on the natural log transformed scale are given in Table 3 and 4.

Note that the data in this small pilot sample provided no evidence of any difference between the two bleaching groups in terms of either ΔE or H₂O₂ penetration level (p ≈ 0.70 and p ≈ 0.40, respectively, Wilcoxon Rank Sum test.).

3.6.2. Sample Size Estimation

Estimates of required sample size were obtained based upon information from the pilot study, specifying α =0.05 and 80% power. Natural log transformations were used to normalize the data. Sample sizes were set at 40 experimental units per treatment. With these sample sizes, we expected to be able to detect, with 80% power, differences (on the natural log scale) of 0.44 for ΔE and 0.49 for hydrogen peroxide penetration level. The effect size for ln ΔE corresponds to a detectable treatment difference of approximately 1.0 to 1.5 units of ΔE.
3.6.3. Randomization

Eighty extracted teeth were randomized: Fifty maxillary canines and thirty mandibular canines were used. Randomization to the two treatments, (conventional versus sealed bleaching technique) was performed separately by arch of origin, in permuted blocks of size ten to achieve balance in treatment numbers. The block size was not disclosed by the statistician until after the study data were collected. Treatment groups were labeled “A” or “B” and the statistician was blinded to the treatment allocation during the analysis phase until the time of abstract preparation.

3.6.4. Statistical Methods

Measurements of color change included overall color change ($\Delta E$), as well as changes in lightness ($\Delta L$), the red-green dimension ($\Delta a$), and the blue-yellow dimension ($\Delta b$). Other measures of interest included hydrogen peroxide penetration and tooth thickness.

The nonparametric Wilcoxon Rank Sum (Mann-Whitney) procedure was used to assess whether the two treatment groups differed at baseline with respect to $L^*$, $a^*$, $b^*$ and tooth thickness. This procedure was also used to evaluate group differences in color change and $H_2O_2$ penetration following treatment. The Wilcoxon Signed Rank test was used to assess whether there was significant change relative to baseline within a given treatment group.

Bivariate analysis using Pearson correlation coefficients was used to assess whether color changes ($\Delta E$, $\Delta L$, $\Delta a$, $\Delta b$) were also related to other measures, such as tooth thickness and $H_2O_2$ penetration, and initial baseline color measurements.
Multiple linear regression was used to evaluate factors affecting color change, which was measured as ΔL, Δa, Δb, and ΔE. Candidate covariates entertained in the modeling of a given color change outcome included bleaching technique, tooth thickness, H₂O₂ penetration, and the relevant baseline value(s) of the particular color dimension. Standard residual analyses were carried out to assess validity of assumptions associated with the regression modeling, including residual plots and Shapiro-Wilk tests of normality. In the case of ΔE, a natural logarithmic transformation was needed to achieve conformity to model assumptions.

Throughout, the level of significance was set at α=0.05.

Figure 1. Humidified chamber
Figure 2. Synchronization of before and after pictures with the use of a software analysis program (MHT Software analysis version 2.43).
CHAPTER 4

RESULTS

4.1. Descriptive Statistics

Sample sizes were set at 40 experimental units per treatment group. With these sample sizes, we expected to be able to detect, with 80% power, differences (on the natural log scale) of 0.44 for ΔE and 0.49 for hydrogen peroxide penetration level. The effect size for ln ΔE corresponds to a detectable treatment difference of approximately 1.0 to 1.5 units of ΔE. Descriptive statistics for all key variables are given in Table 5 and 6. L1, A1, B1 are baseline values; L2, A2, B2 are postbleaching values of L*, a*, b*.

4.2. Baseline Comparisons

The conventional and sealed bleaching groups were similar at baseline with respect to the L*, a* and b* color dimensions, as well as tooth thickness. Descriptive statistics are given in Table 7, as well as results of the Wilcoxon Rank Sum procedure, which was used to assess whether the two groups differed at baseline with respect to any of these four measurements. No evidence was found of any baseline difference between treatment groups (p>0.50 in all instances). Figures 3-7 give box plots illustrating the similarity of the two groups with respect to baseline measures.

4.3. Color Change within Bleaching Group

Following bleaching treatment, each of the two groups showed significant color change relative to baseline in each of the four color change variables (p<0.0001 in all
instances, Wilcoxon Signed Rank test). Descriptive statistics for the four measures of color change (ΔL, Δa, Δb, and ΔE) are given in Table 8.

4.4. Group Comparison of Crude Color Change and Hydrogen Peroxide Penetration

The two bleaching groups were compared with respect to the four measures of color change using the Wilcoxon Rank Sum procedure. Following bleaching treatment, the two groups were similar in terms of color change relative to baseline; descriptive statistics are given in Table 8. Based upon the Wilcoxon Rank Sum test, there was no evidence that the two groups differed for any color change measurement. Figures 7-10 give box plots illustrating the similarity of the two groups with respect to baseline measures.

In contrast, the two groups were found to differ significantly in the level of hydrogen peroxide penetration following bleaching (p<0.0001, Wilcoxon Rank Sum Test). In the conventional bleaching group, the mean level of H\textsubscript{2}O\textsubscript{2} penetration was higher, i.e., 0.54 μg (median 0.50) with a standard deviation of 0.20. In the sealed bleaching group, the mean level of penetration was significantly lower, 0.33 μg (median 0.31) with a standard deviation of 0.16. The distribution of hydrogen peroxide penetration levels is illustrated in Figure 11.

4.5. Correlates of Color Change

There was interest in whether color change was also related to other measures, such as tooth thickness and H\textsubscript{2}O\textsubscript{2} penetration. Pearson correlation coefficients were used as
measures of bivariate association, and are given in Table 9. None of the four color change measures appeared to be correlated with hydrogen peroxide penetration (Figure 12-15), but a number of other interesting relationships were identified.

Particularly noteworthy are the highly significant correlations of $\Delta L$ with baseline lightness ($r=-0.32$, $p=0.0038$) and the correlation of $\Delta a$ with baseline values of $a^*$ ($r=-0.42$, $p<0.0001$). In addition, changes in the red-green color dimension ($\Delta a$) were strongly correlated with tooth thickness ($r=0.37$, $p=0.0009$).

