TOOTH DISCOLORATION BY 3MIX-MP AS INTRACANAL MEDICATION

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ABSTRACT

The aims of this study were to investigate the effects of 3Mix-MP as an intracanal medicament on the discoloration of teeth and to examine the different methods of placement of 3Mix-MP by lentulo spiral and MTA gun with and without bonding agent in order to prevent or reduce the discoloration effect. Finally, to examine the efficacy of bleaching agents on 3Mix-MP stained teeth.

Part1: Twenty extracted permanent maxillary central and lateral incisors were prepared. Ten teeth were used as control. On the other ten teeth, 3Mix-MP was used as intra canal medication. Teeth from each group were immersed in normal saline in dark individual containers, and placed in an incubator at 37 ˚C with 95% humidity for 22 days. Tooth color was measured from day 1 to day 5, day 8, day 15, and day 22. Part 2: Fifty extracted permanent maxillary central and lateral incisors were prepared. Ten teeth were used as control. For each 20 teeth, lentulo spiral and MTA gun was used to place the medication. Then each method was sub-divided into two groups. The root portion of ten teeth were placed with 3Mix-MP without dentin bonding coated in the pulp chamber and the root portion of the other ten teeth with 3Mix-MP were placed with dentin bonding coated in the pulp chamber. Teeth from each group were stored the same as in part one for 21 days. Tooth color was measured at day 1, day 7, day 14, and day 21. Part 3: 22 extracted permanent maxillary central and lateral incisors which were stained by 3Mix-MP were used in this part. Two teeth were used as the control group. Ten teeth were treated with sodium perborate mixed with 30% hydrogen peroxide and another ten teeth were treated with opalescence endo. Teeth of each group were stored the same as in part one for 21 days. Tooth color was measured at day 7, day 14, and day 21.

The results of part 1, in terms of L, a, and b color spaces, indicate the samples that were filled with 3Mix-MP had negative ΔL^*, Δa^*, and Δb^* values both at the cervical and middle parts, which means teeth became darker, greenish, and blueish and the color change was significantly different ($P<.05$) over a period of 22 days. The ΔE^* value was greater than two in the first day at the cervical part and on the second day at the middle part and the values increased significantly ($P<.05$) over a period of 22 days. For Part 2, the statistical analysis found that only dentine bonding affected the ΔL^*, Δa^*, and Δb^* values, while for the ΔE^* value, dentine bonding and method of placement by MTA gun affected the color difference significantly ($P<.05$). Regarding Part 3, teeth with both sodium perborate and opalescence endo had the positive ΔL^* and Δa^* values for both the cervical and middle parts, which means teeth became white and less green, while the Δb^* value had a negative value, which means teeth became blue after bleaching was finished and the change of ΔL^* value was significantly different ($P<.05$). The ΔE^* values at days 7, 14, and 21 after bleaching increased significantly ($P<.05$) and the values increased to the same level as pre-stained on day 14 for both sodium perborate and opalescence endo groups.

In conclusion, 3Mix-MP could stain teeth dark-green. The discoloration could be detected since the first day after introducing the medication and the discoloration became darker over the period of study. Dentine bonding and method of placement by MTA gun could reduce the overall color change but did not prevent it. Sodium perborate mixed with 30% hydrogen peroxide and opalescence endo could restore tooth color and the bleaching procedure can be done in 14 days for acceptable results.

KEY WORDS: DISCOLORATION / INTRACANAL / MEDICATION / TOOTH

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LIST OF ABBREVIATIONS

˚C	Degree Celsius
CEJ	Cemento-enamal junction
CIE	International Commission on Illumination
cm	Centimeter
DEJ	Dento-enamel junction
EDTA	Ethylene diamine tetraacetic acid
g	Gram
h	Hour
H_2O_2	Hydrogen peroxide
LSTR	Lesion Sterilization and Tissue Repair
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeters
mm^2	Square millimeter
MP	Macrogol and propylene glycol
NaOCl	Sodium hypochlorite
SD	Standard deviation
wk	Week

CHAPTER I
INTRODUCTION

Recently, 3Mix has been introduced as a new intracanal medication. The medication has been introduced by Hoshino et al. in 1996, who found that the medicament could disinfect infected root dentin, infected pulps and periapical lesions (1). The mixture of metronidazole, ciprofloxacin and minocycline is known as 3Mix or 3Mix-MP if macrogol and propylene glycol are used as a vehicle. This medication is used for the concept of revascularization of necrotic pulps for young permanent teeth with immature roots. Recently, some case reports and series have reported using 3Mix in infected necrotic immature roots to obtain revascularization (2-6) and endodontic treatment of a large cyst-like periradicular lesion (7). However, the protocol has potential clinical and biological complications, such as teeth discoloration, development of resistant bacterial strains and allergic reaction to the intracanal medication.

This 3Mix material consisting of minocycline, a semi-synthetic tetracycline derivative, is believed to produce color change in teeth, although reported teeth discoloration associated with its use is rare in the literature. Kim et al. 2000, reported the effect of Ledermix paste as an intracanal medicament on discoloration of immature and mature teeth and they found that Ledermix paste could cause discoloration of crowns if the paste was left on the walls of access cavities or teeth were exposed to sunlight. They concluded that the discoloration could be a result of demethylchlortetracycline, which is a tetracycline antibiotic (8, 9). The role of light in the process of discoloration was demonstrated by Wallman and Hilton in 1962, where an extracted pigmented tooth was split longitudinally. One half was exposed to light and the other kept in the dark. After the study, they found that the pigment exposed to light became in brown, while the unexposed to light remained yellow. Their assumption was the deposition of tetracycline in teeth is probably due to its chelating properties and possibly to the formation of a tetracycline-calcium orthophosphate

complex. They suggested that the brown color may be due to an oxidation product of tetracycline, the formation of which is hastened by light (10). Reynolds et al. have reported a case where the pulp was revascularized using a modified novel technique to eliminate potential coronal discoloration by 3Mix. They found that the modified protocol of using bonding agent and flowable composite to the inner surface of access could prevent crown discoloration (5).

Discoloration of tetracycline-stained teeth causes an esthetic problem that requires effective treatment. The severity of discoloration depends on time, duration of administration, type of tetracycline and the amount of dosage (11-13). Several methods including vital and non vital bleaching have been used to lighten the color of tetracycline-stained teeth. Commonly used bleaching agents are solutions of hydrogen peroxide of different strengths, sodium perborate, and carbamide peroxide. Hydrogen peroxide and carbamide peroxide are mainly indicated for external bleaching, whereas sodium perborate is mostly used for internal bleaching (14).

To date, little information exist on how to prevent the discoloration of teeth caused by 3Mix and the effectiveness of bleaching agents on tetracycline-stained teeth due to this intracanal medication. Despite this fact, this present study is conducted to determine the technique that can prevent crown discoloration and the most effective bleaching agent on tetracycline-stained teeth by 3Mix.

CHAPTER II
LITERATURE REVIEW

Important literature related to this thesis was reviewed as follows:

2.1 3Mix

The medicament, 3Mix is an intracanal antibiotic, which the Cariology Research Unit of the Niigata University School of Dentistry has developed for the concept of Lesion Sterilization and Tissue Repair (LSTR) therapy that utilizes a mixture of antibacterial drugs such as metronidazole, ciprofloxacin, and minocycline to disinfect of dentinal, pulpal and periapical lesions (15, 16).

2.1.1 Composition of 3Mix

The concentrations of each antibiotic drug in 3Mix are metronidazole (500 mg), ciprofloxacin (200 mg), and minocycline (100 mg) (17).

2.1.1.1 Metronidazole is a nitroimidazole compound that exhibits a broad spectrum of activity against protozoa and anaerobic bacteria. Known for its strong antibacterial activity against anaerobic cocci, as well as gram negative and gram-positive bacilli, it has been used both systemically and topically in the treatment of periodontal disease. Metronidazole readily permeates bacterial cell membranes. It then binds to the DNA, disrupting its helical structure and leads to very rapid cell death (3). It is converted in anaerobic organism by the redox enzyme pyruvate-ferredoxin oxidoreductase. The nitro group of metronidazole is chemically reduced by ferredoxin (or a ferredoxin-linked metabolic process) and the products are responsible for disrupting the DNA helical structure, thus inhibiting nucleic acid synthesis.

2.1.1.2 Ciprofloxacin is a synthetic floroquinolone, which the FDA approved ciprofloxacin in October 1987. It has a bactericidal mode of action. It acts through the inhibition of DNA gyrase, resulting in degradation of the DNA by exonucleases. This bactericidal activity persists not only during the multiplication

phase, but also during the resting phase of the bacterium. Ciprofloxacin has very potent activity against gram-negative pathogens but very limited activity against gram-positive bacteria. Most anaerobic bacteria are resistant to ciprofloxacin; therefore, it is often combined with metronidazole in the treatment of mixed infections (3). Side effects of ciprofloxacin have been reported; however, Black et al. (18) found the medicine to be clinically safe when applied in low doses. When applied as an intra-canal medicament in low doses, adverse systemic side effects should be minimized.

2.1.1.3 Minocycline is a semisynthetic tetracycline derivative introduced in 1967. It has a broad-spectrum of antimicrobial activity including aerobic and anaerobic gram-positive, gram-negative and spirochete, which inhibits protein synthesis, causing bacteriostasis. It also reduces fatty acids in sebum, and exhibits an anti-inflammatory effect (19). It gains access to bacterial cells by passive diffusion through the outer membrane followed by active transport through the inner membrane. They then act by inhibiting protein synthesis on the surfaces of ribosomes (3).

2.2 Protocol to preparation of 3Mix

The protocol of preparation is the powdered forms of each drug are mixed in a ratio of 1:1:1 (1), and then mixed with a vehicle. Macrogol and propylene glycol (MP) is a recommended vehicle and previous studies have shown that propylene glycol could quickly and efficiently deliver dye through the root canal system when compared to distilled water (20). The 3Mix-MP can be prepared in two consistencies. The first preparation is mixed with MP in a ratio of 1:5 (MP:3Mix) to be a creamy consistency and the second preparation is to mix with MP in a ratio of 1:7 (MP:3Mix) to be a smear consistency (17). In addition, 3Mix can also be mixed with sterile saline when the MP is inaccessible. Thibodeau and Trope in 2007 (21) have found that 3Mix with sterile saline as a vehicle could disinfect immature dog root canals. Clinical recommendation for using 3Mix has been proposed by Trop 2006 that the medication could be placed in the canal for 28 d (17).

2.3 Effectiveness of 3Mix as root canal medication

Sato et al. 1996, have investigated the potential of a mixture of ciprofloxacine, metronidazole, and minocycline to kill bacteria in the deep layers of root canal dentine. The amount of 0.5 mg of each drug was mixed and placed in the infected root canal. They have found that no bacteria were recovered from the deep layers of the root canal dentine wall after 24h application (22). Trop et al. in 2005 have studied the efficacy of 3Mix in the disinfection of immature dog teeth with apical periodontitis. Before placing the antimicrobial drugs into canals, all sample cultures showed 100% positive for bacterial and after the application of the 3Mix paste for two wk, the positive culture dropped to 30%. This result demonstrated the effectiveness of 3Mix to disinfect root canals of immature dog teeth with apical periodontitis (3).

2.4 Application of 3Mix in endodontics

2.4.1 Lesion Sterilization and Tissue Repair (LSTR)

Takushige and Hoshino in 2004 (23) investigated the use of 3Mix in endodontic treatment of primary teeth. The medicament 3Mix was mixed with macrogol and propylene glycol (MP) or root canal sealer and was placed in orifices or on the bottom of the pulp chamber of infected root canals of 56 patients who had physiologic root resorption and periradicular lesions. After the investigation, they have that clinical symptoms disappeared after treatment, permanent teeth erupted without any disorder, and the mean function time of primary teeth was 680 d. From this result, they concluded that primary teeth with periradicular lesions with or without physiologic root resorption were treated successfully by the LSTR endodontic therapy.

2.4.2 Revascularization

Banchs and Trope have reported a case that used 3Mix as a intracanal medicament in the immature permanent teeth with apical periodontitis. After 26 d of placement of the medication, a blood clot was created and covered by MTA and the tooth composite was restored. At six mo to two y follow-up, they found that the

patient was asymptomatic, thickening of dentinal wall was obvious and the pulp responded to the cold test (2). Ding et al. reported a clinical study of revascularization of 12 patients with immature permanent teeth with chronic or acute apical periodontitis. They found that teeth exhibited complete root development and positive response to pulp testing (4). Moreover, the report of a case series by Jung et al. also had similar results when using 3Mix as a intracanal medication in immature permanent teeth with pulpal necrosis and apical periodontitis, after the follow-up ranging from ten mo to five y (6).

2.4.3 Large cystic lesions

Ozan and Kursat have described the endodontic treatment of a large cyst-like periradicular lesion approximately 3.5 cm in diameter. The mixture of antibiotic drugs (3Mix) was prepared and spun down into the canal by a lentulo spiral. Two mo recall revealed significant healing; the tooth had no symptoms and bone expansion had stopped. The antibiotic was removed and the canal was obturated. The last control visit (seven mo) revealed the radiolucent area was absent; trabecular bone was forming with no symptoms, and soft tissues were healthy (7).

2.5 Mechanism of minocycline stained teeth

Teeth discoloration is one of the complications using 3Mix because these antibiotic drugs contain minocycline, a semisynthetic tetracycline derivative. The common side effect of minocyclin is tissue hyper pigmentation including the oral mucosa, sclera, skin, thyroid, nail, bone and teeth (19). Minocycline staining is characterized by blue-gray to gray hue darkening of the crown and black or green darkening of the roots of erupted teeth (24, 25). Staining of the adult dentition appears to occur in 3 to 6% of patients taking long-term minocycline at > 100 mg daily (26, 27). The onset of discoloration can occur any time from one mo to many years after the initiation of treatment (25, 27) but this side effect will happen when taking minocycline orally.

The exact mechanism that minocycline causes tooth discoloration is still controversial, but currently three possible theories have been proposed (19, 28, 29). The first is the extrinsic theory, based on the fact that minocycline is excreted in a high concentration in the gingival fluid and has the ability to mineralize enamel. This process could allow minocycline to stain or etch the enamel by diffusing through the pulp or by affecting odontogenesis (26, 30, 31). The second is the intrinsic theory proposing that, when minocycline is absorbed, it is bound to plasma proteins and distributed to various tissues in the body. It is especially deposited in collagen-rich tissues such as pulp, dentin, cementum and bone because these tissues have a high affinity for minocycline. It then oxidizes slowly over time and is transformed to a pigmented byproduct (32, 33). The third concept is theory iron theory stating that haemosiderin, a breakdown product of minocycline, binds to calcium ions via chelation to form insoluble complexes, and is incorporated into the tooth matrix causing the discoloration (25, 34, 35).

2.6 Prevention of intracanal medicament stained teeth

To prevent or minimize intracanal medicament stained teeth. Kim et al. have suggested that the placement of medicament should be restricted to the root rather than fill in pulp chamber (8, 9). Reynolds et al. have reported a case where the pulp was revascularized using a modified novel technique to eliminate potential coronal discoloration by 3Mix. The technique was employed that before placing 3Mix into the root canal, the inner surfaces of the coronal access were etch and rinsed and a bonding agent was applied and cured. Then a root canal projector with a size 20 k-file inside the projector was placed into the prepared access. The space between the projector and the coronal dentin was sealed with flow able composite to avoid contact between 3Mix and dentinal wall. Using this protocol, they have found that it could prevent crown discoloration (5).

2.7 Bleaching of tetracycline stained teeth

2.7.1 Causes of tooth discoloration

Tooth discoloration occurs during or after enamel or dentine formation. Some discoloration appears after tooth eruption, and others are the result of dental procedures. Tooth discoloration varies in etiology, appearance, location, severity, and affinity to tooth structure (36). Tooth discoloration can be classified as intrinsic, extrinsic or a combination of both, according to its location and etiology (37).

2.7.1.1 Extrinsic causes, the principal extrinsic causes are chromogens derived from habitual intake of dietary sources, such as coffee, tea, licorice, chocolate, or from tobacco, mouth rinses, or plaque on the tooth surface (38).

2.7.1.2 Intrinsic causes can be classified into two types: Systemic causes are drug-related (tetracycline), metabolic, and genetic. Local causes are pulp necrosis, intrapulpal hemorrhage, pulp tissue remnants after endodontic therapy, endodontic materials, coronal filling materials, root resorption, and aging (38).

2.7.2 Bleaching agents

Bleaching agents act as either oxidizing or reducing agents. Most bleaching agents are oxidizers, and many preparations are available. The most commonly used for whitening teeth are carbamide peroxide, hydrogen peroxide and sodium perborate (14, 38).