These bivariate correlations indicate that those teeth that were initially darker tended to show greater increases in lightness after bleaching treatment (Figure 16). In the case of the $a^*$ dimension, the changes were primarily negative, that is toward the green end of the red-green dimensional scale. Those teeth that showed the greatest change (i.e., negative change, shifting toward the green end of the scale) tended to be those that had the highest baseline $a^*$ levels (were more red), (Figure 17) and the smallest tooth thicknesses (Figure 18).

Change in the yellow-blue color dimension ($\Delta b$) was also correlated with tooth thickness ($r=0.25$, $p=0.025$). These changes were also overwhelmingly negative, that is, shifted toward the blue end of the yellow-blue dimensional scale. Those teeth that showed the greatest change (i.e., negative change, shifting toward the blue end of the scale) tended to be those that had the smallest tooth thicknesses (Figure 19).

As seen in Table 10, several of the color change measures themselves were highly correlated. Not surprisingly, $\Delta E$, which is a function of the other three dimensions, was strongly correlated with all three change measures, and with a number of other factors.
with baseline $a^*$ ($r=0.39$, $p=0.0004$, Figure 20). There was also an association with baseline $L^*$ ($r=-0.29$, $p=0.010$, Figure 21), and a suggestion of an association with tooth thickness ($r=-0.19$, $p=0.084$, Figure 22). Table 11 indicates that baseline $a^*$ was highly correlated with the other two baseline measures, $L^*$ ($r=-0.80$, $p<0.0001$) and $b^*$ ($r=0.47$, $p<0.0001$). Note that for these exploratory correlation analyses, no attempt was made to adjust for multiple comparisons.

The issue of group comparisons was therefore revisited in the context of multiple linear regression, which made it possible to reassess group differences after adjustment for covariates.

4.6. Multiple Linear Regression Modeling

Multiple linear regression was used to model the four expressions of color change: $\Delta L$, $\Delta a$, $\Delta b$, $\Delta E$. In modeling of $\Delta L$, $\Delta a$, and $\Delta b$, the possible effects of hydrogen peroxide penetration level, tooth thickness, treatment group and the corresponding baseline color measure were assessed. In modeling of $\Delta E$, covariates entertained in the model included hydrogen peroxide penetration level, tooth thickness, treatment group and all three baseline color measures, i.e., $L^*$, $a^*$, and $b^*$. As part of the modeling process, standard residual analyses were carried out to assess validity of assumptions associated with the regression modeling, such as normality, variance homogeneity, and linearity of relationships. Procedures included residual plots and Shapiro-Wilk tests of normality.

No concerning results were identified, except in the case of $\Delta E$, where a natural logarithmic transformation was needed to achieve conformity to model assumptions.
4.6.1. Modeling of Change in L*

Linear modeling of the change in lightness (ΔL) showed that the increase in lightness tended to be greater for teeth with lower initial L* values (r = -0.32, p=0.004, Figure 23). After adjustment for initial L*, there was no evidence that ΔL differed with bleaching technique (p=0.27). The mean difference in ΔL between the two treatment groups was 0.36 lightness units. The adjusted (for baseline L) means for ΔL were 2.38 for the conventional bleaching group and 2.02 for the sealed bleaching group. No other covariate was significantly associated with change in lightness. The R² value, which gives the proportion of the variability in the outcome explained by the model was 10.2%; details of the regression model are given in Table 12.

4.6.2. Modeling of Change in a*

Linear modeling of the change in the red-green color dimension (Δa) yielded a more complex picture. All four covariates were significantly related to change in the a* color dimension (Table 13). The majority of change in a* was in the negative direction, that is, toward the green end of the a* scale, as was illustrated in Figures 17 and 18. This change was more marked in teeth that had greater baseline values of a*, i.e., that were originally more red (p<0.0001). This trend in Δa was also less marked as tooth thickness increased (p<0.0001) and for those teeth that had greater H₂O₂ penetration (p=0.004).

After adjusting for the effects of the other three factors: initial value of a*, tooth thickness, and level of H₂O₂ penetration, the effect of treatment group was highly significant (p=0.002). The mean difference in Δa between the two treatment groups was 0.34 a* units. The adjusted means for Δa were -0.97 for the conventional bleaching group
and -0.63 for the sealed bleaching group. After adjustment for the effects of the other covariates, the average extent of change in Δa was .34 units greater (in the negative or “greener” direction) in the conventional bleaching group. The value of R^2 for this model was 43.7%.

After adjustment of the Δa results for bleaching group, tooth thickness and baseline value of a*, there was still a relationship with hydrogen peroxide penetration, i.e., the greater (negative) change in the red-green color dimension tended to occur in those teeth with lower hydrogen peroxide penetration (Figure 24).

The adjustment made was to the levels of the conventional bleaching group, and mean levels of thickness and baseline a* based upon the entire group. The value of the partial correlation (correlation of hydrogen peroxide penetration with Δa after adjustment for the other covariates) is 0.268, which is modest. Adding in hydrogen peroxide penetration to the model increases the R-square value by about 6.6% (i.e., relative to the model which already had group, thickness, and baseline a*).

4.6.3. Modeling of Change in b*

Linear modeling of the change in the yellow-blue color dimension (Δb) indicated that only a single variable, tooth thickness, was associated with Δb, the change in the yellow-blue color dimension (r = 0.25, p=0.025, Figure 25). The changes observed were overwhelmingly in the negative direction (toward the blue end of the scale). These results showed that this change was less marked in those teeth with greater thickness. The value of R^2 for this model was 6.3%; details of the regression model are given in Table 14. None of the other variables considered (baseline value of b*, H₂O₂ penetration, or
treatment group) were significantly associated with Δb (p>0.05) after adjustment for tooth thickness.

4.6.4. Modeling of Overall Color Change

Linear modeling of the natural logarithm of overall color change (ΔE) identified significant relationships with treatment group, tooth thickness and baseline a* (Table 15). Since ΔE is a composite measure of color change, it is unsurprising that many of the same covariates identified in previous models were significant predictors of overall color change. Note that these results are on the natural log scale, since a logarithmic transformation was needed to achieve conformity to model assumptions.

Change in overall color was more marked in teeth of lesser thickness (p=0.007), and in those teeth that had greater (more red) values of a* at baseline (p<0.0001). These relationships were previously depicted in Figures 22 and 20, respectively. Greater color change was seen in the conventional group (p=0.014). Adjusted means for ln ΔE were 1.23 for the conventional bleaching group and 1.00 for the sealed bleaching group. These correspond to ΔE values of 3.42 and 2.72, respectively. The value of R² for this model was 27.1%.
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<thead>
<tr>
<th>Table 1. Descriptive statistics based upon raw data from pilot study: Conventional bleaching group</th>
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<tbody>
<tr>
<td><strong>Variable</strong></td>
</tr>
<tr>
<td>----------------</td>
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<tr>
<td>Delta E</td>
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<td>H₂O₂ penetration</td>
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<td>Ln H₂O₂ penetration</td>
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<table>
<thead>
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<th>Table 4. Descriptive statistics based upon natural log transformed data from pilot study: Sealed bleaching group</th>
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</thead>
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</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>Ln Delta E</td>
</tr>
<tr>
<td>Ln H₂O₂ penetration</td>
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Table 5. Descriptive statistics based upon raw data from final study: Conventional bleaching group

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<tr>
<th>Variable</th>
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<th>Mean</th>
<th>Std dev</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
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</thead>
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<td>Thickness (mm)</td>
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<td>0.1964584</td>
<td>2.6350000</td>
<td>2.2300000</td>
<td>3.0000000</td>
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<tr>
<td>L1</td>
<td>40</td>
<td>70.1990000</td>
<td>3.4213574</td>
<td>70.4050000</td>
<td>62.0500000</td>
<td>77.8200000</td>
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<td>A1</td>
<td>40</td>
<td>2.9330000</td>
<td>1.5800669</td>
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<td>-0.2200000</td>
<td>6.4800000</td>
</tr>
<tr>
<td>B1</td>
<td>40</td>
<td>23.7085000</td>
<td>2.6189815</td>
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<td>17.5200000</td>
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<tr>
<td>L2</td>
<td>40</td>
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<td>3.0848472</td>
<td>72.2900000</td>
<td>64.1900000</td>
<td>81.5800000</td>
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<td>Delta L</td>
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<td>1.5816006</td>
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<td>-0.2200000</td>
<td>6.3700000</td>
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<td>-2.1300000</td>
<td>-5.0200000</td>
<td>1.0300000</td>
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<tr>
<td>Delta E</td>
<td>40</td>
<td>3.5977403</td>
<td>1.3734878</td>
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<td>1.6411581</td>
<td>6.6142271</td>
</tr>
<tr>
<td>H₂O₂ Pen(μg)</td>
<td>40</td>
<td>0.5374750</td>
<td>0.2019327</td>
<td>0.4980000</td>
<td>0.2390000</td>
<td>1.2720000</td>
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</table>
Table 6. Descriptive statistics based upon raw data from final study: Sealed bleaching group

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<tr>
<th>Variable</th>
<th>N</th>
<th>Mean</th>
<th>Std dev</th>
<th>Median</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickness (mm)</td>
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<td>2.6442500</td>
<td>0.1872183</td>
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<td>40</td>
<td>69.7510000</td>
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<td>69.5650000</td>
<td>62.1900000</td>
<td>77.3300000</td>
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<td>A1</td>
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<td>1.6489884</td>
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<td>0.7000000</td>
<td>7.4600000</td>
</tr>
<tr>
<td>B1</td>
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<td>28.8800000</td>
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<tr>
<td>L2</td>
<td>40</td>
<td>71.8045000</td>
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<td>2.4907500</td>
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<td>Delta L</td>
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<td>0.4437682</td>
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<td>Delta A</td>
<td>40</td>
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<td>1.1929945</td>
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<td>Delta B</td>
<td>40</td>
<td>3.1149932</td>
<td>1.4778032</td>
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<td>0.7491996</td>
<td>6.9836667</td>
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<tr>
<td>H$_2$O$_2$ Pen (μg)</td>
<td>40</td>
<td>0.3340750</td>
<td>0.1555474</td>
<td>0.3095000</td>
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<td>0.8230000</td>
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Table 7. Comparison of baseline data for conventional and sealed bleaching groups

<table>
<thead>
<tr>
<th>Baseline Measure</th>
<th>Conventional Bleaching</th>
<th>Sealed Bleaching</th>
<th>P-Value*</th>
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<tr>
<td></td>
<td>Mean (SD)/ Median</td>
<td>Mean (SD)/ Median</td>
<td></td>
</tr>
<tr>
<td>L*</td>
<td>70.2 (3.43) 70.4</td>
<td>69.8 (3.78) 69.6</td>
<td>0.56</td>
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<tr>
<td>a*</td>
<td>2.9 (1.58) 2.7</td>
<td>3.2 (1.65) 3.0</td>
<td>0.54</td>
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<tr>
<td>b*</td>
<td>23.7 (2.62) 23.7</td>
<td>24.0 (2.40) 24.2</td>
<td>0.51</td>
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<tr>
<td>Tooth Thickness</td>
<td>2.6 (0.20) 2.64</td>
<td>2.64 (0.19) 2.64</td>
<td>0.95</td>
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</table>

*Significance probability associated with the Wilcoxon Rank Sum test; there was no evidence that the two groups differed at baseline with respect to any of these initial measurements.

Table 8. Descriptors of color change within each bleaching group

<table>
<thead>
<tr>
<th>Color Change Measure **</th>
<th>Conventional Bleaching</th>
<th>Sealed Bleaching</th>
<th>P-Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)/ Median</td>
<td>Mean (SD)/ Median</td>
<td></td>
</tr>
<tr>
<td>ΔL</td>
<td>2.35 (1.58) 2.00</td>
<td>2.05 (1.49) 2.11</td>
<td>0.62</td>
</tr>
<tr>
<td>Δa</td>
<td>-0.87 (0.60) -0.77</td>
<td>-0.73 (0.44) -0.70</td>
<td>0.66</td>
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<tr>
<td>Δb</td>
<td>-2.06 (1.23) -2.13</td>
<td>-1.83 (1.19) -1.87</td>
<td>0.38</td>
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<tr>
<td>ΔE</td>
<td>3.60 (1.37) 3.36</td>
<td>3.11 (1.48) 2.92</td>
<td>0.15</td>
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</table>

*Significance probability associated with the Wilcoxon Rank Sum test of the null hypothesis that the two groups did not differ in the distribution of the specified color change measure. There was no evidence that the two groups differed for any change measurement based upon the Wilcoxon Rank Sum test.