2.7.2.1 Carbamide peroxide is an organic white crystalline compound and is formed by urea and hydrogen peroxide and used in different concentrations between 3% and 15%; the average pH is 5 to 6.5. In a hydrophilic environment, it breaks down into approximately 3% hydrogen peroxide and 7% urea (14). Carbamide peroxide is mostly used for external bleaching and has been associated with varying degrees of damage to teeth and surrounding mucosa. Therefore, these materials must be used with caution and usually under strict supervision of the dentist. The chemical reaction of carbamide peroxide is $H_2NCONH_2 \cdot H_2O_2 \rightarrow H_2NCONH_2 + H_2O_2$ (in water) (36).

2.7.2.2 Hydrogen peroxide (H_2O_2) is a powerful oxidizer that is available in various strengths. The most common concentration used is 30% to 35% in stabilized solutions. At a high concentration, hydrogen peroxide is caustic, burns tissues on contact and can release free radicals. High concentration solutions must be handled with care because they are unstable, lose oxygen quickly and may explode unless they are refrigerated and stored in a dark container. Its low molecular weight can penetrate dentin and can release oxygen that breaks the double bonds of the organic and inorganic compounds inside the dentinal tubules (14). The chemical reaction of hydrogen peroxide is shown below (36, 39):

1) $H_2O_2 \rightarrow 2HO^{\cdot}$

   $2HO^{\cdot} + H_2O_2 \rightarrow H_2O + HO^{\cdot}_2$

   $HO^{\cdot}_2 \leftrightarrow H^+ + O_2^{\cdot}$

2) $2H_2O_2 \leftrightarrow 2H_2O + 2\{O\} \longleftrightarrow 2H_2O + O_2$

3) $H_20_2 \leftrightarrow H^+ + HOO^-$

2.7.2.3 Sodium perborate $NaBO_3(H_2O)_n$ (n=1-4) is an oxidizing agent available in powder form or in various commercial proprietary combinations. When fresh, it contains about 95% perborate and 9.9% available oxygen. It is stable when dry but it decomposes to form sodium metaborate, hydrogen peroxide and nascent oxygen in the presence of acid, warm air or water. Sodium perborate preparation is available in three types: monohydrate, trihydrate and tetrahydrate. Sodium perborate is easily controlled and safer than hydrogen peroxide solutions; thus it is the material of choice for internal bleaching (14). The chemical reaction of sodium perborate is shown below (36, 39):

$Na_2(B_2(O_2)_2(OH)_4) + 2H_2O \rightarrow 2NaBO_3 + 2H_2O_2$

Stewart (40) and Warren et al. (41) investigated in vitro conditions of sodium perborate combined with superoxol and found that sodium perborate and superoxol were effective in discolored teeth. Moreover, Weiger et al. (42) compared the effectiveness of three different types of sodium perborate mixed with 30% fresh hydrogen peroxide and water on discolored teeth and found no difference between the three types of sodium perborate mixed with 30% H_2O_2. On the other hand, sodium perborate mixed with 30% H_2O_2 was more effective than that

mixed with water. Correspondingly, Rotstein et al. (43) reported a success rate of 80% using sodium perborate mixed with superoxol.

2.8 Mechanism of bleaching agents

The bleaching process is designed to enable the oxidizing agent to reach sites within the enamel and dentin to allow chemical reaction to occur. The intention of bleaching action is to deliver the active ingredient to the discolored segments of the tooth to dislodge or decolorize chromatic particles.

In general, the bleaching process becomes feasible because of the permeability offered by tooth structure. Hydrogen peroxide has the capacity to diffuse through the tooth structure, causing oxidation or reduction of staining molecules. In contact with the tissue, the hydrogen peroxide molecule breaks up and forms highly unstable and reactive oxygen and peridroxil free radicals. These free radicals can open the highly pigmented carbon ring compounds and convert them to chains (hydrophilic nonpigmented structures) that are lighter in color. The bleaching process continues to the extent that all the original pigment is rendered colorless and totally removed (44-48). The bleaching process is shown in Figure 2.1 (48).

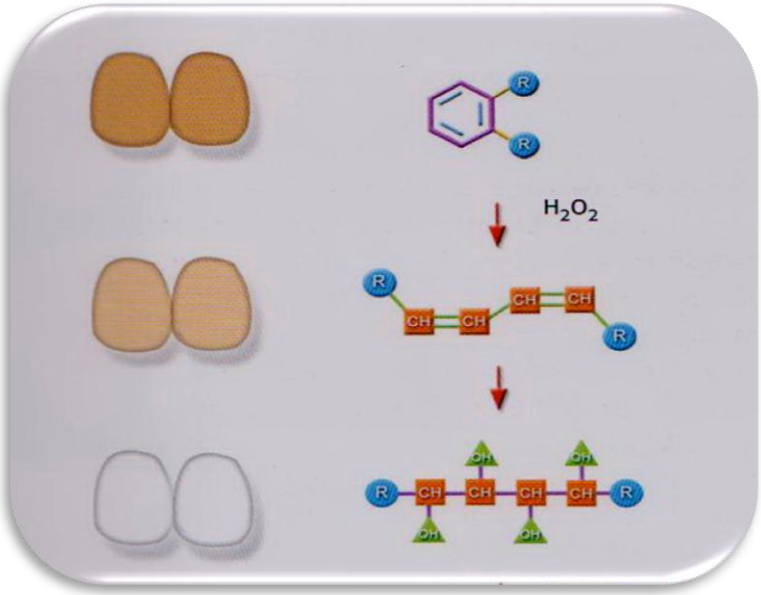


Figure 2.1 Mechanism of tooth bleaching

2.9 Bleaching techniques of nonvital teeth

2.9.1 Thermocatalytic technique

The thermocatalytic technique has been proposed for many years as one of techniques to bleach nonvital teeth by using hydrogen peroxide and heat. Because when heat is applied, a reaction produces foam and releases the oxygen present in the preparation. This technique involves placement of 30% to 35% hydrogen peroxide in cotton pellets in the pulp chamber followed by heat application by electric heating devices or heat spatula. Heat is repeated three or four times with fresh bleaching agent. At the end of appointment, the bleaching agent is sealed in the pulp chamber for additional bleaching between appointments as in the walking bleach technique (14, 38, 48). The disadvantage of this technique is the possibility of irritation to the cementum and periodontal ligament by the oxidizing agent in combination with heat; thus, causing external cervical root resorption. Its effectiveness is less than the walking bleach technique and requires more time, so this technique is not recommended (14, 48).

2.9.2 Walking bleach technique

The walking bleach technique was introduced by Spasser in 1961 (49). This technique is used in situations requiring internal bleaching. It is an effective technique and requires the least chair time. The first introduction of a mixture of sodium perborate and distilled water for this bleaching technique was reported by Salvas (50) and to improve the bleaching effectiveness, the modified use of 30% hydrogen peroxide instead of water was advocated by Nutting and Poe (51). In brief the procedures of the walking bleach technique involve after root canal treatment. The root filling material will be removed to the apical level of gingival margin and a protective layer is applied on the obturating material but not extending into the incisal of the gingival margin to minimize leakage of bleaching agents. Then the mixture of bleaching agent is left in the pulp cavity and the access is sealed with temporary cement for seven d (14, 48). The need to repeat the bleaching technique depends on the severity of the discoloration. Many studies have demonstrated that the walking bleach technique is a safe and successful technique for discolored teeth caused by

tetracycline (52), a mixture of sodium perborate and water or hydrogen peroxide continues to be used today and has been described many times as a successful technique for intracoronal bleaching.

2.10 Method of measurement of tooth color and stain

2.10.1 Systems of measurement

Two systems are widely used for describing the color of teeth, i.e., The Munsell System and the International Commission on Illumination (CIE) color system (53, 54).

2.10.1.1 Munsell system compares the perceived color of an object with a standardized and orderly arrangement of color chips. It describes colors in a three dimensional coordinate system of the color wheel including dimensions of hue, value, and chroma (Figure 2.2). Hue is the pigment or color tone of a tooth or dental restoration, for example, red, blue and green. Value refers to the lightness or darkness of a color. The scale of value ranges from a low of 0 for pure black to a high of 10 for pure white. Chroma indicates saturation or intensity of the color tone (53-55).

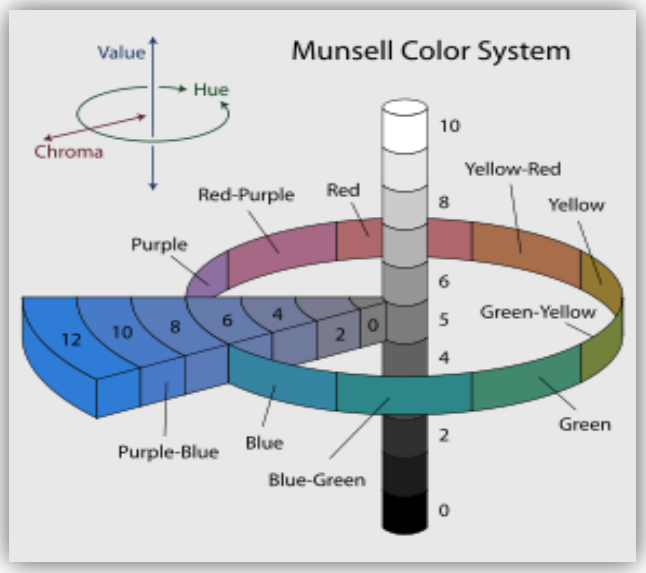


Figure 2.2 Munsell Color System

2.10.1.2 CIE system is the international standard for color measurements. Its concept is that all colors can be matched by mixing relative amounts of three primary colors: red (X), green (Y) and blue (Z). The X, Y, Z can be mathematically converted to the CIE L* a* b* color space (56) (Figure 2.3).

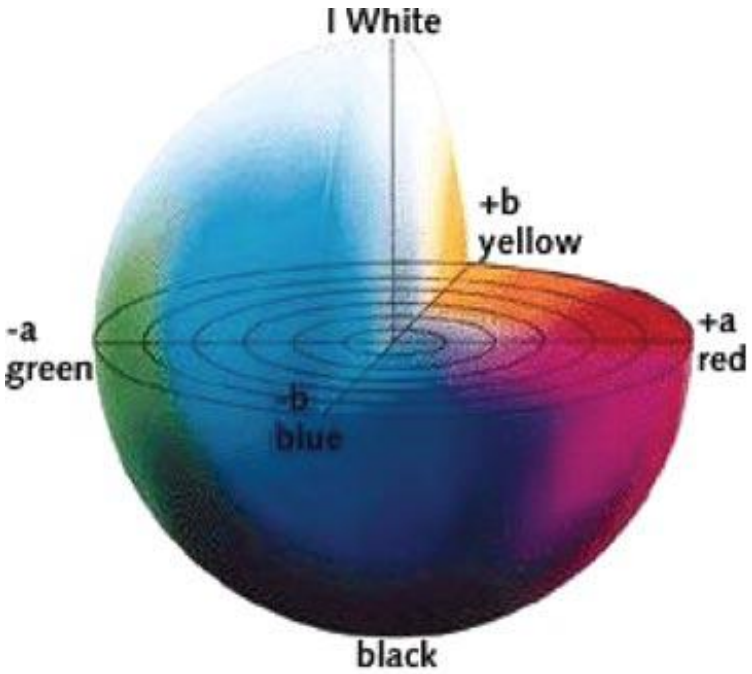


Figure 2.3 CIE Lab Color Space

The L* represents lightness and darkness from white to black, ranging from 0, perfect black to 100, perfect white (Figure 2.4).

L=0 **L=100**

Figure 2.4 L color

The a* corresponds to the red-green; a positive value (+127) indicates pure red, whereas a negative value (-127) indicates pure green (Figure 2.5).

Figure 2.5 A color

The b*corresponds to the yellow-blue; a Positive value (+127) indicates pure yellow, whereas a negative value (-127) indicates pure blue (Figure 2.6).

Figure 2.6 B color

The color difference metric known as ΔE has been used effectively to quantify color difference in a wide range of industries. It is computed by the following formula: $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$. The ΔL*, for example, denotes the difference in L* between the two samples and the same in Δa*and Δb*. The values of ΔL*, Δa*, Δb* are given by the following equations:

$\Delta L^* = L_1 - L_2$

$\Delta a^* = a_1 - a_2$

$\Delta b^* = b_1 - b_2$

In general, an ΔE value less than 1 is considered excellent (57, 58). If the color difference is $1 \leq \Delta E \geq 2$, the match is clinically acceptable and if the value is greater than 2, the match is considered clinically unacceptable (59). Ruyter et al. in 1987 reported that color differences with ΔE lower than approximately 3.3 were acceptable (60). Seghi et al. in 1989 found that a color difference value of greater than 2 ΔE units was correctly judged by observers 100% of the time. Incorrect judgments were made when color difference fell within 1 to 2 (61). In addition, Johnston and Kao in 1989 (62) have recommended the extended visual rating scale for appearance match. The rate 0 indicates an excellent esthetic match; 2 to 4 indicates very slight to obvious mismatch, but within an acceptable range for most patients and 6 to 10 indicate poor to totally unacceptable esthetics.

2.10.2 Methods of measurement

Tooth color and stain measurement are assessed using a wide range of measurement methods such as subjective (visual) and objective (instrumental) assessment (54, 63).

2.10.2.1 Subjective assessment, the visual shade matching is the most frequently applied method in dentistry. A shade guide composes of a set of tooth-shaped porcelain tabs intended to cover the range of colors present in human teeth and using the Munsell Color System to measure tooth color. Shade matching has been used to determine shade differences in natural teeth and to assess changes in tooth whiteness (63). The process is the tooth and the shade guide are observed simultaneously under the same light condition. General variables such as external light conditions, experience, age, and fatigue of the human eye and physiological variables such as color blindness may lead to inconsistencies and bias (64, 65).

The disadvantages have been described such as the range of shades available is inadequate and does not cover the complete color space of natural tooth color; the shades are not systematic in their color space (66, 67), a lack of consistency is found among and within individual dentists in matching colors; the results cannot be transformed into the CIE Lab color scale, and none of the commercially available shade guides are identical (68).

2.10.2.2 Objective assessment involves the use of digital image analysis systems, spectrophotometers and colorimeters (54, 55, 63).

Digital image analysis systems (RGB devices) assess tooth color including stain and whiteness. The devices acquire red, green and blue image information to create a color image. RGB devices and digital cameras represent the basic approach to electronic shade taking (55, 63).

The advantages of this device are a decrease in subjectivity and improved reliability; the database of the image can be analyzed and re-investigated at a later date, does not require a clinician, involves no contact with the tooth surface, but assesses the whole tooth surface (63). The disadvantages of this systems are the device is not a measurement instrument, rather it infers the color properties of the captured image and is useful for providing lab technicians with a reference point, but should not be relied upon to determine the shade of teeth (55).

Spectrophotometers measure and record the amount of visible radiant energy reflected or transmitted by an object one wavelength and have been used to measure the visible spectra of extracted and vital teeth (69, 70).

The disadvantages of spectrophotometers are the equipment is complex, expensive and more importantly, it is difficult to measure the color of teeth in vivo with these machines. Another problem is that only small areas are assessed at one time, leading to results that may be unrepresentative of the whole tooth surface (53).

Colorimeters are engineered to directly measure color as perceived by human eyes. A colorimeter filters light in three or four areas of the visible spectrum to determine the color of an object and is generally designed to measure color in X, Y, Z terms or in CIE Lab values (54, 55). Normally, colorimeters have been used and have shown good repeatability of tooth color measurements in vitro and in vivo (53, 71) and also have been used to measure color changes of extracted teeth undergoing bleaching treat- ment (72).

The disadvantages of colorimeters for measuring tooth color include: the instruments are designed to measure flat surfaces; teeth are often not flat and can have surface anomalies, and small aperture colorimeters are prone to significant edge-loss effects so color determinations will be subject to errors (73, 74).

2.11 Location of measurement

The distribution of tooth color relies on different regions of the tooth, which can be divided in three regions: gingival, middle, and incisal. William et al. in 1997 (75) measured 95 extracted anterior teeth using a spectrophotometer. The study measured three regions of teeth and found that the color of the gingival and middle regions was not significantly different while gingival and incisor regions showed significant differences. Goodkind and Schwabacher (76), measured three regions of 2,830 anterior teeth in vivo using a colorimeter, and concluded that the middle site appears to present tooth color best, while the incisor and cervical sites appear to be more affected by their surroundings.

CHAPTER III
OBJECTIVE

The aims of this study were to:

1. investigate the effects of 3Mix as an intracanal medicament on the discoloration of teeth;
2. examine whether the discoloring effects, if present, were related to the method of placement;
3. examine the applying bonding could prevent or reduce the discoloration effect; and
4. examine the efficacy of bleaching agents on 3Mix stained teeth.

HYPOTHESES

First Hypothesis

H_0 : Tooth discoloration by 3Mix-MP does not increase over time.

H_1 : Tooth discoloration by 3Mix-MP increases over time.

Second Hypothesis

H_0: The methods of 3Mix-MP placement by lentulo spiral and MTA gun cause discoloration of teeth equally.

H_1: The methods of 3Mix-MP placement by lentulo spiral and MTA gun cause the discoloration of teeth differently.

Third Hypothesis

H_0: Teeth are coated with bonding agent and teeth not coated with bonding agent have discoloration by 3Mix-MP equally.

H_1: Teeth coated with bonding agent and teeth not coated with bonding agent have discoloration by 3Mix-MP differently.

Fourth Hypothesis

H_0: The Opalescence Endo (35% hydrogen peroxide) and sodium perborate mixed with 30% hydrogen peroxide can whiten 3Mix-MP stained teeth equally.

H_1: The Opalescence Endo (35% hydrogen peroxide) and sodium perborate mixed with 30% hydrogen peroxide can whiten 3Mix-MP stained teeth differently.

CHAPTER IV
MATERIALS AND METHODS

This thesis is composed of three parts: duration of discoloration, comparison of methods to prevent tooth discoloration by 3Mix-MP and efficacy of bleaching agents on 3Mix-MP stained teeth.

4.1 Preparation of 3Mix-MP

The protocol of preparing 3Mix-MP follows the protocol of Martin Trope in 2006 (17).

4.1.1 Antibiotics (3Mix) and vihecle

metronidazole 400 mg (Flagyl®), ciprofloxacin 500 mg (Cifloxin®) and minocycline 100 mg. Macrogol ointment and propylene glycol were used as vehicle.

Sugar coatings were removed from tablets with blade or opened capsules and then crush individually in separate mortars. Grind each antibiotic to a fine powder and combine equal amounts of antibiotics (1:1:1) on a mixing pad (0.02g of each antibiotic). Then mixed equal amounts of macrogol ointment and propylene glycol (1:1) by clean spatula on a pad to became opaque. Finally, 3Mix (0.06g) and MP (0.035g) were mixed together to creamy consistency.

4.2 Preparation of teeth

4.2.1 Inclusion criteria

The inclusion criteria included extracted permanent maxillary central and lateral incisors, which free of caries and any restoration. Where the age and sex of donors were unknown

4.2.2 Exclusion criteria

The exclusion criteria included extracted permanent maxillary central and lateral incisors with cracks or fractures and teeth with previous endodontic treatment.

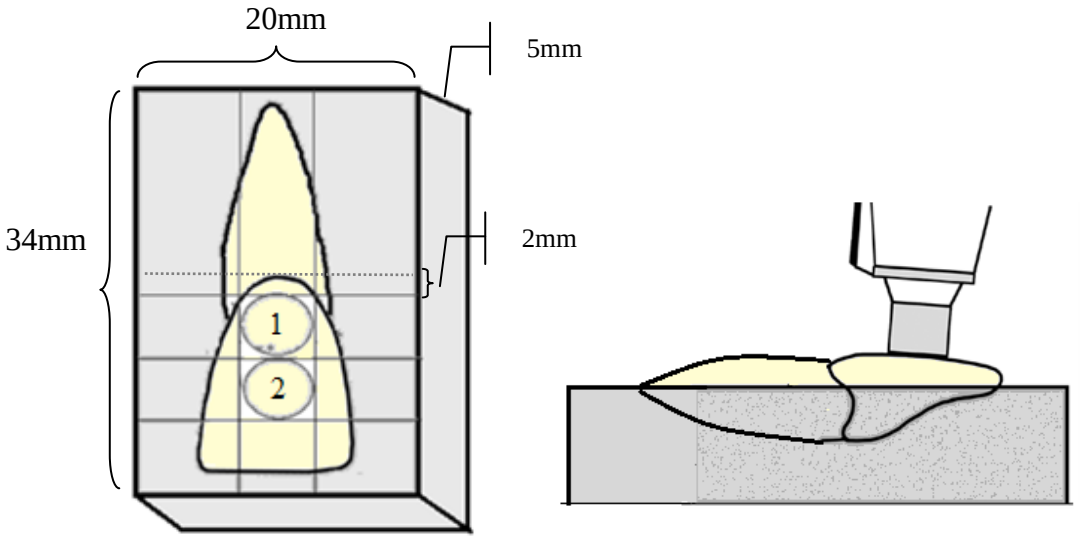
4.2.3 Teeth preparation

All teeth were stored in 1% thymol solution at 4 ˚C until used. Calculus and stain were removed using a hand scaler or ultrasonic instument. Access preparations were prepared and teeth were radiographic examined in a mesio-dital view to measure the thickness of the dentin. Pulp tissue was removed with barbed broaches . The root canal was irrigated with 5 ml of 2.5% sodium hypochlorite without mechanical instrumentation, 5 ml of 17% EDTA and finally rinsed with 5ml of normal saline. Then canals were dried with paper point, the apical areas were sealed with dentin bonding and resin composite for 2 mm thickness and three layers of nail varnish (REVLON® Pure Pearl color, USA) was coated to the roots 3 mm below CEJ.

4.3 Tooth color measurement

The tooth color change was measured with a colorimeter (ShadeEye NCC, Shofu Dental, Menlo Park, CA, USA). The optical capture device of ShadeEye NCC was located on the tip of the instrument, which is 3 mm^2 in diameter. The colorimeter was calibrated on its own white ceramic block before tooth color was measured. The technique of tooth color measurement followed the technique of Panagiotis et al. in 2009 (77). All teeth were fixed individually in transparent polyvinyl siloxane (MEMOSIL®2 Heraeus Kulzer, LLC. Hanau, Germany). This siloxane impression was used as a custom made jig to ensure the position of measurement was the same and then five guiding lines were drawn on the siloxane jig. The first line was 2 mm above the CEJ, the second line was 3 mm above the first line, the third line was 3 mm above the second line and the fourth and the fifth lines were 3 mm from misial and distal site of the tooth and parallel to the long axis of the tooth. The cervical and middle sites were used as regions to measure tooth color. These two regions of each tooth were measured by placing the elastic tip of the instrument perpendicular to and in complete

contact with the labial surface of the tooth and each region was measured three times (Fig. 4.1). Tooth color was measured after opened access to serve as baseline.



A Labial view shows measurement area and guiding lines

B Side view shows tooth and tip positioning

1. Cervical site

2. Middle site

Figure 4.1. Location of tip placement.

4.4 Data collection

Teeth were evaluated for shade alteration with a colorimeter. Each tooth was measured in the same room and at the time, which had the same light intensity as possible. The evaluation was based on the CIE Lab System. Three measurements for each region (cervical and middle sites of labial surface of tooth) were obtained and the mean was calculated for each tooth. The effects of 3Mix-MP on discoloration of teeth was evaluated by comparison between variations in L*, a* and b* values (ΔL*, Δa*, Δb*) at each period and the baseline and the ΔE* values were used to assess the color differences between two samples by the following formulas (56, 78):

$$\Delta L^* = L_1 - L_2$$

$$\Delta a^* = a_1 - a_2$$

$$\Delta b^* = b_1 - b_2$$

$$\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$$

4.5 Part 1: Duration of discoloration

4.5.1 Preparation of teeth

Twenty teeth were used in this part; all teeth were prepared as described previously. Teeth were randomly divided into two groups as follows:

Group 1 (control group): 10 teeth

Moist cotton pellets were placed in the pulp chamber and access cavities were sealed with temporary filling (CAVITON, Hydraulic temporary restorative. GC Corporation. Tokyo, Japan)

Group 2 (3Mix-MP):10 teeth

The 0.02 g of 3Mix-MP was used as intra-canal medication and using lentulo spiral size 30 (LENTULO® Paste carrier, Densply Maillefer, CH-1338 Ballaigues, Switzerland) to spin the medication into the canals. The medication was limited to the root portion of the canal and after placement, the access cavities were sealed with temporary filling (CAVITON, Hydraulic temporary restorative. GC Corporation. Tokyo, Japan).

Teeth of each group were immersed in normal saline in dark individual containers and placed in an incubator at 37 ˚C with 95% humidity for 22 days. The summary of the study design is shown in the Figure 4.2.

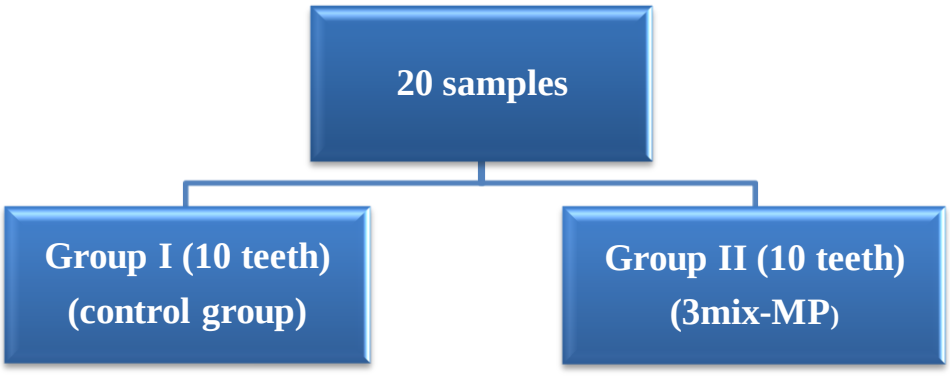


Figure 4.2 Flowchart summarizing the study design

4.5.2 Tooth color measurement

Tooth color was measured after opened access to serve as baseline and day 1 to day 5 and then at day 8, 15 and 22 after placing 3Mix-MP.

4.5.3 Statistic analysis

Repeated-measures analysis was used to identify the different color changes for 22 days at P-value < .05 by using SPSS 13.0 analytic software.

4.6 Part II: The comparison of methods to prevent tooth discoloration by 3Mix-MP

4.6.1 Preparation of teeth

Fifty teeth were used in this part; all teeth were prepared as described previously and teeth were randomly divided into five groups as follow:

Group 1 (Control group): 10 teeth

Each canal was left with a moist cotton pellet in the pulp chamber and the access cavities were seal with temporary filling (CAVITON, Hydraulic temporary restorative. GC Corporation. Tokyo, Japan).

Group 2 (Lentulo group): 20 teeth

2.1 No bonding 10 teeth

3Mix-MP was used as intra-canal medication using lentulo spiral (LENTULO® Paste carrier, Densply Maillefer.CH-1338 Ballaigues, Switzerland) size 35 to spin 0.02 g of 3Mix-MP into each root canal. The lentulo spiral was inserted 4 mm below the orifice. The medication was limited to the root portion of the canal and after placement, the access cavities were sealed with temporary filling (CAVITON, Hydraulic temporary restorative. GC Corporation. Tokyo, Japan).

2.2 With bonding (10 teeth)

Each tooth was coated with bonding (CLEARFIL™ SE BOND X, Kuraray Medical INC, Okayama, Japan) into the pulp chamber before placing 3Mix-MP. A cotton pellet was placed in the orifice and dentine bonding was applied according to manufacturer instructions. Primer was applied in brush motion, left for 20 seconds and then used mild air flow to dry the primer. After that, bonding was applied in brush motion, followed by mild air flow and light cured for ten seconds and the cotton pellet was removed. The lentulo spiral was used to spin 0.02g of 3Mix-MP into root canal the same as in group 2, the access cavities were sealed with temporary filling (CAVITON, Hydraulic temporary restorative. GC Corporation. Tokyo, Japan).

Group 3 (MTA gun group) 20 teeth

3.1 No bonding 10 teeth

Each tooth placed 0.02 g of 3Mix-MP into the canal using a massing gun (MTA Gun System, Densply Maillefer. CH-1338 Ballaigues, Switzerland) without

bonding coating in the pulp chamber. The medication was placed within the root canal below the canal orifice and the access cavities were sealed with temporary filling (CAVITON, Hydraulic temporary restorative. GC Corporation. Tokyo, Japan).

3.2 With bonding 10 teeth

Each tooth was coated with bonding into the pulp chamber as described in group 2.2 before placing 0.02g of 3Mix-MP with a massing gun and the access cavities were sealed with temporary filling (CAVITON, Hydraulic temporary restorative. GC Corporation. Tokyo, Japan).

Teeth of all groups were immersed in normal saline in dark individual containers and placed in an incubator at 37 ˚C with 95% humidity for 21 days. The summary of the study design is shown in Figure 4.3

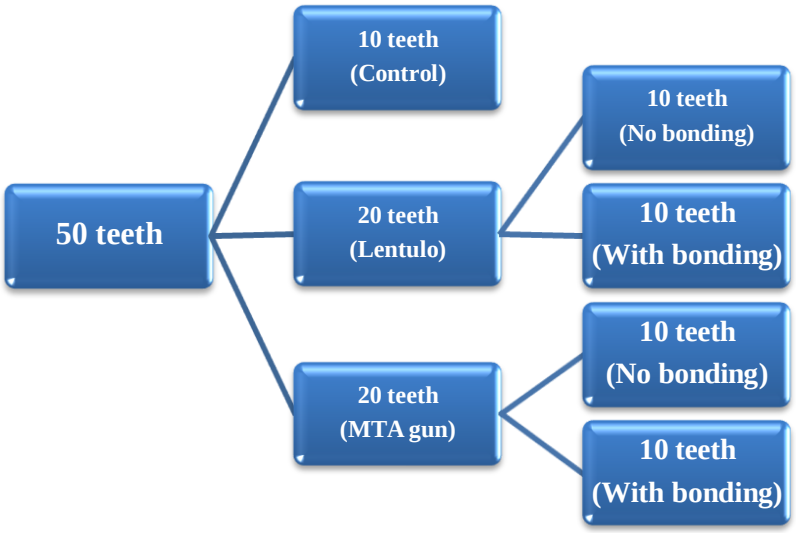


Figure 4.3 Flowchart summarizing the study design

4.6.2 Tooth color measurement

Tooth color was measured after opened access to serve as baseline and day 1, 7, 14 and 21 after placing 3Mix-MP.

4.6.3 Statistic analysis

Factorial design was used to identify the factors which effected color changes at day 1, 7, 14 and 21 at P-value < .05 by using SPSS 13.0 analytic software.

4.7 Part III: The efficacy of bleaching agents on 3Mix-MP stained teeth

4.7.1 Preparation of teeth

Thirty teeth were used in this part; teeth were prepared as described previously, then all teeth were stained with 0.02g 3Mix-MP using lentulo spiral size 30 into each root canal and the access cavities were sealed with cavit. After that, teeth were immerse in normal saline in dark containers and place in an incubator at 37 ˚C with 95% humidity for 21 days. After completely staining teeth, the 3Mix-MP was removed with 20 ml of 2.5% NaOCl. The canals were dried with paper point, then cotton pellets were placed into root canals and a 2 mm thick base of glassiomoner cement (VitreBond ^{TM}, 3M ESPE, USA) was placed at 2 mm below conforminf to CEJ. Any remnant of base material covering the walls of the access cavity was removed with a carbide bur and teeth were randomly divided into three groups as follow:

Group1 (Control group): 2 teeth

Each pulp chamber was left with a moist cotton pellet instead of bleaching agent and the access cavities were sealed with temporary filling (CAVITON, Hydraulic temporary restorative. GC Corporation. Tokyo, Japan).

Group 2: 10 teeth

The amount of 2g sodium perborate was mixed with 1ml of superoxal (30% Hydrogen peroxide) as a thick paste and placed in the pulp chambers of teeth by spatula and the access was sealed with temporary filling (CAVITON, Hydraulic temporary restorative. GC Corporation. Tokyo, Japan).

Group 3: 10 teeth

The 35% hydrogen peroxide gel (Opalescence Endo, Ultradent Products, Inc., Utah, USA) was loaded with syringe into the pulp chamber of the teeth and the access was sealed with temporary filling (CAVITON, Hydraulic temporary restorative. GC Corporation. Tokyo, Japan).

The teeth were immersed in normal saline in dark individual containers and placed in an incubator at 37 ˚C with 95% humidity. The bleaching agents were

changed every seven days and the teeth were bleached for total of 21 days. The summary of the study design is shown in Figure 4.4

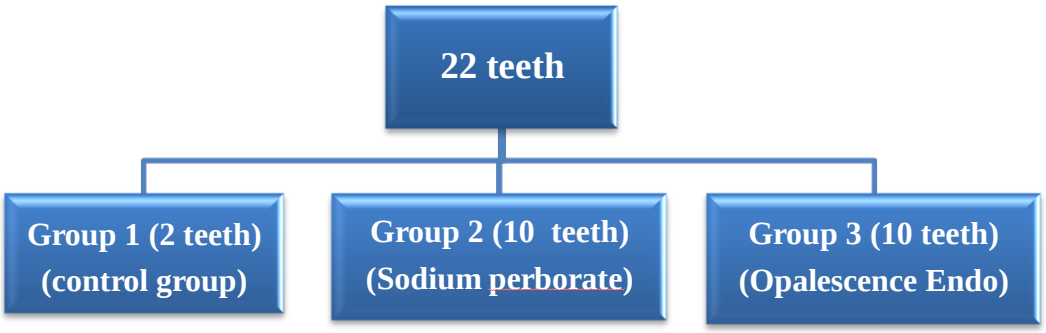


Figure 4.4 Flowchart summarizing the study design

4.7.2 Tooth color measurement

Tooth color was measured before stained, stained (as baseline) and after bleaching at day 7, 14 and 21.