** Significant change relative to baseline was found for each of the four color change measures, within each the conventional and the sealed bleaching groups (p<0.0001 in all instances, Wilcoxon Signed Rank Test).
Table 9. Correlations of color change measures with baseline color measurements, tooth thickness, and level of hydrogen peroxide penetration

<table>
<thead>
<tr>
<th></th>
<th>Thickness</th>
<th>H₂O₂ Pen</th>
<th>L1</th>
<th>A1</th>
<th>B1</th>
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<tbody>
<tr>
<td>Delta L</td>
<td>-0.02630</td>
<td>0.16073</td>
<td>-0.31987</td>
<td>0.41322</td>
<td>0.06695</td>
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<td></td>
<td>0.8169</td>
<td>0.1544</td>
<td>0.0038</td>
<td>0.0001</td>
<td>0.5551</td>
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<td>Delta A</td>
<td>0.36540</td>
<td>-0.01521</td>
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<td></td>
<td>0.0009</td>
<td>0.8935</td>
<td>0.0187</td>
<td>&lt;0.0001</td>
<td>0.0469</td>
</tr>
<tr>
<td>Delta B</td>
<td>0.25065</td>
<td>0.09269</td>
<td>-0.03786</td>
<td>-0.02182</td>
<td>-0.16964</td>
</tr>
<tr>
<td></td>
<td>0.0249</td>
<td>0.4135</td>
<td>0.7388</td>
<td>0.8477</td>
<td>0.1325</td>
</tr>
<tr>
<td>Delta E</td>
<td>-0.19438</td>
<td>0.12335</td>
<td>-0.28503</td>
<td>0.38823</td>
<td>0.15060</td>
</tr>
<tr>
<td></td>
<td>0.0840</td>
<td>0.2757</td>
<td>0.0104</td>
<td>0.0004</td>
<td>0.1824</td>
</tr>
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</table>

*Pearson correlation coefficients, N=80, Prob > |r| under HO: Rho=0

Table 10. Correlations among color change measures

<table>
<thead>
<tr>
<th></th>
<th>Delta L</th>
<th>Delta A</th>
<th>Delta B</th>
<th>Delta E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delta L</td>
<td>1.00000</td>
<td>-0.51357</td>
<td>-0.04836</td>
<td>0.80805</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>0.6701</td>
<td>0.0027</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Delta A</td>
<td>-0.51357</td>
<td>1.00000</td>
<td>0.33140</td>
<td>-0.67735</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>0.0027</td>
<td>1.00000</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Delta B</td>
<td>-0.04836</td>
<td>0.33140</td>
<td>1.00000</td>
<td>-0.53842</td>
</tr>
<tr>
<td></td>
<td>0.6701</td>
<td>0.0027</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta E</td>
<td>0.80805</td>
<td>-0.67735</td>
<td>-0.53842</td>
<td>1.00000</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Pearson correlation coefficients, N=80, Prob > |r| under HO: Rho=0

Table 11. Correlations among baseline color measures

<table>
<thead>
<tr>
<th></th>
<th>L1</th>
<th>A1</th>
<th>B1</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>1.00000</td>
<td>-0.79447</td>
<td>-0.21593</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>0.0544</td>
</tr>
<tr>
<td>A1</td>
<td>-0.79447</td>
<td>1.00000</td>
<td>0.47171</td>
</tr>
<tr>
<td></td>
<td>&lt;0.0001</td>
<td></td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>B1</td>
<td>-0.21593</td>
<td>0.47171</td>
<td>1.00000</td>
</tr>
<tr>
<td></td>
<td>0.0544</td>
<td>&lt;0.0001</td>
<td></td>
</tr>
</tbody>
</table>

*Pearson correlation coefficients, N=80, Prob > |r| under HO: Rho=0
Table 12. Linear regression modeling of change in lightness (ΔL)

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>Estimated β</th>
<th>S.E.</th>
<th>t-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>11.766</td>
<td>3.212</td>
<td>3.66</td>
<td>0.0005</td>
</tr>
<tr>
<td>Baseline L*</td>
<td>-0.137</td>
<td>0.046</td>
<td>-2.98</td>
<td>0.0038</td>
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</table>

Table 13. Linear regression modeling of change in the red-green color dimension (Δa)

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>Estimated β</th>
<th>S.E.</th>
<th>t-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-3.838</td>
<td>0.673</td>
<td>-5.70</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Conventional Group</td>
<td>-0.343</td>
<td>0.107</td>
<td>-3.19</td>
<td>0.0021</td>
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<tr>
<td>Tooth Thickness</td>
<td>1.292</td>
<td>0.247</td>
<td>5.22</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>H₂O₂ Penetration</td>
<td>0.800</td>
<td>0.269</td>
<td>2.97</td>
<td>0.0040</td>
</tr>
<tr>
<td>Baseline a*</td>
<td>-0.178</td>
<td>0.030</td>
<td>-6.01</td>
<td>&lt;0.0001</td>
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</table>

Table 14. Linear regression modeling of change in the yellow-blue color dimension (Δb)

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>Estimated β</th>
<th>S.E.</th>
<th>t-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>-6.136</td>
<td>1.837</td>
<td>-3.34</td>
<td>0.0013</td>
</tr>
<tr>
<td>Tooth Thickness</td>
<td>1.587</td>
<td>0.694</td>
<td>2.29</td>
<td>0.0249</td>
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</table>
Table 15. Linear regression modeling of the natural log of overall change (ΔE)