4.7.3 Statistic analysis

Repeated-measures analysis of variance was used to identify the different within group and the unpaired t-test was used to compare the means of two bleaching agents on day 7, 14 and 21 at P-value < .05 by using SPSS 13.0 analytic software.

CHAPTER V
RESULTS

5.1 Part 1: Duration of discoloration

The day after applying 3Mix-MP, a dark-green shade began to appear in the cervical part of the teeth of group two and discoloration became darker over time, while in the control group, the tooth color remained the same for all 22 days (Figure 5.1.1).

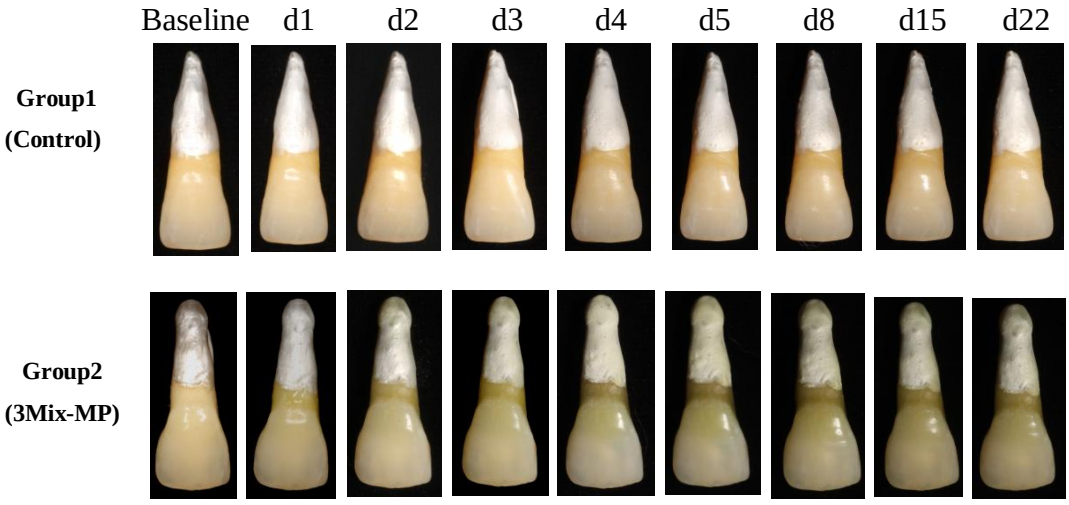


Figure 5.1.1. Photographs of representative teeth at the time intervals after 3Mix-MP application.

The results of color change of all groups at cervical and middle parts are demonstrated in Table 5.1.1 and figure 5.1.2. For the control group, the ΔL*, Δa*, Δb* and ΔE* values remained the same along the period of this study.

5.1.1 L* value

The changes in brightness (ΔL*) of the 3Mix-MP group at cervical and middle parts were similar. The ΔL* decreased sharply to black axis in the first two days and continued to decrease over 22 days. For the cervical part, the dark color (ΔL*) increased significantly for the first two days (P<.05) then slowly increased until day 4. At day 5, the color continued to the dark axis significantly until day 22 (P<.05). For the middle part, the dark color (ΔL*) increased significantly from day 1 until day 22 (P<.05).

5.1.2 A* value

The mean changes in the red-green color (Δa*) at the cervical and middle parts of the 3Mix-MP group increased in green color sharply for the first four days and then slowly increased until day 8. At day 15 and 22, the green color started to decrease.

5.1.3 B* value

The mean changes in the yellow-blue axis (Δb*) at the cervical and middle parts of the 3Mix-MP group were similar in pattern. The color increased to yellow in the first three days, then the color began to decrease to yellowish color from day 4 to day 15 and finally decreased to blue axis at day 22.

5.1.4 E* value

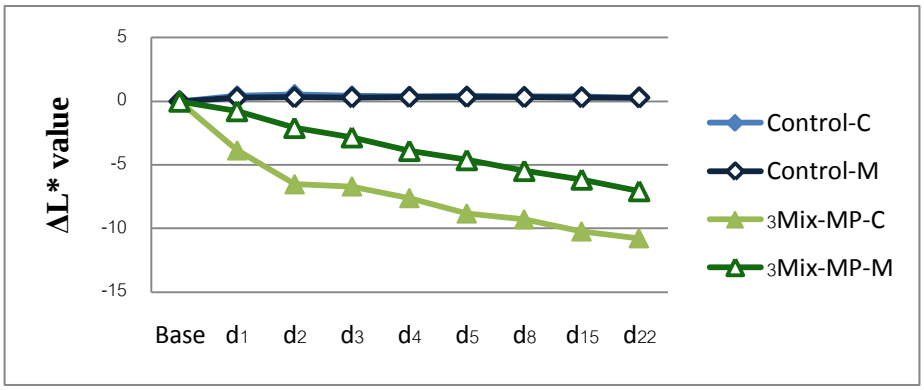
The ΔE* value shown was greater than two in the first day at the cervical part and in the second day at the middle part after introducing 3Mix-MP and the values were increased significantly (P<.05) over a period of 22 days.

Table 5.1.1 Mean change in the CIE Lab parameters between baseline and each day after introduction of 3Mix-MP at the cervical and middle part (mean ± SD).

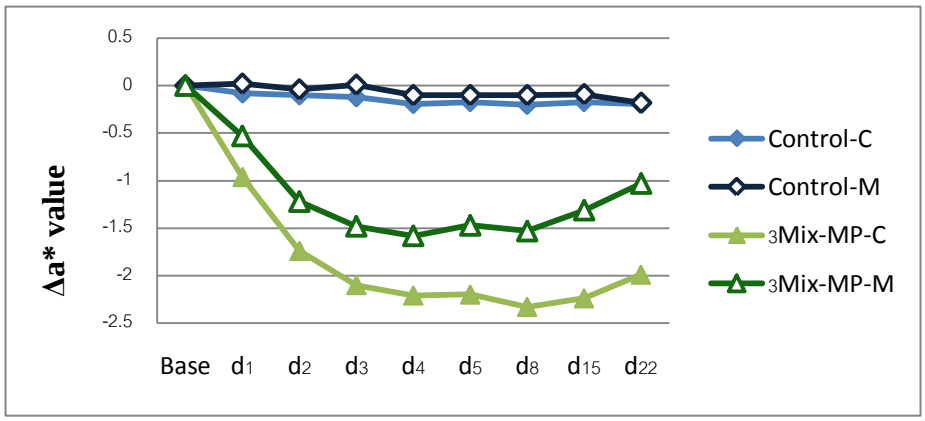
	Group	Value	d1	d2	d3	d4	d5	d8	d15	d22
Cervical	Control	ΔL*	0.42±0.35	0.54±0.34	0.43±0.39	0.40±0.56	0.43±0.36	0.40±0.51	0.37±0.54	0.22±0.64
		ΔE*	0.60±0.25	0.66±0.31	0.75±0.28	0.78±0.31	0.77±0.29	0.79±0.29	0.81±0.24	0.86±0.37
	3Mix-MP	ΔL*	-3.86±2.42	-6.25±3.02	-6.69±3.19	-7.61±3.02	-8.82±2.66	-9.28 ±2.58	10.83±3.20	-10.77±3.53
		ΔE*	4.68±2.42	7.40±2.85	7.79±3.16	8.40±2.85	9.35±2.76	10.00±2.59	-10.23±3.21	11.29±3.76
Middle	Control	ΔL*	0.29±0.73	0.31±0.84	0.37±0.63	0.33±0.64	0.34±0.44	0.32±0.50	0.37±0.55	0.37±0.69
		ΔE*	0.82±0.30	0.98±0.53	0.77±0.40	0.79±0.34	0.75±0.34	0.64±0.38	0.76±0.41	0.77±0.47
	3Mix-MP	ΔL*	1.77±0.78	-2.07±1.77	-2.81±1.31	5.15±1.47	5.55±2.20	-5.54± 1.99	-6.13 ± 2.11	-7.04 ± 2.45
		ΔE*	-0.75±0.68	3.40±1.02	4.66±1.10	-3.88±1.76	-4.60±2.82	6.29±1.99	6.79±2.17	7.87±2.61

Figure 5.1.2. Mean changes in the CIE Lab parameters between each time interval
(a) ΔL* value (b) Δa* value (c) Δb* value (d) ΔE* value

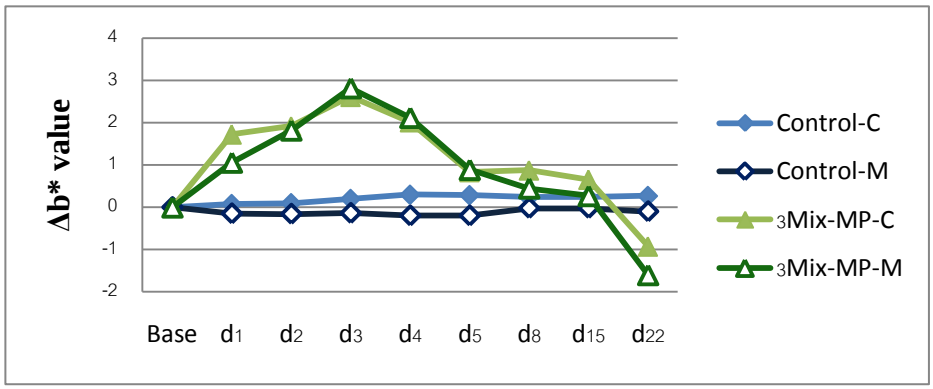
(a)



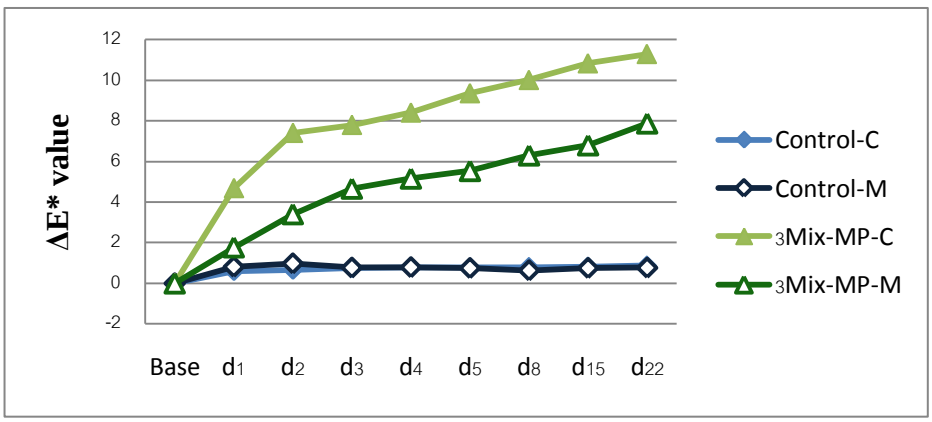
(b)



(c)



(d)



5.2 Part 2: The comparison of methods to prevent tooth discoloration by 3Mix-MP

The day after the placing of 3Mix-MP, a dark green color began to appear in the cervical part of the teeth without bonding both in groups 2 and 3 and the color became darker over time. In contrast, teeth with bonding in group 2 and 3 had a minor change in color at day 21 on the cervical part of the teeth (Figure 5.2.1).

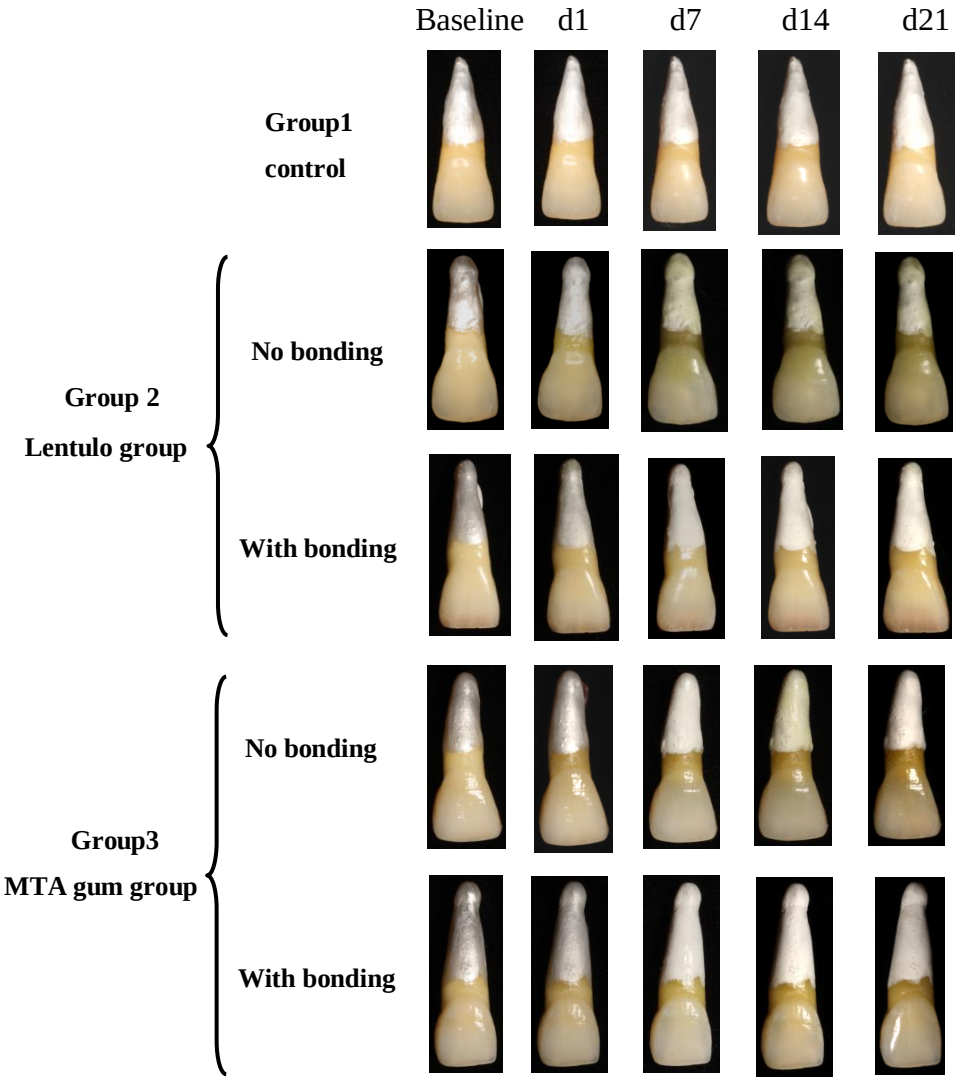


Figure 5.2.1. Photographs of teeth at each time intervals after 3Mix-MP application

The results of color change of experimental groups at the cervical and middle parts are described below. For the control group, the ΔL*, Δa*, Δb* and ΔE* values remained the same along the period of this study.

5.2.1 L* value

Statistical analysis found methods of placing 3Mix-MP were not affected the ΔL*, which meant that tooth color became dark in a similar fashion regardless of the method of placement. In contrast, the bonding agent affected the ΔL*, significantly (P<.05).

At the cervical part, teeth with bonding coating in the pulp chamber were less dark in color than teeth without bonding at days 1, 14 and 21, significantly (P<.05), while at the middle part, no difference in dark color was observed between teeth with and without bonding. The data are shown in Tables 5.2.1 to 5.2.4.

Table 5.2.1. Mean change the ΔL* between methods and bonding agent at day 1 after introduction of 3Mix-MP at the cervical and middle parts (Mean±SD).

Location	**Methods of placment**			
	Bonding	**Lentulo**	**MTA gun**	**Total**
Cervical	**No**	-3.86 ± 2.42	-1.96 ± 2.85	-2.91 ± 2.75
	Yes	-0.66 ± 0.42	-0.98 ± 0.71	-0.82 ± 0.59*
	Total	-2.26 ± 2.35	-1.47 ± 2.08	-1.86 ± 2.23
	Bonding	**Lentulo**	**MTA gun**	**Total**
Middle	**No**	-0.75 ± 0.68	-1.34 ± 1.66	-1.04 ± 1.27
	Yes	-0.46 ± 0.39	-0.55 ± 0.28	-0.50 ± 0.33
	Total	-0.60 ± 0.56	-0.94 ± 1.22	-0.77 ± 0.96

* Significantly different P<.05

Table 5.2.2. Mean change in ΔL* between methods and bonding agent at day 7 after introduction of 3Mix-MP at the cervical and middle parts (Mean±SD).