<table>
<thead>
<tr>
<th>VARIABLE</th>
<th>Estimated β</th>
<th>S.E.</th>
<th>t-Value</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>2.395</td>
<td>0.632</td>
<td>3.79</td>
<td>0.0003</td>
</tr>
<tr>
<td>Conventional Group</td>
<td>0.226</td>
<td>0.090</td>
<td>2.51</td>
<td>0.0143</td>
</tr>
<tr>
<td>Tooth Thickness</td>
<td>-0.667</td>
<td>0.239</td>
<td>5.22</td>
<td>0.0063</td>
</tr>
<tr>
<td>Baseline a*</td>
<td>0.119</td>
<td>0.028</td>
<td>4.20</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Figure 3. Box plots for baseline lightness (L*) by bleaching group
Figure 4. Box plots for baseline red-green value (a*) by bleaching group

Figure 5. Box plots for baseline yellow-blue value (b*) by bleaching group
Figure 6. Box plots for tooth thickness by bleaching group

Figure 7. Box plots for ΔL (Change in lightness L* relative to baseline) by bleaching group
Figure 8. Box plots for $\Delta a$ (Change in red-green color dimension $a^*$ relative to baseline) by bleaching group

Figure 9. Box plots for $\Delta L$ (Change in yellow-blue color dimension $b^*$ relative to baseline) by bleaching group
Figure 10. Box plots for ΔE (overall color change relative to baseline) by bleaching group

Figure 11. Box plots for hydrogen peroxide penetration level by bleaching group
Figure 12. Scatterplot showing relationship of change in lightness ($\Delta L$) with hydrogen peroxide penetration levels (µg): Although the sealed bleaching group tended to have lower hydrogen peroxide penetration levels, the distribution of the change scores were similar.

Figure 13. Scatterplot showing relationship of change in chroma in the a-axis ($\Delta a$) with hydrogen peroxide penetration levels (µg): Although the sealed bleaching group tended to have lower hydrogen peroxide penetration levels, the distribution of the change scores were similar.
Figure 14. Scatterplot showing relationship of change in chroma in the b-axis ($\Delta b$) with hydrogen peroxide penetration levels (μg): Although the sealed bleaching group tended to have lower hydrogen peroxide penetration levels, the distribution of the change scores were similar.

Figure 15. Scatterplot showing relationship of change in overall color ($\Delta E$) with hydrogen peroxide penetration levels (μg): Although the sealed bleaching group tended to have lower hydrogen peroxide penetration levels, the distribution of the change scores were similar.
Figure 16. Scatterplot showing relationship of change in lightness (ΔL) with baseline lightness (L*): The greatest increases in lightness tended to occur in the teeth that were darkest at baseline (r = -0.32, p=0.0038).

Figure 17. Scatterplot showing relationship of change in the red-green color dimension (Δa) with baseline a*: The greatest (negative) changes were associated with higher (more red) baseline levels of a* (r = -0.42, p<0.0001).
Figure 18. Scatterplot showing relationship of change in the red-green color dimension ($\Delta a$) with tooth thickness: The greatest (negative) changes were associated with lesser tooth thickness ($r=0.37$, $p=0.0009$).

Figure 19. Scatterplot showing relationship of change in the yellow-blue color dimension ($\Delta b$) with tooth thickness: The greatest (negative) changes were associated with lesser tooth thickness ($r=0.25$, $p=0.025$).
Figure 20. Scatterplot showing relationship of overall color change (ΔE) with baseline $a^*$: Greater overall color change was associated with greater (more red) values of $a^*$ at baseline ($r=0.39$, $p=0.0004$).

Figure 21. Scatterplot showing relationship of overall color change (ΔE) with baseline $L^*$: Greater overall color change tended to be associated with lower baseline values of $L^*$ ($r=-0.29$, $p=0.010$).
Figure 22. Scatterplot showing relationship of overall color change ($\Delta E$) with tooth thickness: There was a suggestion that greater overall color change was associated with lesser tooth thickness ($r=-0.19$, $p=0.084$).

Figure 23. Linear regression of change in lightness ($\Delta L$) on baseline $L^*$. *Note that there was no evidence of an association with bleaching treatment.*
Figure 24. Scatterplot showing relationship of change in the red-green color dimension (Δa) adjusted for group, tooth thickness, and baseline a* with hydrogen peroxide penetration levels: The greater (negative) change in the red-green color dimension tended to occur in those teeth with lower hydrogen peroxide penetration.

Figure 25. Linear regression of change in yellow-blue color dimension (Δb) on tooth thickness.

* Note that there was no evidence of an association with bleaching treatment.
This study compared the relationship of the amount of hydrogen peroxide penetration into the pulp cavity between two different in-office bleaching application methods and correlated penetration levels with the color change of the tooth.

Bowles and Ugwuneri\textsuperscript{128} were the first to show, that in extracted teeth exposed to hydrogen peroxide significant levels of hydrogen peroxide could be detected in the pulp cavity. Many studies followed after Bowles and Ugwuneri’s study adopting the newly introduced \textit{in-vitro} model to investigate various factors that might influence the amount of hydrogen peroxide penetration into the pulp cavity. Studies have shown that higher concentrations of hydrogen peroxide\textsuperscript{128}, heat and prolonged bleaching time\textsuperscript{72}, light activation\textsuperscript{76}, altered surface due to restorations\textsuperscript{73} and characteristics like large open dentinal tubules of young teeth\textsuperscript{68} facilitate the diffusion and penetration of hydrogen peroxide molecules from the outer tooth surface into the pulp cavity.

However, to the best of our knowledge the relationship of the amount of hydrogen peroxide penetration has not been yet correlated with the color change of the tooth. That is, does a larger amount of hydrogen peroxide penetration also demonstrate superior bleaching or would it be more beneficial to have less penetration but more hydrogen peroxide retention in the tooth structure? The author of this thesis hypothesized that by controlling the diffusion gradient between the outer tooth surface and the pulp cavity, the diffusion of the hydrogen peroxide molecules could be slowed down so that the active agents were more likely to interact with the stain molecules rather than penetrating into
the pulp. This could not only enhance the bleaching efficacy but also decrease the irritation of the pulp due to less exposure to hydrogen peroxide.

5.1. Baseline Comparisons

Knowledge of human tooth color and its distribution are important in understanding the prognostic factors of tooth bleaching. The color of human teeth shows gradation from the gingival side to the incisal region. O’Brien\textsuperscript{129} determined the color distribution in three regions in a sample of human teeth and expressed the results in CIE 1976 L* a* b* values and detected significant differences in the color parameters of the three distinct regions in teeth. The average L*, a*, and b* values in the middle region of evaluated anterior teeth were 72.4, 1.2, and 16.2, respectively.

Compared to O’Brien’s study, the teeth used in our study were slightly lower in value and higher in chroma. The mean L*, a*, b* values for the conventional and sealed bleaching group being 70.2, 2.9, 23.7, and 69.8, 3.2, 24.0, respectively. This difference might be attributed to the fact that only canine teeth were used in this study whereas all anterior teeth were selected for O’Brien’s color distribution study. In this thesis canines were selectively chosen, because their size is overall larger than central and lateral incisors making it more feasible to encompass a certain amount of acetate buffer.

Based on the Wilcoxon Rank Sum Test, the conventional and sealed bleaching groups were similar at baseline with respect to L*, a* and b* color dimensions as well as tooth thickness, which illustrates well the successful randomization into the two treatment groups by the use of permuted blocks of size ten to achieve balance in treatment numbers.
5.2. Conventional In-Office Bleaching versus Sealed In-Office Bleaching

In-office bleaching is generally preferred by both dentists and patients that the responsibility for procedure of bleaching teeth is taken in the office\textsuperscript{130}. In-office bleaching produces immediate bleaching results and can also be used as a kick start so that patients comply better with home bleaching procedures.

However, irritation to the nasal mucosa, inadvertent exposure to the highly concentrated bleaching gel as well as inconvenience and increased costs associated with required multiple replenishment of bleaching gel during one bleaching session have been pointed out as disadvantages of conventional in-office bleaching procedures\textsuperscript{127}. In order to prevent the evaporation and desiccation of the active agents, Kwon\textsuperscript{127} proposed placing a linear low density polyethylene (LLDPE) wrap onto the power whitening gel. This made the sealed bleaching procedure safer, simpler and more economical compared to the conventional in-office bleaching procedure.

Since, the bleaching agent is not replenished in the sealed bleaching technique, the diffusion gradient is kept more constant than in the conventional in-office bleaching procedure so that less hydrogen peroxide penetration levels are expected.

5.3. Group Comparison of Crude Color Change

The two bleaching groups were compared with respect to the four measures of color change using the Wilcoxon Rank Sum procedure. Following bleaching treatment, the two groups were similar in terms of color change relative to baseline. The mean of color change parameters $\Delta L$, $\Delta a$, $\Delta b$, $\Delta E$ for the conventional and sealed bleaching group were 2.35, -0.87, -2.06, 3.60 and 2.05, -0.73, -1.83, 3.11, respectively. This change of $\Delta E$
in both groups is considered to be discernible to the naked eye and reflects the clinical relevance of this study.

Thus, the first null hypothesis presented that the color changes (\(\Delta E\), \(\Delta L\), \(\Delta a\), \(\Delta b\)) will not differ according to the bleaching protocol employed was accepted.

It is also interesting to point out the relevance of \(\Delta E\) values of both groups, to an in-vivo pilot study. Matis et al.\(^8\) comparing eight different in-office bleaching products showed that contact time was important while concentration was not. Color measurements were made with a colorimeter at baseline, immediately after treatment and one, two, four and six weeks after bleaching. The mean \(\Delta E\) measured immediately after bleaching was 6.77. And at one and six weeks after bleaching the mean \(\Delta E\) regressed to 3.31 and 2.34, respectively.

The \(\Delta E\) values of our study measured two hours post-bleaching corresponded to the \(\Delta E\) values of Matis et al.’s study\(^8\) measured one week after bleaching. This might suggest that dehydration that occurs during the bleaching procedure and which often is attributed to false interpretation of the bleaching result is mostly reversed after two hours.

Matis et al.\(^8\) showed that the in-office products with the lower concentrations (15~25%) had the highest \(\Delta E\) values immediately after bleaching. The authors suggested that contact time is more important than the concentration and speculated that other agents added to the product must have catalyzed the peroxide reaction with stain molecules.

Contrary to Matis et al.\(^8\) who pointed out the influence of other agents added to the product that might have activated the peroxide, we speculate the importance of the diffusion gradient that is lower in lower concentrations (15~25%) of hydrogen peroxide.
bleaching agents. There seems to be an optimal concentration for in-office bleaching that balances diffusion into the tooth structure as well as interaction with stain molecules. That is, the higher the concentration the better and faster the bleaching efficacy, might not be applicable after a certain percentage of hydrogen peroxide.

The group comparison of crude color changes in our study which showed no differences between the two treatment groups strongly supports our hypothesis that the diffusion gradient is an important factor to consider for the efficacy of bleaching in terms of color change.

5.4. Group Comparison of Crude Hydrogen Peroxide Penetration Levels

The two groups were found to differ significantly in the level of hydrogen peroxide penetration following bleaching which was highly expected. In the conventional bleaching group, the mean level of $\text{H}_2\text{O}_2$ penetration was higher, i.e., 0.54 μg. In the sealed bleaching group, the mean level of penetration was significantly lower, 0.33 μg.

Based upon the Wilcoxon Rank Sum test, we rejected the second null hypothesis that the hydrogen peroxide penetration levels will not differ according to the bleaching protocol used.

The mean hydrogen peroxide penetration levels of both groups are much lower than in a previous study by Bowles and Ugwuneri\textsuperscript{128} who applied 30% hydrogen peroxide for 15 minutes at 37°C and detected hydrogen peroxide levels of 25.4±8.5μg. This difference can be explained by the difference of cavity preparation, tooth selection, hydrogen peroxide delivery method and temperature settings.
In our study, care was taken to avoid preparation of the labial side of the pulp cavity to leave the labial part of the tooth as intact as possible. Instead cavity preparation was performed towards the lingual part in order to encompass 30 μl of acetate buffer that would act as a stabilizing agent for the diffused hydrogen peroxide. However, Bowles and Ugwuneri\textsuperscript{128} enlarged the pulp cavity uniformly to encompass 100 μl of acetate buffer which left only a small part of labial tooth structure intact.