Location	**Methods of placment**			
	Bonding	**Lentulo**	**MTA gun**	**Total**
Cervical	**No**	-9.28 ± 2.58	-8.42 ± 3.50	-8.85 ± 3.02
	Yes	-7.48 ± 4.36	-6.40 ± 3.92	-6.94 ± 4.07
	Total	-8.38 ± 3.60	-7.41 ± 3.76	-7.89 ± 3.67
	Bonding	**Lentulo**	**MTA gun**	**Total**
Middle	**No**	-5.45 ± 1.99	-5.17 ± 2.76	-5.31 ± 2.76
	Yes	-4.39 ± 2.44	-4.34 ± 1.81	-4.36 ± 2.09
	Total	-4.92 ± 2.24	-4.75 ± 2.73	-4.83 ± 2.46

Table 5.2.3. Mean change in ΔL* between methods and bonding agent at day 14 after introduction of 3Mix-MP at the cervical and middle parts (Mean±SD).

Location	**Methods of placment**			
	Bonding	**Lentulo**	**MTA gun**	**Total**
Cervical	**No**	-10.23 ± 3.21	-10.34 ± 3.58	-10.28 ± 3.31
	Yes	-8.88 ± 2.96	-7.37 ± 3.90	-8.12 ± 3.46*
	Total	-9.55 ± 3.08	-8.85 ± 3.95	-9.25 ± 3.51
	Bonding	**Lentulo**	**MTA gun**	**Total**
Middle	**No**	-6.13 ± 2.11	-6.15 ± 3.36	-6.14 ± 2.73
	Yes	-5.53 ± 2.31	-4.80 ± 1.89	-5.16 ± 2.09
	Total	-5.83 ± 2.17	-5.47 ± 2.74	-5.65 ± 2.45

* Significantly different P<.05

Table 5.2.4. Mean change in ΔL* between methods and bonding agent at day 21 after introduction of 3Mix-MP at the cervical and middle parts (Mean±SD).

Location	**Methods of placment**			
	Bonding	**Lentulo**	**MTA gun**	**Total**
Cervical	**No**	-10.77 ± 3.53	-11.13 ± 3.68	-10.95 ± 3.51
	Yes	-9.71 ± 2.86	-7.65 ± 3.84	-8.68 ± 3.46*
	Total	-10.24 ± 3.17	-9.39 ± 4.07	-9.81 ± 3.63
	Bonding	**Lentulo**	**MTA gun**	**Total**
Middle	**No**	-7.04 ± 2.45	-6.53 ± 3.31	-6.78 ± 2.84
	Yes	-5.89 ± 2.25	-4.86 ± 1.83	-5.37 ± 2.07
	Total	-6.46 ± 2.36	-5.69 ± 2.74	-6.08 ± 2.56

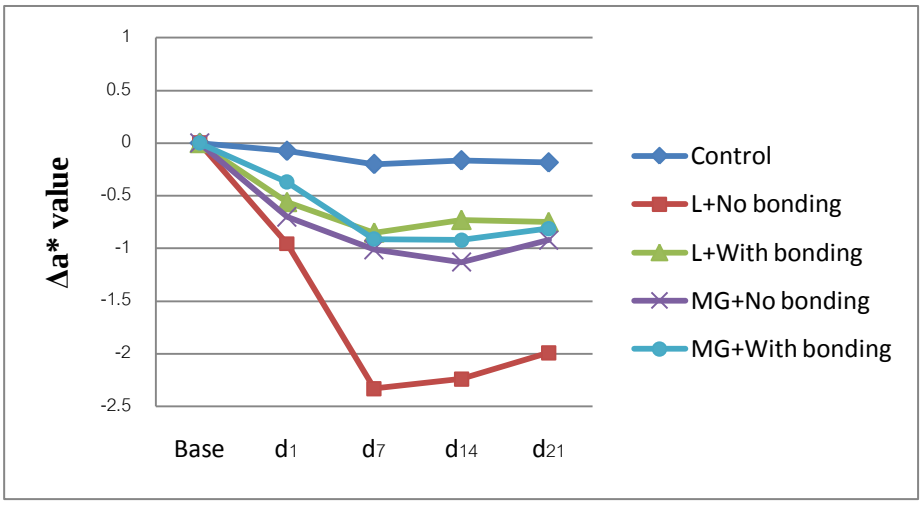
* Significantly different P<.05

5.2.2 A* value

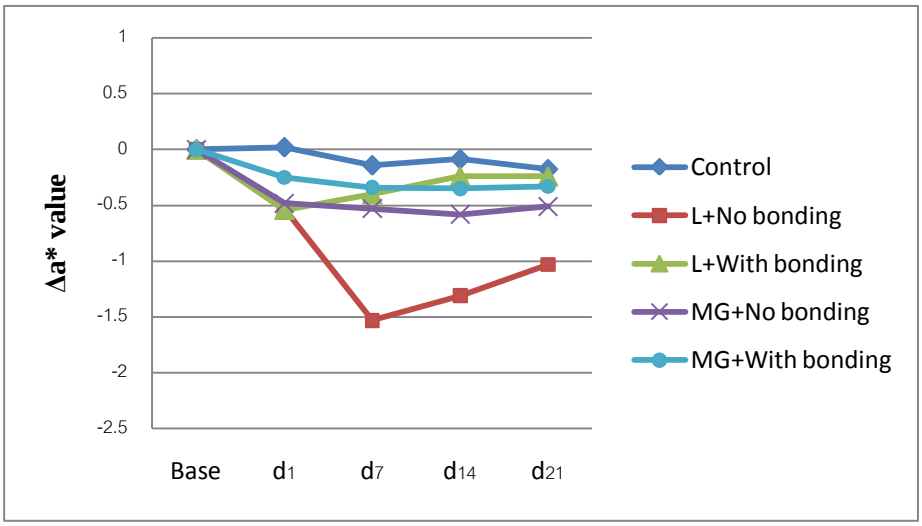
Teeth without bonding coating in the pulp chamber, placed with 3Mix-MP with lentulo spiral, had the highest negative values, which meant stronger green color, while teeth with bonding, placed with 3Mix-MP with MTA gun and lentulo spiral had minor green color. The data is shown in Figures 5.2.2 a, b.

Figure 5.2.2. Mean changes in Δa* value at (a) cervical and (b) middle parts at each time interval

(a)



(b)

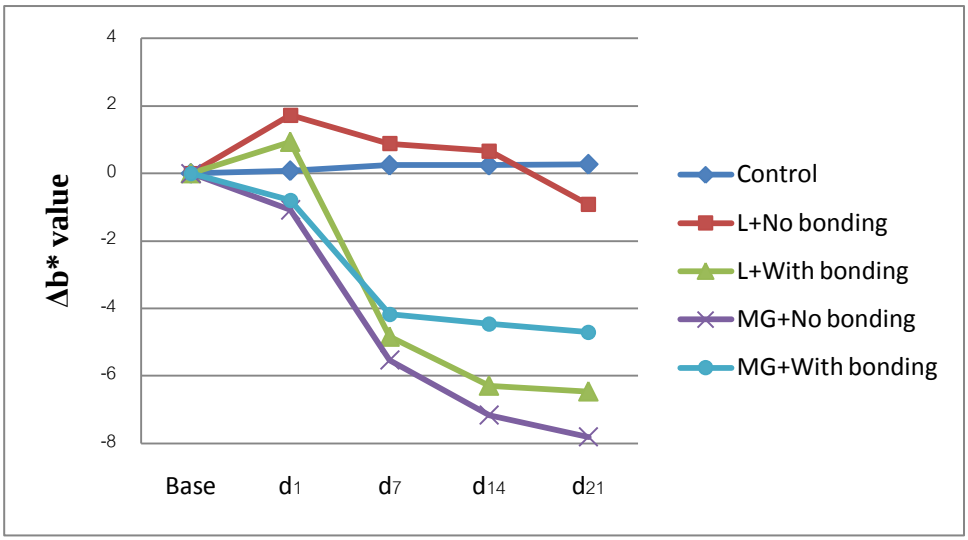


5.2.3 B* value

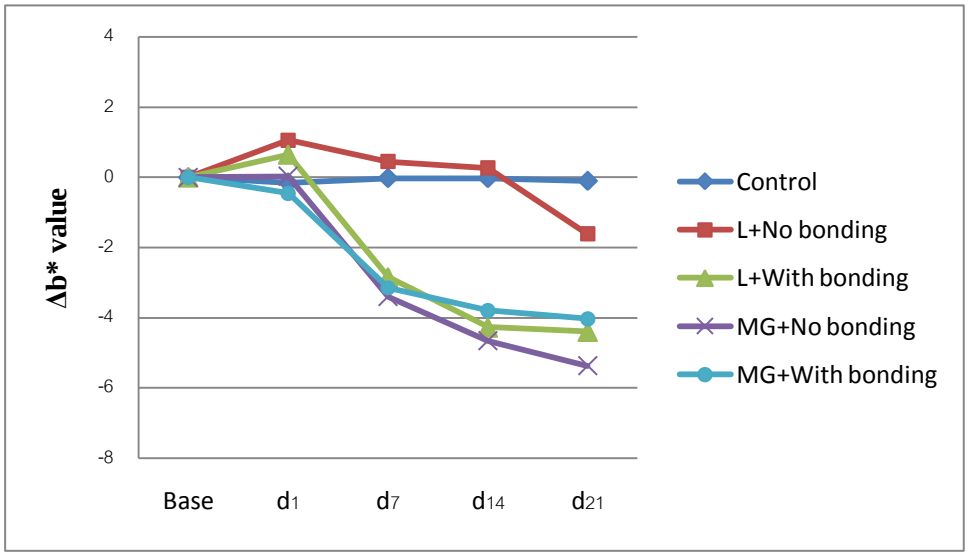
Teeth with bonding coating in the pulp chamber, placed with 3Mix-MP with MTA gun and lentulo spiral had stronger blue color than teeth without bonding placed with 3Mix-MP by lentulo spiral. The data is shown in Figures 5.2.3 a, b.

Figure 5.2.3. Mean changes in Δb* value at (a) cervical and (b) middle parts at each time interval

(a)



(b)



5.2.4 E* value

At the cervical part, teeth with dentin bonding, placed with 3Mix-MP with MTA gun showed less value than teeth without dentin bonding, placed with 3Mix-MP with lentulo. While at the middle part, the ΔE* value was not different between the two groups. The data is shown in Tables 5.2.5 to 5.2.8

Table 5.2.5. Mean change in the ΔE* between methods and bonding agent at day 1 after introduction of 3Mix-MP at the cervical and middle parts (Mean±SD).

Location	**Methods of placment**			
	Bonding	**Lentulo**	**MTA gun**	**Total**
Cervical	**No**	4.68 ± 2.42	3.58 ± 2.89	4.13 ± 2.65
	Yes	1.91 ± 1.50	1.57 ± 0.26	1.74 ± 1.17*
	Total	3.29 ± 2.42	2.58 ± 2.30	2.93 ± 2.36
	Bonding	**Lentulo**	**MTA gun**	**Total**
Middle	**No**	1.77 ± 0.78	2.05 ± 1.93	1.91 ± 1.44
	Yes	1.40 ± 1.04	0.98 ± 0.69	1.19 ± 0.88
	Total	1.58 ± 0.91	1.52 ± 1.51	1.55 ± 1.23

* Significantly different P<.05

Table 5.2.6. Mean change in the ΔE* between methods and bonding agent at day 7 after introduction of 3Mix-MP at the cervical and middle parts (Mean±SD).

Location	**Methods of placement**			
	Bonding	**Lentulo**	**MTA gun**	**Total**
Cervical	**No**	10.00 ± 2.59	10.33 ± 3.70	10.17 ± 3.12
	Yes	9.32 ± 4.05	7.99 ± 3.69	8.66 ± 3.83
	Total	9.66 ± 3.33	9.16 ± 3.79	9.41 ± 3.53
	Bonding	**Lentulo**	**MTA gun**	**Total**
Middle	**No**	6.29 ± 1.99	6.49 ± 3.10	6.39 ± 2.54
	Yes	5.56 ± 2.21	5.64 ± 1.57	5.60 ± 1.87
	Total	5.93 ± 2.08	6.06 ± 2.43	6.00 ± 2.24

Table 5.2.7. Mean change in the ΔE* between methods and bonding agent at day 14 after introduction of 3Mix-MP at the cervical and middle parts (Mean±SD).

Location	**Methods of placment**			
	Bonding	**Lentulo**	**MTA gun**	**Total**
Cervical	**No**	10.83 ± 3.20	12.73 ± 3.57	11.78 ± 3.44
	Yes	11.24 ± 2.05	8.94 ± 3.73*	10.09 ± 3.16
	Total	11.03 ± 2.62	10.84 ± 4.05	10.94 ± 3.37
	Bonding	**Lentulo**	**MTA gun**	**Total**
Middle	**No**	6.79 ± 2.17	7.98 ± 2.82	7.39 ± 2.52
	Yes	7.22 ± 1.91	6.39 ± 1.60	6.80 ± 1.77
	Total	7.01 ± 2.00	7.18 ± 2.37	7.09 ± 2.17

* Significantly different P<.05

Table 5.2.8. Mean change in the ΔE* between methods and bonding agent at day 21 after introduction of 3Mix-MP at the cervical and middle parts (Mean±SD).

Location	**Methods of placment**			
	Bonding	**Lentulo**	**MTA gun**	**Total**
Cervical	**No**	11.29 ± 3.76	13.73 ± 3.62	12.51 ± 3.80
	Yes	12.02 ± 1.85	9.31 ± 3.52*	10.67 ± 3.07
	Total	11.65 ± 2.91	11.52 ± 4.15	11.59 ± 3.54
	Bonding	**Lentulo**	**MTA gun**	**Total**
Middle	**No**	7.87 ± 2.61	8.72 ± 2.64	8.30 ± 2.59
	Yes	7.53 ± 1.76	6.57 ± 1.57	7.05 ± 1.70
	Total	7.70 ± 2.17	7.65 ± 2.38	7.67 ± 2.25

* Significantly different P<.05

5.3 Part 3: The efficacy of bleaching agents on 3Mix-MP stained teeth

Figure 5.3.1 shows the photo of teeth of the control group and two experimental groups before stained, after staining and after applying two bleaching agents. Teeth of two experimental groups whitened both the cervical and the middle parts after bleaching finished. As seen in the photo below, the dark green color of teeth in the sodium perborate group disappeared on day 14 and became bright on day 21, while in the opalescence group, the dark green color disappeared on day 21. For teeth in the control group, the shade remained the same as after staining.

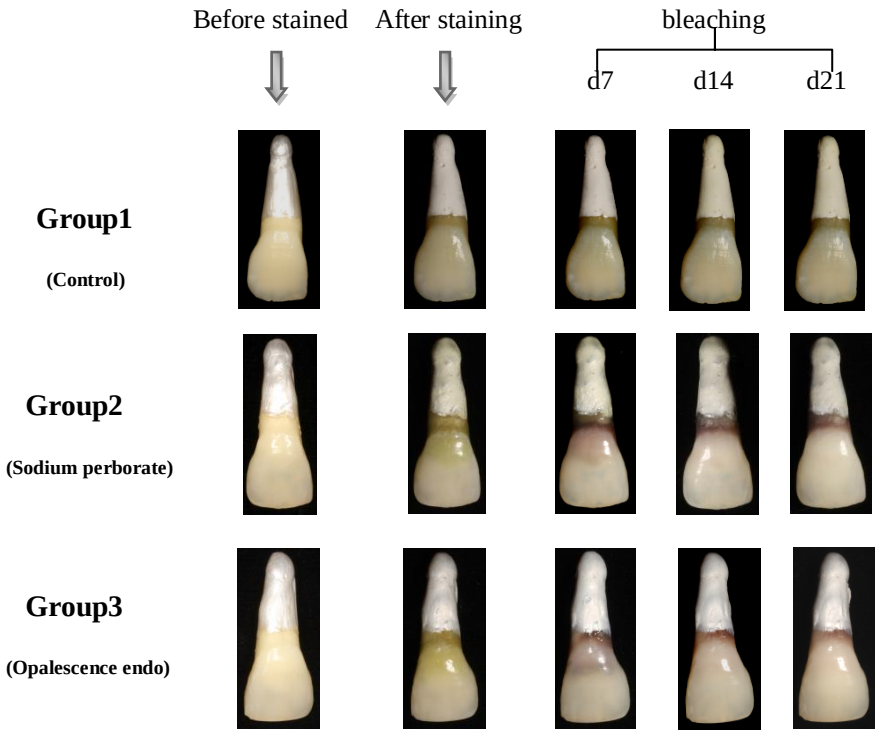


Figure 5.3.1. Photographs of teeth at time intervals after bleaching application

The results of color change of the experimental groups at the cervical and middle parts are demonstrated in figures 5.3.2 and 5.3.3. For the control group after tooth staining, the ΔL*, Δa*, Δb* and ΔE* values remained the same along the period of this study.