Canines were selectively chosen for this study, because they exhibit lower lightness values and higher chroma values. Since canines are also thicker than other anterior teeth used in Bowles and Ugwuneri’s study\textsuperscript{128} one might expect lower penetration levels associated with our study.

Bowles and Ugwuneri\textsuperscript{128} used hydrogen peroxide liquid and higher temperatures (37°C), whereas a viscous hydrogen peroxide gel was used at room temperature (23°C) in our study, probably influencing the amount of hydrogen peroxide penetration.

There are many factors affecting the hydrogen penetration level and the levels seem to differ according to the experimental protocol employed. However, it is important to understand the clinical significance of hydrogen peroxide penetration into the pulp cavity and the possible risk associated with significant levels of hydrogen peroxide penetration.

It is logical to think that higher hydrogen peroxide penetration levels will also result in greater color change. However the group comparison of crude color change and hydrogen peroxide penetration levels in this study has shown that although the hydrogen peroxide penetration level was significantly higher in the conventional bleaching group, there was no difference in terms of color change between the two groups.
Hydrogen peroxide penetration levels have not been associated with the frequency and severity of hypersensitivity that is one of the most frequently reported side effect of tooth bleaching. The effects of bleaching and hydrogen peroxide penetration at the molecular level within the cells and connective tissues of the pulp, is still not well established. Little is known about the effects of hydrogen peroxide on metabolic processes in the pulp, such as glucose metabolism and protein synthesis, especially synthesis of collagen.

Bowles and Thompson have shown that a number of pulpal enzymes (alkaline phosphatase, aldolase, glucose 6-phosphate dehydrogenase (G6-PDH), serum glutamic-oxaloacetic transaminase (SGOT), isocitrate dehydrogenase, malate dehydrogenase, and phosphohexose isomerase) are quite sensitive to the combination of hydrogen peroxide and heat. However, calculations made from their data show that the actual quantity of hydrogen peroxide that inhibited enzyme activity was relatively large (in the range of 50mg) while the present and previous studies revealed only microgram quantities of hydrogen peroxide penetration into the pulp cavity.

Despite, the high threshold for pulpal enzyme inhibition it seems to be prudent to minimize hydrogen peroxide penetration into the pulp cavity without compromising bleaching efficacy.

5.5. Correlates of Color Change

The main purpose of this study was to correlate color change with other measures, such as tooth thickness and hydrogen peroxide penetration, since no previous study had explored these correlations. Pearson correlation coefficients were used as measures of
bivariate association and interesting correlations were found between $\Delta L$ and baseline lightness ($r=-0.32$, $p=0.0038$), $\Delta a$ and baseline a* values ($r=-0.42$, $p<0.0001$), and strong correlation in the changes of $\Delta a$ with tooth thickness ($r=0.37$, $p=0.0009$).

These correlations indicate that those teeth that were initially darker tended to show greater increases in lightness after bleaching treatment. In the case of a* dimension, the changes were primarily negative, that is toward the green end of the red-green dimensional scale. Those teeth that showed the greatest change toward the negative direction tended to be those that had the highest baseline a* levels (were more red) and the smallest tooth thicknesses.

Based on the bivariate analysis the third null hypothesis that there will be no correlation between color change parameters ($\Delta E$, $\Delta L$, $\Delta a$, $\Delta b$) and hydrogen peroxide penetration levels was accepted. However, the fourth null hypothesis stated that there will be no correlation between color change parameters ($\Delta E$, $\Delta L$, $\Delta a$, $\Delta b$) and tooth thickness had different outcomes depending on the specific color parameter. Change in lightness ($\Delta L$) and change in the red-green color dimension ($\Delta a$) were related to tooth thickness, so that the null hypotheses for these parameters were rejected. Overall color change ($\Delta E$) and change in the yellow-blue color dimension ($\Delta b$) was not related to tooth thickness leading to the acceptance of the null hypotheses for the two parameters. Since overall color change is a composite of all three color parameters, it was suggestive that it was also related to tooth thickness ($p=0.08$).

The fifth null hypothesis presented that there will be no correlation between color change parameters ($\Delta E$, $\Delta L$, $\Delta a$, $\Delta b$) and initial baseline color measurements ($L^*$, $a^*$, $b^*$), had twelve sub-null hypotheses. Six of them were rejected and six accepted. It is
noteworthy to point out that change in lightness was strongly related to initial lightness value and that change in the red-green color dimension was related to initial a* values. Overall color change was related to both, initial lightness value and the initial a* value.

The color of human teeth shows gradation from the gingival to the incisal region as was documented by O’Brien\textsuperscript{129}. The correlations of baseline lightness and a* values with tooth color change might explain why teeth after bleaching exhibit a less accentuated gradation and show a more uniform color within the same tooth.

Wetter et al.\textsuperscript{133} evaluated the color differences of canines and incisors in a comparative long-term clinical trial and showed that a significantly stronger overall increase in lightness was observed for canines after bleaching when compared with incisors resulting in a more homogenous lightness values within the arch. Wetter et al.’s findings\textsuperscript{133} also support the correlation found in our study.

The bivariate analysis did not reveal any correlation between the four color change measures and hydrogen peroxide penetration level. However, in order to address the correlations and reassess group differences, multiple linear regression was performed after adjustment for covariates.

5.6. Modeling of Change in L*

Linear modeling of the change in lightness (ΔL) showed that the increase in lightness tended to be greater for teeth with lower initial L* values ($r = -0.32$, $p=0.004$). After adjustment for initial L*, there was no evidence that ΔL differed with bleaching technique ($p=0.27$). The mean difference in ΔL between the two treatment groups was 0.36 lightness units, which clinically is not discernible to the naked eye.
Bleaching involves a series of complex changes that alter a set of separate color parameters of which L* is generally regarded as the primary one and also the most used to assess the effectiveness of a bleaching procedures $^{133}$.

Modeling of $\Delta$L showed that it was not affected by other covariates except for initial lightness values, which seem to make it a consistent measure for evaluating bleaching efficacy.

Based on the linear modeling of $\Delta$L, the null hypothesis stated that there will be no predictors for change in lightness was rejected.

It is also noteworthy to point out the importance of taking initial baseline values into consideration when comparing different treatment groups in bleaching studies, since they affect the amount of change in lightness ($\Delta$L).

5.7. Modeling of Change in a*

Linear modeling of the change in the red-green color dimension ($\Delta$a) yielded a more complex picture. All four covariates were significantly related to change in the red-green color dimension. Thus the null hypothesis that there will be no predictors for the color change in the red-green color dimension was rejected.

After adjusting for the effects of the other three factors: initial value of a*, tooth thickness, and level of H$_2$O$_2$ penetration, the effect of treatment group was highly significant ($p=0.002$). The mean difference in $\Delta$a between the two treatment groups was 0.34 a* units. The adjusted means for $\Delta$a were -0.97 for the conventional bleaching group and -0.63 for the sealed bleaching group. After adjustment for the effects of the other covariates, the average extent of change in $\Delta$a in the negative direction was 0.34
units greater in the conventional bleaching group. How important or significant this difference would be clinically, has not been discussed in the literature. The threshold of perceptibility of color change has been reviewed and evaluated in terms of ΔE values\textsuperscript{112}. However, the threshold perceptibility of Δa and Δb separately has not been investigated yet.

After adjustment of the Δa results for bleaching group, tooth thickness and baseline value of a*, there was still a relationship with hydrogen peroxide penetration, i.e., the greater (negative) change in the red-green color dimension tended to occur in those teeth with lower hydrogen peroxide penetration.

5.8. Modeling of Change in b*

Linear modeling of the change in the yellow-blue color dimension (Δb) indicated that only a single variable, tooth thickness, was associated with Δb, the change in the yellow-blue color dimension (r = 0.25, p=0.025). These results showed that this change was more marked in those teeth with thinner thickness.

The null hypothesis that there will be no predictors for the change in the yellow-blue color dimension was thus rejected.

5.9. Modeling of Overall Color Change

Linear modeling of the natural logarithm of overall color change (ΔE) identified significant relationships with treatment group, tooth thickness and baseline a*. Since ΔE is a composite measure of color change, it is unsurprising that many of the same
covariates identified in previous models were significant predictors of overall color change.

The null hypothesis that there will be no predictors for the overall color change was also rejected.

5.10. Limitations of the Study

This in-vitro model is representative of the in-vivo process, although it is not known how closely it would compare to the in-vivo absorption of hydrogen peroxide in teeth with vital pulps during the bleaching process. There are at least two forces that might work against the penetration of hydrogen peroxide molecules towards the pulp: the positive pulpal pressure and osmotic pressure of the gels.

The preparation of the pulp cavity to encompass a certain amount of buffer is another factor that will be different under in-vivo conditions. However, the author made great effort to keep the labial part of the tooth as intact as possible and instead extend the cavity towards the lingual part.

Tooth dehydration has been pointed out as an important factor to consider when making shade measurements on teeth for proper color selection of restorative materials and for evaluation of color to compare bleaching efficacy.

Tooth dehydration could occur during the appointment because of the long time spent with the mouth open. This results in alteration of tooth lightness and chroma; more than 24 hours is needed to regain the normal tooth coloration. Generally it is recommended clinically to select the shade prior to performing any restorative work,
since it is not practical to wait 24 hours. Besides “24 hours” was not based on any research data but was rather proposed out of clinical experience.

Russell et al.\textsuperscript{136} evaluated the dehydration component associated with restorative procedures \textit{in-vivo} and found that teeth become brighter and less color saturated after rubber dam application (15 min) and brighter after impression taking. The original values as measured by CIELAB color parameters (L*, a*, b*) were regained after 30 minutes.

So far there have been no studies evaluating color changes in bleaching treatments due to the dehydration component. However, there seems to be a need for consensus on the proper time for postbleaching shade measurements \textit{in-vitro} and \textit{in-vivo}. The authors in this study used 2 hours postbleaching for the shade measurement and found comparable ΔE values to Matis et al.’s study\textsuperscript{82} that measured the shade one week after bleaching.

Another limitation of this study was that it did not consider the accumulative effect of color change with repeated conventional versus sealed bleaching technique. Although an \textit{in-vitro} study by Rosenstiel et al.\textsuperscript{79} has shown that color changes beyond the first in-office bleaching treatment were small, repeated bleaching with a different bleaching regimen might result in other findings.

\textbf{5.11. Future Directions}

This study explored the diffusion aspect of the mechanism of bleaching by comparing two different in-office bleaching treatments and the findings supported the hypothesis that controlling the diffusion gradient affected the hydrogen peroxide penetration levels into the pulp cavity. Furthermore, the group comparison of crude color
change showed that the two groups were similar in terms of color change relative to baseline.

Based on these findings further studies should be employed to evaluate different bleaching agent concentrations, various delivery methods on hydrogen peroxide penetration levels as well as color change and ultimately suggest a bleaching regimen with minimal hydrogen peroxide penetration without compromising bleaching efficacy.

The diffusion aspect of hydrogen peroxide into the tooth structure should be evaluated not only in terms of quantitative outcome of penetration into the pulp cavity, but also in terms of penetration patterns into the enamel and dentin. Knowledge of penetration patterns of hydrogen peroxide into tooth structure combined with knowledge on diffusion properties will assist in understanding the mechanism of bleaching.

Hydrogen peroxide penetration levels have not been correlated with hypersensitivity associated with tooth bleaching. Further clinical studies should be performed to address the relationship between hydrogen peroxide penetration levels into the pulp cavity with the severity of hypersensitivity experienced.
CHAPTER 6

CONCLUSION

This study compared the relationship of the amount of hydrogen peroxide penetration into the pulp cavity between two different in-office application methods and correlated penetration levels with the color change of the tooth.

The findings supported the hypothesis that controlling the diffusion gradient affected the hydrogen peroxide penetration levels into the pulp cavity. Furthermore, the group comparison of crude color change showed that the two groups were similar in terms of color change relative to baseline.

Within the limitation of this study, the sealed bleaching technique compared to the conventional in-office technique exhibited lower hydrogen peroxide levels without compromising bleaching efficacy in terms of color change.

The sealed bleaching technique may be suggested as an alternative in-office bleaching regimen to minimize hydrogen peroxide penetration into the pulp.
REFERENCES