5.3.1 L* value

The ΔL*of two the experimental groups before staining, after staining and after applying the bleaching agents at days 7, 14 and 21 at the cervical and middle parts are listed in Table 5.3.1. The mean changes in brightness (ΔL*) of different periods are depicted in Figures 5.3.2a and 5.3.3a.

At the cervical part of the sodium perborate group after bleaching, the white color of each day was increased significantly (P<.05). At day 7, teeth were whitened the same as the pre-stained, while at days 14 and 21, teeth were more whitened than the pre-stained. For the opalescence endo group after bleaching, the white color of each day was also increased significantly (P<.05), but teeth color whitened the same as the pre-stained at day 21. Comparing the mean changes of ΔL* between the two experimental groups, teeth bleached with sodium perborate at days 7, 14 and 21 did not show any difference in brightening when compared with teeth bleached with opalescence endo at days 7, 14 and 21.

At the middle part of sodium perborate group after bleaching at day 7, 14 and 21, teeth were more whiten than pre-stained (P<.05). Opalescence endo group, teeth at day 7 and 14 were improved to white the same as pre-stained, while day 21 teeth were more whiten than pre-stained significantly (P<.05). When compared the mean changes of L value (ΔL*) between two experiment groups, teeth were bleached with sodium perborate at day 7, 14 and 21 were more whiten than teeth were bleached with opalescence endo at day 7, 14 and 21, significantly (P<.05).

Table 5.3.1. Mean change in the L value (ΔL*) between pre-stained and 7, 14 and 21 days after introduction of bleaching agents at the cervical and middle parts (mean ± SD).

	Group	Before stained	After Staining	Day 7	Day 14	Day 21
Cervical	Sodium perborate	5.27 ± 2.96	0.00 ± 0.00	4.32 ± 1.40	7.04 ± 2.74	8.97 ± 4.47
	Opalescence Endo	8.74 ± 4.77	0.00 ± 0.00	3.23 ± 2.88	6.21 ± 2.75	8.37 ± 2.98
Middle	Sodium perborate	2.16 ± 1.65	0.00 ± 0.00	5.05 ± 2.32	5.80 ± 2.10	6.83 ± 2.75
	Opalescence Endo	3.57 ± 2.58	0.00 ± 0.00	2.73 ± 1.45	4.06 ± 0.98	5.06 ± 2.02

5.3.2 A* value

The Δa*of the two experimental groups before stained, after staining and after applying the bleaching agents at days 7, 14 and 21 at the cervical and middle parts are shown in Figures 5.3.2 b and 5.3.3 b. For the two experimental groups Δa* was increased to the red axis and nearly reached the same level of those pre stained on day 7 after bleaching and again slightly decreased but still stayed within the red axis at days 14 and 21.

5.3.3 B* value

The Δb*of the two experimental groups before stained, after staining and after applying the bleaching agents at days 7, 14 and 21 at the cervical and middle parts are shown in Figures 5.3.2 c and 5.3.3 c. For the sodium perborate group after bleaching, the Δb* was slightly decreased to the blue axis from day 7 to day 21. In contrast, the Δb* of the opalescence endo group suddenly dropped to the blue axis after the first time of bleaching and it continued to decrease over time.

5.3.4 E* value

The value of color differences (ΔE*) of teeth pre stained and after bleaching at days 7, 14 and 21 of the cervical and middle parts are listed in Table 5.3.2 and shown in Figures 5.3.2 d and 5.3.3 d.

At the cervical part of the sodium perborate group, the ΔE* value at days 7, 14 and 21 after bleaching were increased significantly (P<.05), while the change of ΔE* value of the opalescence endo group did not show any difference among days 7, 14 and 21. When comparing the mean changes of the ΔE* value between the two experimental groups, the ΔE* values of the opalescence endo group at days 7, 14 and 21 higher than the values of the sodium perborate group at days 7, 14 and 21, significantly (P<.05).

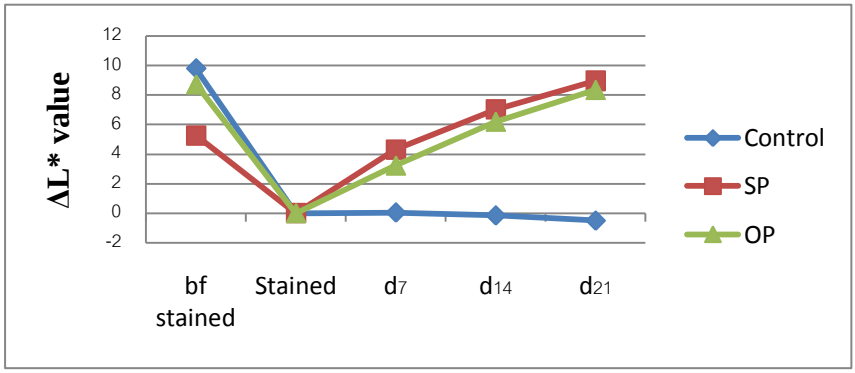
At the middle part, the results of statistic are the same as the cervical part.

Table 5.3.2. Mean change in the E value (ΔE*) of pre-stained and 7, 14 and 21 days after introduction of bleaching agents at the cervical and middle parts (mean ± SD).

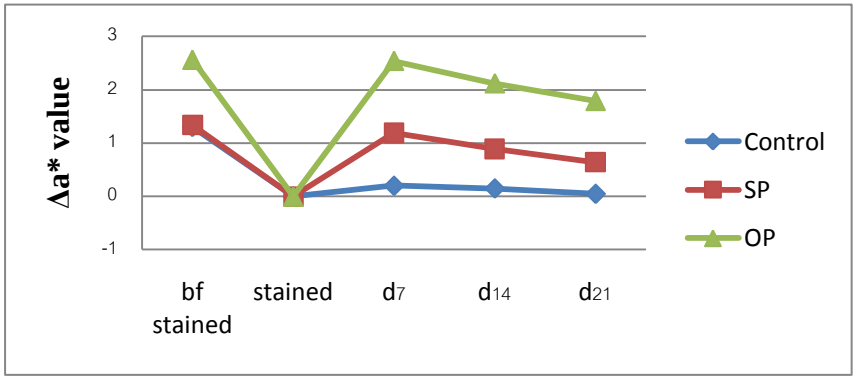
	Group	Before stained	After staining	Day 7	Day 14	Day 21
Cervical	Sodium perborate	6.18 ± 3.11	0.00 ± 0.00	5.30 ± 2.32	7.70 ± 3.21	9.33 ± 4.57
	Opalescence Endo	10.11± 5.18	0.00 ± 0.00	9.09 ± 4.08	10.69± 3.80	12.22± 3.81
Middle	Sodium perborate	2.87 ± 1.62	0.00 ± 0.00	5.24 ± 2.31	5.98 ± 2.08	6.98 ± 2.72
	Opalescence Endo	4.12 ± 2.77	0.00 ± 0.00	4.09 ± 1.44	5.49 ± 1.69	6.65 ± 2.28

Figure 5.3.2. Mean changes in CIE Lab parameters at each time interval at the cervical part. (a) ΔL* value (b) Δa* value (c) Δb* value (d) ΔE* value

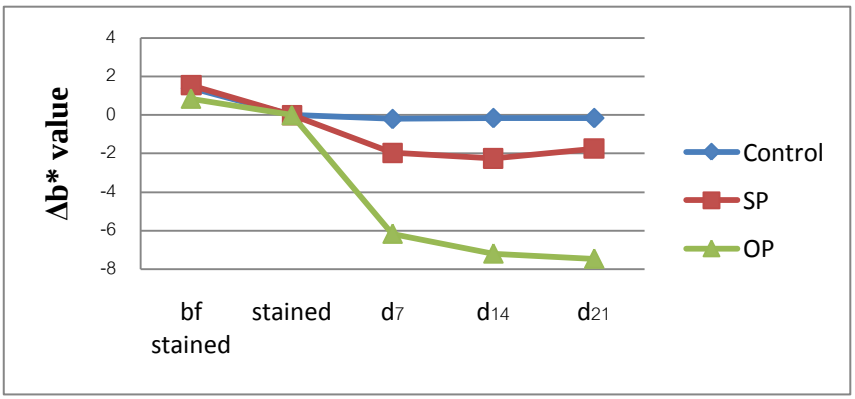
(a)



(b)



(c)



(d)

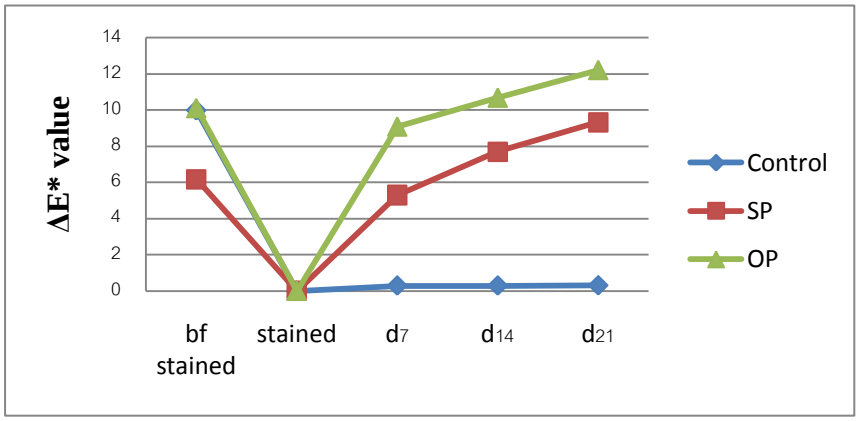
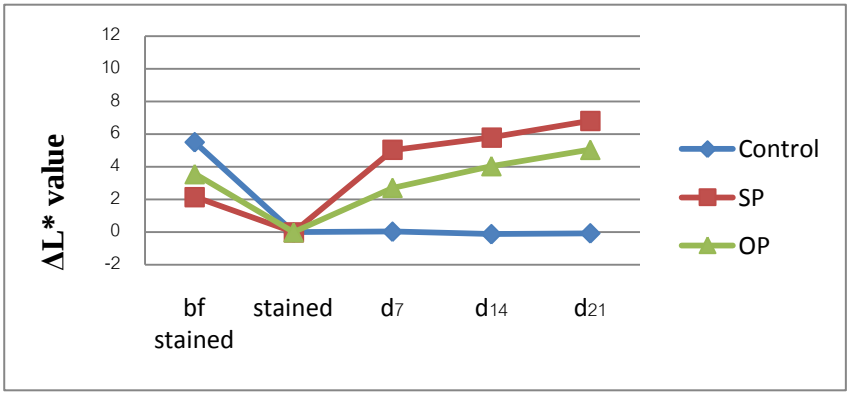
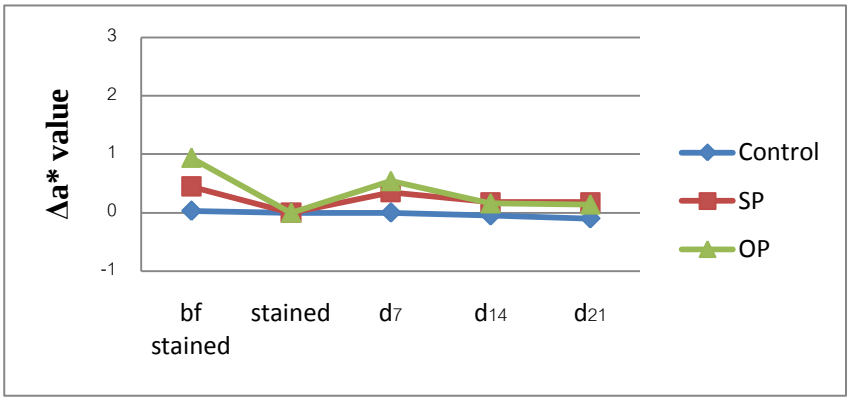


Figure 5.3.3. Mean changes in CIE Lab parameters at each time interval at the middle part. (a) ΔL* value (b) Δa* value (c) Δb* value (d) ΔE* value

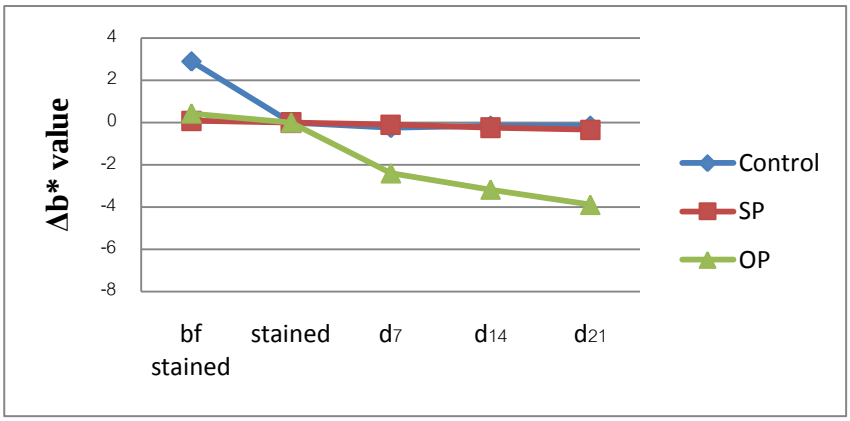
(a)



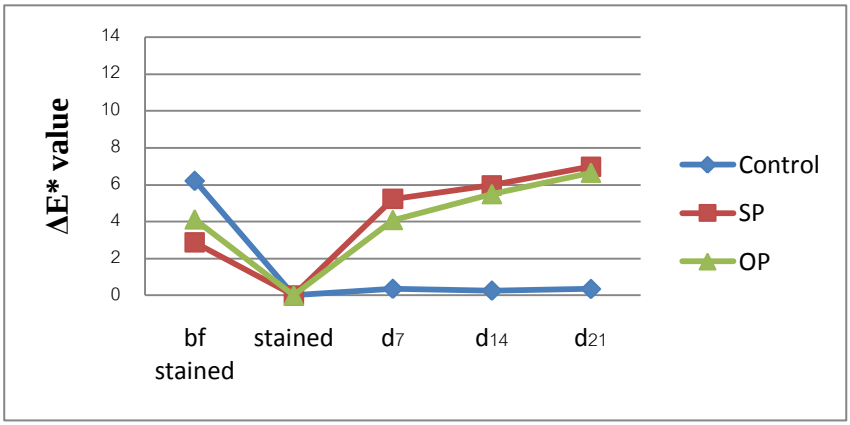
(b)



(c)



(d)



CHAPTER VI
DISCUSSION

6.1 Part 1: Duration of discoloration by 3Mix-MP as intracanal medication

The result of this part shows that the 3Mix-MP was caused of tooth discoloration, while the color of teeth in the control group did not change along the period of study. The tooth color changes could be detected after the first day of introducing 3Mix-MP, which corresponded to the study of Kim et al. in 2010 (79). That study also reported that only the triple antibiotics and minocycline groups could cause tooth discoloration and concluded that minocycline was the only cause of tooth discoloration. Minocycline is a semisynthetic derivative of tetracycline, for which the common side effect is tissue hyper pigmentation. The cause of tooth discoloration may be explained by the iron theory (25, 34, 35), where haemosiderin, a breakdown product of minocycline, binds to calcium ions via chelation to form an insoluble complex and incorporation into the tooth matrix causes discoloration.

The results from this study, in terms of L, a, b color space, indicate the samples that were filled with 3Mix-MP had a negative ΔL* and Δa* value both at the cervical and middle parts, which means teeth became darker and greenish. Although the results of the ΔL* and Δa* value in this study corresponded with Kim et al. in 2010, the change in Δb* value was slightly different. In Kim's study, the Δb* value of the triple antibiotic group had the positive value, which means teeth became yellowish along the period of study, while in this study, the Δb* value was at the yellow axis until day15 and finally decreased to the blue axis at day 22. This difference might be due to different locations of tooth color measurement and duration of study. Kim's study measured the color on the root surface and the duration of study was only 14 days, with similar results. After 14 days, this study showed that the Δb* values changed to blue color.

The dark green color appeared on the tooth even though the medication was limited to the root portion of the canal and the discoloration of the cervical part was strongly increased over time. This result could be explained by the direction of the dentinal tubule, which is an inverted cone with the smallest dimensions at the DEJ and the largest dimensions at the pulp and travelled in an incisal direction from the canal to the enamel surface (80-83). Additionally, the vehicle that was used in this study was macrogol and propylene glycol (MP), and previous study has shown that propylene glycol could quickly and efficiently deliver dye through the root canal system (20).

In addition, the color differences (ΔE*) used to determine the changes in the overall shade are perceptible by a human observer and showed the value of the cervical part was 4.68 on the first day of introducing 3Mix-MP and the middle part the ΔE* value was 3.40 on the second day. The interpretation of the color difference is based on Seghi et al. in 1989 (61) which reported that sample pairs producing a measured color-different value of greater than 2, ΔE* units were correctly judged by the observer 100% of the time. Incorrect judgments were made when the measured color-difference fell within 1 to 2. The result of this study indicated that the color difference could be detected on the first and second day for the cervical and middle parts respectively, after introducing 3Mix-MP. This result corresponded to the study of Kim et al. in 2010 (79), but in this study tooth color was measured at the crown rather than the root, which will be more relevant in clinical situations.

6.2 Part 2: The comparison of methods to prevent tooth discoloration by 3Mix-MP

Tooth discoloration maybe caused by a breakdown product of minocycline binding to calcium ions via chelation to form insoluble complexes, and incorporated into the tooth matrix causing the discoloration (25, 34, 35). Therefore, 3Mix-MP cannot stain the tooth unless it comes in contact with the coronal dentin. Based on this hypothesis, some studies and case reports present how to prevent tooth discoloration by 3Mix-MP. Kim et al. 2000 have suggested that the placement of medicament should be restricted to the root rather than fill the pulp chamber (8, 9). Reynolds et al. 2009 recommended the use of the projector and the inner surfaces of the coronal dentin to be sealed with flow able composite to avoid contact between 3Mix and the dentinal wall (5). Because Reynolds's technique requires a special appliance, dentine bonding and method of placement 3Mix-MP using an MTA gun and lentulo spiral were used in this study to examine the efficiency of these techniques in the prevention of tooth discoloration. The result of this study showed that dentine bonding was the only factor that could affect darkness in color (ΔL* value). Although the statistic showed bonding could affect the ΔL* significantly, teeth of the bonding group also had negative values, which meant that dentine bonding agent could not prevent the change in darkness of teeth, completely. Moreover, the Δa* value also showed the negative value both at the cervical and middle parts, which meant the teeth became green. However, teeth with bonding and teeth which used the MTA gun showed slightly more green coloration than teeth without bonding and using a lentulo spiral. Therefore, dentine bonding was effective in preventing the green coloration and this result corresponded to the study of Kim et al. in 2010 (79). For the Δb* value, teeth with the bonding group showed the negative value, which indicated that the teeth had become blue. This result conflicted with the study of Kim et al. in 2010 (79). Their samples obtained the positive Δb*, which indicated that the samples were yellowish. This difference might be due to different locations of tooth color measurement and duration of study. Kim's study measured the color on the root surface and the duration of study was only 14 days.

The overall changes in these three color coordinates can be expressed in the ΔE* value, the result of bonding group which placed with 3Mix-MP by MTA gun

showed the value less than the value of the non bonding group which placed with 3Mix-MP by lentulo spiral. This indicated that dentine bonding and method of placement by MTA gun could reduce the overall color change but did not prevent it. This result might be due to the resin tag and hybridized complex (84) being to cover up the dentinal tubule as well as the coronal dentin, which made the 3Mix-MP not come in contact with the coronal dentin directly and Clearfil SE Bond was used in this study because there were studies have found that Clearfil SE Bond had less microleakage when compared to others self-etch adhesives and performed equivalently to the sealing performance of the total-etch (85, 86).

6.3 Part 3: The efficacy of bleaching agents on 3Mix-MP stained teeth

The bleaching efficacy of sodium perborate mixed with superoxol (30% hydrogen peroxide) was compared with Opalescen Endo (35% hydrogen peroxide gel). The results indicated that sodium perborate with superoxol could whiten tooth color better than opalescent Endo. Because this study used new sodium perborate with fresh superoxol, which corresponded to the Ho and Goerig studied (40). That study found that new sodium perborate with fresh superoxol was most effective with a 93% success rate when compared to new sodium perborate with old superoxal and distilled water. Rotstein et al. 1991, also found 80% success using sodium perborate with 30% hydrogen peroxide (43) when compared with sodium perborate mixed with 3% hydrogen peroxide and water. Moreover, hydrogen peroxide concentration and pH are the factors that affect bleaching. The highest concentration generally used is 35%, but when in gel form, the concentration is reduced to 25% (87). The opalescence endo contains 35% hydrogen peroxide, but the result shows the efficacy to whitening teeth was lower than sodium perborate mixed with 30% hydrogen peroxide. This might be due to the fact that the opalescence endo is in gel form, which made its effectiveness low.

The optimum pH for hydrogen peroxide to have an oxidation effect is pH 9.5 to 10.8 (88), while opalescence endo has a pH 3.0 to 5.0, which produced a less effective result in this study. In contrast, Roland et al. 1993 (89), found that the pH ranged from 10 to 11 when sodium perborate was mixed with hydrogen peroxide in different concentrations. This might be the reason why sodium perborate mixed with superoxal in this study could produced the better result than opalescence endo.

The overall changes in these three color coordinates can be expressed in the ΔE^* value. For the sodium perborate group at the cervical and the middle part, the result showed the value nearly the same as that of the pre stained after post bleaching. As seen in Table 5.3.2, the ΔE^* values were 5.30, 7.70 and 9.33 at the cervical part at days 7, 14 and 21, respectively and at the middle part, the values were 5.24, 5.98 and 6.98 at days 7, 14 and 21 respectively. This result indicated that the bleaching procedure should be stopped at day14 because the value at the two parts nearly reached the same level as the pre stained and when compared with Figure 5.3.1 at day 14, the tooth shade was already improved and resembled the pre stained tooth shade.

However, the successful results of bleaching agents obtained in this study may be due to several factors, i.e., the etiology of discoloration. In the present study teeth were discolored by intracanal medication, which define as the local cause and this type of discoloration is more amenable to bleach and provides better result than teeth discoloration by systemic cause like tetracycline. In addition, the duration of discoloration was suspect bleaching is more likely to be successful in recently stained teeth than in teeth with long standing discoloration (90). The discoloration produced in this study lasted only 21 days whereas in most clinical cases, it was usually longer.

CHAPTER VII
CONCLUSIONS

In conclusion, 3Mix-MP could stain tooth color to become dark-green. The discoloration could be detected since the first day after placed the medication and the discoloration became darker over the period of study.

Dentine bonding and technique of placement by MTA gun could reduce the overall color change, but did not prevent it. For Clinical application, dentine bonding should coat in the pulp chamber before placing 3Mix-MP into the root portion by MTA gun.

Sodium perborate mixed with 30% hydrogen peroxide and opalescence endo have the efficacy to whiten tooth discolored by 3Mix-MP as intracanal medication and the bleaching procedure could be done in 14 days for acceptable result.

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APPENDIX

STATISTICAL ANALYSIS

1. Part I. Duration of discoloration by 3Mix-MP as intracanal Medication

1.1 L value

Cervical part

Descriptive Statistics

	Mean	Std. Deviation	N
d1	-3.8600	2.42129	10
d2	-6.5200	3.02666	10
d3	-6.6900	3.19251	10
d4	-7.6100	3.02855	10
d5	-8.820	2.6619	10
d8	-9.2800	2.58577	10
d15	-10.2300	3.21457	10
d22	-10.7700	3.53005	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	70.756	1	70.756	23.053	.001
	Level 2 vs. Level 3	.289	1	.289	.304	.595
	Level 3 vs. Level 4	8.464	1	8.464	4.092	.074
	Level 4 vs. Level 5	14.641	1	14.641	11.173	.009
	Level 5 vs. Level 6	2.116	1	2.116	11.342	.008
	Level 6 vs. Level 7	9.025	1	9.025	8.901	.015
	Level 7 vs. Level 8	2.916	1	2.916	10.738	.010
Error(day)	Level 1 vs. Level 2	27.624	9	3.069		
	Level 2 vs. Level 3	8.561	9	.951		
	Level 3 vs. Level 4	18.616	9	2.068		
	Level 4 vs. Level 5	11.794	9	1.310		
	Level 5 vs. Level 6	1.679	9	.187		
	Level 6 vs. Level 7	9.125	9	1.014		
	Level 7 vs. Level 8	2.444	9	.272		

Middle part

Descriptive Statistics

	Mean	Std. Deviation	N
d1	-.7500	.68597	10
d2	-2.0700	1.17714	10
d3	-2.8100	1.31652	10
d4	-3.8800	1.76811	10
d5	-4.6000	2.28352	10
d8	-5.4500	1.99958	10
d15	-6.1300	2.11715	10
d22	-7.0400	2.45366	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	17.424	1	17.424	27.824	.001
	Level 2 vs. Level 3	5.476	1	5.476	4.092	.074
	Level 3 vs. Level 4	11.449	1	11.449	6.743	.029
	Level 4 vs. Level 5	5.184	1	5.184	6.078	.036
	Level 5 vs. Level 6	7.225	1	7.225	30.600	.000
	Level 6 vs. Level 7	4.624	1	4.624	4.222	.070
	Level 7 vs. Level 8	8.281	1	8.281	10.484	.010
Error(day)	Level 1 vs. Level 2	5.636	9	.626		
	Level 2 vs. Level 3	12.044	9	1.338		
	Level 3 vs. Level 4	15.281	9	1.698		
	Level 4 vs. Level 5	7.676	9	.853		
	Level 5 vs. Level 6	2.125	9	.236		
	Level 6 vs. Level 7	9.856	9	1.095		
	Level 7 vs. Level 8	7.109	9	.790		

1.2 E value

Cervical part

Descriptive Statistics

	Mean	Std. Deviation	N
d1	4.68267	2.420760	10
d2	7.40314	2.855397	10
d3	7.79746	3.169483	10
d4	8.40372	2.857746	10
d5	9.35697	2.761597	10
d8	10.00966	2.599382	10
d15	10.83100	3.202250	10
d22	11.29017	3.766456	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	74.010	1	74.010	43.442	.000
	Level 2 vs. Level 3	1.555	1	1.555	1.062	.330
	Level 3 vs. Level 4	3.675	1	3.675	1.517	.249
	Level 4 vs. Level 5	9.087	1	9.087	6.922	.027
	Level 5 vs. Level 6	4.260	1	4.260	17.172	.003
	Level 6 vs. Level 7	6.746	1	6.746	8.168	.019
	Level 7 vs. Level 8	2.108	1	2.108	3.558	.092
Error(day)	Level 1 vs. Level 2	15.333	9	1.704		
	Level 2 vs. Level 3	13.183	9	1.465		
	Level 3 vs. Level 4	21.800	9	2.422		
	Level 4 vs. Level 5	11.814	9	1.313		
	Level 5 vs. Level 6	2.233	9	.248		
	Level 6 vs. Level 7	7.433	9	.826		
	Level 7 vs. Level 8	5.333	9	.593		

Middle part

Descriptive Statistics

	Mean	Std. Deviation	N
d1	1.77497	.783141	10
d2	3.40810	1.021643	10
d3	4.66497	1.105826	10
d4	5.15963	1.472514	10
d5	5.55328	2.207384	10
d8	6.29943	1.997975	10
d15	6.79687	2.172071	10
d22	7.87285	2.616565	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	26.671	1	26.671	71.569	.000
	Level 2 vs. Level 3	15.797	1	15.797	15.793	.003
	Level 3 vs. Level 4	2.447	1	2.447	1.194	.303
	Level 4 vs. Level 5	1.550	1	1.550	1.229	.296
	Level 5 vs. Level 6	5.567	1	5.567	7.321	.024
	Level 6 vs. Level 7	2.474	1	2.474	2.874	.124
	Level 7 vs. Level 8	11.577	1	11.577	6.593	.030
Error(day)	Level 1 vs. Level 2	3.354	9	.373		
	Level 2 vs. Level 3	9.002	9	1.000		
	Level 3 vs. Level 4	18.441	9	2.049		
	Level 4 vs. Level 5	11.345	9	1.261		
	Level 5 vs. Level 6	6.844	9	.760		
	Level 6 vs. Level 7	7.749	9	.861		
	Level 7 vs. Level 8	15.804	9	1.756		

2. Part II. The comparison of methods to prevent teeth discoloration by 3Mix-MP

2.1 L value

Cervical part

Descriptive Statistics

Dependent Variable: d1

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	-3.8600	2.42129	10
	Yes	-.6600	.42216	10
	Total	-2.2600	2.35716	20
MTA gun	No	-1.9600	2.85003	10
	Yes	-.9800	.71461	10
	Total	-1.4700	2.08380	20
Total	No	-2.9100	2.75220	20
	Yes	-.8200	.59436	20
	Total	-1.8650	2.23211	40

Tests of Between-Subjects Effects

Dependent Variable: d1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	62.243(a)	3	20.748	5.656	.003
Intercept	139.129	1	139.129	37.925	.000
Methods	6.241	1	6.241	1.701	.200
Bonding	43.681	1	43.681	11.907	.001
Methods * Bonding	12.321	1	12.321	3.359	.075
Error	132.068	36	3.669		
Total	333.440	40			
Corrected Total	194.311	39			

a R Squared = .320 (Adjusted R Squared = .264)

Descriptive Statistics

Dependent Variable: d7

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	-9.2800	2.58577	10
	Yes	-7.4800	4.36012	10
	Total	-8.3800	3.60899	20
MTA gun	No	-8.4200	3.50105	10
	Yes	-6.4000	3.92145	10
	Total	-7.4100	3.76352	20
Total	No	-8.8500	3.02785	20
	Yes	-6.9400	4.07384	20
	Total	-7.8950	3.67249	40

Tests of Between-Subjects Effects

Dependent Variable: d7

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	46.011(a)	3	15.337	1.150	.342
Intercept	2493.241	1	2493.241	186.998	.000
Methods	9.409	1	9.409	.706	.406
Bonding	36.481	1	36.481	2.736	.107
Methods * Bonding	.121	1	.121	.009	.925
Error	479.988	36	13.333		
Total	3019.240	40			
Corrected Total	525.999	39			

a R Squared = .087 (Adjusted R Squared = .011)

Descriptive Statistics

Dependent Variable: d14

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	-10.2300	3.21457	10
	Yes	-8.8800	2.96416	10
	Total	-9.5550	3.08809	20
MTA gun	No	-10.3400	3.58212	10
	Yes	-7.3700	3.90699	10
	Total	-8.8550	3.95348	20
Total	No	-10.2850	3.31302	20
	Yes	-8.1250	3.46302	20
	Total	-9.2050	3.51939	40

Tests of Between-Subjects Effects

Dependent Variable: d14

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	58.117(a)	3	19.372	1.641	.197
Intercept	3389.281	1	3389.281	287.131	.000
Methods	4.900	1	4.900	.415	.523
Bonding	46.656	1	46.656	3.953	.054
Methods * Bonding	6.561	1	6.561	.556	.461
Error	424.942	36	11.804		
Total	3872.340	40			
Corrected Total	483.059	39			

a R Squared = .120 (Adjusted R Squared = .047)

Descriptive Statistics

Dependent Variable: d21

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	-10.7700	3.53005	10
	Yes	-9.7100	2.86800	10
	Total	-10.2400	3.17720	20
MTA gun	No	-11.1300	3.68391	10
	Yes	-7.6500	3.84744	10
	Total	-9.3900	4.07765	20
Total	No	-10.9500	3.51643	20
	Yes	-8.6800	3.46769	20
	Total	-9.8150	3.63368	40

Tests of Between-Subjects Effects

Dependent Variable: d21

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	73.395(a)	3	24.465	1.995	.132
Intercept	3853.369	1	3853.369	314.172	.000
Methods	7.225	1	7.225	.589	.448
Bonding	51.529	1	51.529	4.201	.048
Methods * Bonding	14.641	1	14.641	1.194	.282
Error	441.546	36	12.265		
Total	4368.310	40			
Corrected Total	514.941	39			

a R Squared = .143 (Adjusted R Squared = .071)

Middle part

Descriptive Statistics

Dependent Variable: d1

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	-.7500	.68597	10
	Yes	-.4600	.39777	10
	Total	-.6050	.56566	20
MTA gun	No	-1.3400	1.66280	10
	Yes	-.5500	.28382	10
	Total	-.9450	1.22967	20
Total	No	-1.0450	1.27443	20
	Yes	-.5050	.33947	20
	Total	-.7750	.96030	40

Tests of Between-Subjects Effects

Dependent Variable: d1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	4.697(a)	3	1.566	1.803	.164
Intercept	24.025	1	24.025	27.661	.000
Methods	1.156	1	1.156	1.331	.256
Bonding	2.916	1	2.916	3.357	.075
Methods * Bonding	.625	1	.625	.720	.402
Error	31.268	36	.869		
Total	59.990	40			
Corrected Total	35.965	39			

a R Squared = .131 (Adjusted R Squared = .058)

Descriptive Statistics

Dependent Variable: d7

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	-5.4500	1.99958	10
	Yes	-4.3900	2.44470	10
	Total	-4.9200	2.24068	20
MTA gun	No	-5.1700	3.47820	10
	Yes	-4.3400	1.81488	10
	Total	-4.7550	2.73351	20
Total	No	-5.3100	2.76499	20
	Yes	-4.3650	2.09568	20
	Total	-4.8375	2.46844	40

Tests of Between-Subjects Effects

Dependent Variable: d7

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9.335(a)	3	3.112	.491	.691
Intercept	936.056	1	936.056	147.605	.000
Methods	.272	1	.272	.043	.837
Bonding	8.930	1	8.930	1.408	.243
Methods * Bonding	.132	1	.132	.021	.886
Error	228.299	36	6.342		
Total	1173.690	40			
Corrected Total	237.634	39			

a R Squared = .039 (Adjusted R Squared = -.041)

Descriptive Statistics

Dependent Variable: d14

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	-6.1300	2.11715	10
	Yes	-5.5300	2.31087	10
	Total	-5.8300	2.17887	20
MTA gun	No	-6.1500	3.36229	10
	Yes	-4.8000	1.89620	10
	Total	-5.4750	2.74550	20
Total	No	-6.1400	2.73465	20
	Yes	-5.1650	2.09115	20
	Total	-5.6525	2.45304	40

Tests of Between-Subjects Effects

Dependent Variable: d14

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	12.173(a)	3	4.058	.656	.584
Intercept	1278.030	1	1278.030	206.776	.000
Methods	1.260	1	1.260	.204	.654
Bonding	9.506	1	9.506	1.538	.223
Methods * Bonding	1.406	1	1.406	.228	.636
Error	222.507	36	6.181		
Total	1512.710	40			
Corrected Total	234.680	39			

a R Squared = .052 (Adjusted R Squared = -.027)

Descriptive Statistics

Dependent Variable: d21

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	-7.0400	2.45366	10
	Yes	-5.8900	2.25756	10
	Total	-6.4650	2.36938	20
MTA gun	No	-6.5300	3.31262	10
	Yes	-4.8600	1.83618	10
	Total	-5.6950	2.74389	20
Total	No	-6.7850	2.84924	20
	Yes	-5.3750	2.07133	20
	Total	-6.0800	2.56027	40

Tests of Between-Subjects Effects

Dependent Variable: d21

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	26.486(a)	3	8.829	1.387	.262
Intercept	1478.656	1	1478.656	232.292	.000
Methods	5.929	1	5.929	.931	.341
Bonding	19.881	1	19.881	3.123	.086
Methods * Bonding	.676	1	.676	.106	.746
Error	229.158	36	6.366		
Total	1734.300	40			
Corrected Total	255.644	39			

a R Squared = .104 (Adjusted R Squared = .029)

2.2 E value

Cervical part

Descriptive Statistics

Dependent Variable: d1

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	4.68267	2.420760	10
	Yes	1.91581	1.508745	10
	Total	3.29924	2.422539	20
MTA gun	No	3.58936	2.892225	10
	Yes	1.57092	.763922	10
	Total	2.58014	2.304544	20
Total	No	4.13602	2.655699	20
	Yes	1.74336	1.177279	20
	Total	2.93969	2.362006	40

Tests of Between-Subjects Effects

Dependent Variable: d1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	63.819(a)	3	21.273	4.981	.005
Intercept	345.671	1	345.671	80.930	.000
Methods	5.171	1	5.171	1.211	.279
Bonding	57.248	1	57.248	13.403	.001
Methods * Bonding	1.400	1	1.400	.328	.570
Error	153.764	36	4.271		
Total	563.255	40			
Corrected Total	217.584	39			

a R Squared = .293 (Adjusted R Squared = .234)

Descriptive Statistics

Dependent Variable: d7

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	10.00966	2.599382	10
	Yes	9.32693	4.050983	10
	Total	9.66830	3.331160	20
MTA gun	No	10.33931	3.705943	10
	Yes	7.99708	3.698904	10
	Total	9.16820	3.798708	20
Total	No	10.17449	3.120059	20
	Yes	8.66200	3.836622	20
	Total	9.41825	3.535571	40

Tests of Between-Subjects Effects

Dependent Variable: d7

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	32.262(a)	3	10.754	.850	.476
Intercept	3548.135	1	3548.135	280.578	.000
Methods	2.501	1	2.501	.198	.659
Bonding	22.876	1	22.876	1.809	.187
Methods * Bonding	6.885	1	6.885	.544	.465
Error	455.248	36	12.646		
Total	4035.645	40			
Corrected Total	487.510	39			

a R Squared = .066 (Adjusted R Squared = -.012)

Descriptive Statistics

Dependent Variable: d14

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	10.83100	3.202250	10
	Yes	11.24797	2.053525	10
	Total	11.03948	2.626901	20
MTA gun	No	12.73901	3.574377	10
	Yes	8.94747	3.735387	10
	Total	10.84324	4.055163	20
Total	No	11.78501	3.444887	20
	Yes	10.09772	3.162212	20
	Total	10.94136	3.373879	40

Tests of Between-Subjects Effects

Dependent Variable: d14

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	73.133(a)	3	24.378	2.367	.087
Intercept	4788.536	1	4788.536	464.899	.000
Methods	.385	1	.385	.037	.848
Bonding	28.469	1	28.469	2.764	.105
Methods * Bonding	44.279	1	44.279	4.299	.045
Error	370.806	36	10.300		
Total	5232.475	40			
Corrected Total	443.939	39			

a R Squared = .165 (Adjusted R Squared = .095)

Descriptive Statistics

Dependent Variable: d21

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	11.29017	3.766456	10
	Yes	12.02644	1.859701	10
	Total	11.65831	2.915590	20
MTA gun	No	13.73652	3.620932	10
	Yes	9.31631	3.527899	10
	Total	11.52641	4.153036	20
Total	No	12.51334	3.808572	20
	Yes	10.67137	3.076783	20
	Total	11.59236	3.542392	40

Tests of Between-Subjects Effects

Dependent Variable: d21

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	100.576(a)	3	33.525	3.104	.039
Intercept	5375.312	1	5375.312	497.692	.000
Methods	.174	1	.174	.016	.900
Bonding	33.929	1	33.929	3.141	.085
Methods * Bonding	66.473	1	66.473	6.155	.018
Error	388.817	36	10.800		
Total	5864.705	40			
Corrected Total	489.393	39			

a R Squared = .206 (Adjusted R Squared = .139)

Middle part

Descriptive Statistics

Dependent Variable: d1

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	1.77497	.783141	10
	Yes	1.40204	1.043121	10
	Total	1.58850	.917895	20
MTA gun	No	2.05705	1.937792	10
	Yes	.98971	.695603	10
	Total	1.52338	1.519111	20
Total	No	1.91601	1.445738	20
	Yes	1.19587	.888457	20
	Total	1.55594	1.239280	40

Tests of Between-Subjects Effects

Dependent Variable: d1

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.434(a)	3	2.145	1.444	.246
Intercept	96.838	1	96.838	65.207	.000
Methods	.042	1	.042	.029	.867
Bonding	5.186	1	5.186	3.492	.070
Methods * Bonding	1.206	1	1.206	.812	.374
Error	53.463	36	1.485		
Total	156.735	40			
Corrected Total	59.897	39			

a R Squared = .107 (Adjusted R Squared = .033)

Descriptive Statistics

Dependent Variable: d7

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	6.29943	1.997975	10
	Yes	5.56942	2.218250	10
	Total	5.93442	2.088532	20
MTA gun	No	6.49211	3.106725	10
	Yes	5.64288	1.573707	10
	Total	6.06749	2.436138	20
Total	No	6.39577	2.544120	20
	Yes	5.60615	1.872258	20
	Total	6.00096	2.240736	40

Tests of Between-Subjects Effects

Dependent Variable: d7

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	6.448(a)	3	2.149	.409	.748
Intercept	1440.460	1	1440.460	273.841	.000
Methods	.177	1	.177	.034	.855
Bonding	6.235	1	6.235	1.185	.284
Methods * Bonding	.036	1	.036	.007	.935
Error	189.367	36	5.260		
Total	1636.275	40			
Corrected Total	195.815	39			

a R Squared = .033 (Adjusted R Squared = -.048)

Descriptive Statistics

Dependent Variable: d14

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	6.79687	2.172071	10
	Yes	7.22559	1.918589	10
	Total	7.01123	2.006685	20
MTA gun	No	7.98398	2.821545	10
	Yes	6.39053	1.603159	10
	Total	7.18726	2.378374	20
Total	No	7.39043	2.525213	20
	Yes	6.80806	1.773289	20
	Total	7.09924	2.173827	40

Tests of Between-Subjects Effects

Dependent Variable: d14

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	13.924(a)	3	4.641	.981	.413
Intercept	2015.970	1	2015.970	425.982	.000
Methods	.310	1	.310	.065	.799
Bonding	3.391	1	3.391	.717	.403
Methods * Bonding	10.223	1	10.223	2.160	.150
Error	170.371	36	4.733		
Total	2200.265	40			
Corrected Total	184.295	39			

a R Squared = .076 (Adjusted R Squared = -.001)

Descriptive Statistics

Dependent Variable: d21

Methods	Bonding	Mean	Std. Deviation	N
Lentulo	No	7.87285	2.616565	10
	Yes	7.53678	1.762853	10
	Total	7.70482	2.178257	20
MTA gun	No	8.72837	2.644618	10
	Yes	6.57656	1.575807	10
	Total	7.65246	2.389075	20
Total	No	8.30061	2.597806	20
	Yes	7.05667	1.700271	20
	Total	7.67864	2.256754	40

Tests of Between-Subjects Effects

Dependent Variable: d21

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	23.743(a)	3	7.914	1.629	.200
Intercept	2358.460	1	2358.460	485.499	.000
Methods	.027	1	.027	.006	.941
Bonding	15.474	1	15.474	3.185	.083
Methods * Bonding	8.242	1	8.242	1.697	.201
Error	174.881	36	4.858		
Total	2557.085	40			
Corrected Total	198.625	39			

a R Squared = .120 (Adjusted R Squared = .046)

3. Part III. The efficacy of bleaching agents on 3Mix-MP stained teeth

3.1 L value

Cervical part

Sodium perborate

Descriptive Statistics

	Mean	Std. Deviation	N
Before stained	5.2700	2.96509	10
stained	.00	.000	10
d7	4.320	1.4085	10
d14	7.040	2.7415	10
d21	8.970	4.4761	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	277.729	1	277.729	31.590	.000
	Level 2 vs. Level 3	186.624	1	186.624	94.065	.000
	Level 3 vs. Level 4	73.984	1	73.984	20.095	.002
	Level 4 vs. Level 5	37.249	1	37.249	8.730	.016
Error(day)	Level 1 vs. Level 2	79.126	9	8.792		
	Level 2 vs. Level 3	17.856	9	1.984		
	Level 3 vs. Level 4	33.136	9	3.682		
	Level 4 vs. Level 5	38.401	9	4.267		

Opalescence endo

Descriptive Statistics

	Mean	Std. Deviation	N
before stained	8.7400	4.77963	10
stained	.00	.000	10
d7	3.230	2.8806	10
d14	6.210	2.7574	10
d21	8.370	2.9803	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	763.876	1	763.876	33.438	.000
	Level 2 vs. Level 3	104.329	1	104.329	12.573	.006
	Level 3 vs. Level 4	88.804	1	88.804	29.650	.000
	Level 4 vs. Level 5	46.656	1	46.656	35.694	.000
Error(day)	Level 1 vs. Level 2	205.604	9	22.845		
	Level 2 vs. Level 3	74.681	9	8.298		
	Level 3 vs. Level 4	26.956	9	2.995		
	Level 4 vs. Level 5	11.764	9	1.307		

Middle part

Sodium perborate

Descriptive Statistics

	Mean	Std. Deviation	N
Before stained	2.160	1.6595	10
Stained	.00	.000	10
d7	5.050	2.3287	10
d14	5.800	2.1071	10
d21	6.830	2.7560	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	46.656	1	46.656	16.943	.003
	Level 2 vs. Level 3	255.025	1	255.025	47.028	.000
	Level 3 vs. Level 4	5.625	1	5.625	12.155	.007
	Level 4 vs. Level 5	10.609	1	10.609	9.605	.013
Error(day)	Level 1 vs. Level 2	24.784	9	2.754		
	Level 2 vs. Level 3	48.805	9	5.423		
	Level 3 vs. Level 4	4.165	9	.463		
	Level 4 vs. Level 5	9.941	9	1.105		

Opalescence endo

Descriptive Statistics

	Mean	Std. Deviation	N
Before stained	3.570	2.5846	10
Stained	.00	.000	10
d7	2.730	1.4568	10
d14	4.060	.9823	10
d21	5.060	2.0250	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	127.449	1	127.449	19.079	.002
	Level 2 vs. Level 3	74.529	1	74.529	35.117	.000
	Level 3 vs. Level 4	17.689	1	17.689	8.994	.015
	Level 4 vs. Level 5	10.000	1	10.000	6.233	.034
Error(day)	Level 1 vs. Level 2	60.121	9	6.680		
	Level 2 vs. Level 3	19.101	9	2.122		
	Level 3 vs. Level 4	17.701	9	1.967		
	Level 4 vs. Level 5	14.440	9	1.604		

3.2 E value

Cervical part

Sodium perborate

Descriptive Statistics

	Mean	Std. Deviation	N
bfstained bf stained	6.18252	3.116797	10
stained	.00	.000	10
d7	5.30344	2.323440	10
d14	7.70779	3.214549	10
d21	9.33917	4.572245	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	382.235	1	382.235	39.347	.000
	Level 2 vs. Level 3	281.265	1	281.265	52.102	.000
	Level 3 vs. Level 4	57.809	1	57.809	35.969	.000
	Level 4 vs. Level 5	26.614	1	26.614	10.453	.010
Error(day)	Level 1 vs. Level 2	87.430	9	9.714		
	Level 2 vs. Level 3	48.585	9	5.398		
	Level 3 vs. Level 4	14.465	9	1.607		
	Level 4 vs. Level 5	22.915	9	2.546		

Opalescence endo

Descriptive Statistics

	Mean	Std. Deviation	N
bfstained bf stained	10.11953	5.186202	10
stained	.00	.000	10
d7	9.09270	4.089114	10
d14	10.69704	3.802385	10
d21	12.22440	3.819096	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	1024.050	1	1024.050	38.073	.000
	Level 2 vs. Level 3	826.772	1	826.772	49.446	.000
	Level 3 vs. Level 4	25.739	1	25.739	27.702	.001
	Level 4 vs. Level 5	23.328	1	23.328	128.905	.000
Error(day)	Level 1 vs. Level 2	242.070	9	26.897		
	Level 2 vs. Level 3	150.488	9	16.721		
	Level 3 vs. Level 4	8.362	9	.929		
	Level 4 vs. Level 5	1.629	9	.181		

Middle part

Sodium perborate

Descriptive Statistics

	Mean	Std. Deviation	N
bfstained bf stained	2.87914	1.628488	10
stained	.00	.000	10
d7	5.24268	2.317161	10
d14	5.98578	2.083903	10
d21	6.98123	2.723864	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	82.895	1	82.895	31.258	.000
	Level 2 vs. Level 3	274.857	1	274.857	51.191	.000
	Level 3 vs. Level 4	5.522	1	5.522	13.424	.005
	Level 4 vs. Level 5	9.909	1	9.909	9.338	.014
Error(day)	Level 1 vs. Level 2	23.868	9	2.652		
	Level 2 vs. Level 3	48.323	9	5.369		
	Level 3 vs. Level 4	3.702	9	.411		
	Level 4 vs. Level 5	9.551	9	1.061		

Opalescence endo

Descriptive Statistics

	Mean	Std. Deviation	N
bfstained bf stained	4.12330	2.773764	10
stained	.00	.000	10
d7	4.09373	1.442378	10
d14	5.49978	1.698818	10
d21	6.65219	2.285053	10

Tests of Within-Subjects Contrasts

Measure: MEASURE_1

Source	day	Type III Sum of Squares	df	Mean Square	F	Sig.
day	Level 1 vs. Level 2	170.016	1	170.016	22.098	.001
	Level 2 vs. Level 3	167.586	1	167.586	80.553	.000
	Level 3 vs. Level 4	19.770	1	19.770	67.937	.000
	Level 4 vs. Level 5	13.280	1	13.280	19.433	.002
Error(day)	Level 1 vs. Level 2	69.244	9	7.694		
	Level 2 vs. Level 3	18.724	9	2.080		
	Level 3 vs. Level 4	2.619	9	.291		
	Level 4 vs. Level 5	6.151	9	.683		

BIOGRAPHY

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