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# The preventive effectiveness of two dentinal tubule occlusion procedures against tooth discoloration resulted from gray MTA and triple antibiotic paste (A Comparative in vitro Study)

A Thesis
Submitted to the Council of the College of Dentistry, University of Baghdad in partial fulfillment of the requirements for the degree of Master of Science in Conservative Dentistry

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# Linguistic certification

This is to certify that the thesis entitled **"The preventive effectiveness of two dentinal tubule occlusion procedures against tooth discoloration resulted from gray MTA and triple antibiotic paste (A Comparative in vitro Study)"** was prepared under my linguistic supervision. Its language was amended to meet the style of the English language.

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# *Dedication*

*To my greatest mother and father for their love and support throughout my life*

*To the lovely little angels Adam & Ayham*

*To my supervisor for his guidance, help, encouragement and support that made this project Possible…….*

*Farah…*

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# Abstract

Tooth discoloration is still one of the biggest aesthetic problems for teeth having revascularization treatment. This discoloration affect the esthetic outcome when it occurs in the esthetic zone. This study conducted for the assessment of the effectiveness of two dentinal tubule closing procedures (dentin bonding and laser) to inhibit the discoloration resulted from triple-antibiotic paste and Gray Mineral Trioxide Aggregate.

Sixty extracted maxillary first premolars were used in this study. Access cavities were prepared and endodontic instrumentation were performed for the buccal canals. The specimens then were randomly divided into two groups (n = 30) as TAP or GMTA groups. Each of these were further divided into 3 subgroups according to surface treatment for the pulp chamber walls into: dentine bonding, Er,Cr:YSGG laser, or left without treatment as control. The shade assessment were done by the usage of VITA Easyshade Advance at baseline and after 3 weeks, and 4 months incubation periods. Color lightness (L*) and color differences (ΔE*) during different stages of the study were calculated and analyzed.

Although both bonding and laser treatment increase color lightness at the baseline measurements, it decreased with time. At 3 weeks, the bonding subgroup of GMTA illustrated the highest measured color lightness in comparison to others. After 4 months incubation period, all subgroups showed decrease in color lightness with the highest detected values within TAP subgroups.

The preventive capacity of dentin bonding and laser on discoloration produced by TAP and GMTA was time dependent. Therefore, the use of these materials still not recommended within esthetic zones.

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# List of abbreviations

| Symbols | Abbreviations |
|---|---|
| **ΔE*** | Color difference |
| **a *** | Axis a * of chroma |
| **ANOVA** | Analysis of variance |
| **b *** | Axis b * of chroma |
| **Bis-GMA** | Bisphenol A glycol dimethacrylate |
| **°C** | Degree celsius |
| **CIE** | Commession International de 1E clairage |
| **$cm^2$** | Square centimeter |
| **D3MA** | Decandiol dimethacrylite |
| **3D** | Three dimension |
| **Er,Cr:YSSG** | Erbium ,Chromium :yttrium –scandium-gallium-garnet |
| **EDTA** | Ethylenediaminetetraacetic acid |
| **GMTA** | Gray Mineral trioxide aggregate |
| **HCL** | Hydrochloric acid |
| **HEMA** | Hydroxyethanol methacrylate |
| **Hz.** | Hertz |
| **L*** | Lightness |
| **LED** | Light emitting diode |
| **LLLT** | Low level laser therapy |
| **MDP** | Methacryloyloxydecyl Dihydrogen phosphate |
| **min** | Minute |
| **mm** | Millimeter |
| **MTA** | Mineral trioxide aggregate |
| **NAOCL** | Sodium hypochloride |
| **Nd:YAG** | Neodymium adoped yttrium aluminium garnet |
| **nm** | Nanometer |
| **SD** | Standard deviation |
| **SE** | Standard error |
| **SEM** | Scanning electron microscopy |
| **TAP** | Triple antibiotic paste |

| | |
|---|---|
| **µm** | Micrometer |
| **W.** | Watt |
| **WGMTA** | White Mineral trioxide aggregate |
| **Wt.** | Weight |

# *Introduction*

# Introduction

Revascularization is one of the strategies that used for regenerative treatment of pulp-dentin complex. This treatment is a successful substitute for the apexification of immature permanent teeth with necrotic pulp (Petrino et al., 2010), to allow the continuance of physiological growth of the root (Iwaya et al., 2001). One of the most essential phase for this procedure is the disinfection of the root canals (Reynolds et al., 2009). Some research workers have advocated the usage of Triple antibiotic paste (TAP) to kill the microbial flora present in the root canal (Petrino et al., 2010, Tawfik et al., 2013, Bezgin et al., 2014), which basically composed of antibiotic derivatives of tetracycline family. This treatment could adversely be affected tooth esthetic causing severe discoloration (Kim et al., 2010, Santos et al., 2017). Since it unites to the calcium ions which present in the dentine by process of chelation and inducing dental discoloration (Kim et al., 2010).

Mineral trioxide aggregate (MTA) is one of the biocompatible repairing substance that have biologic and positive sealing effect. This material was advantageous when applied in various dental operations which include: root-end sealing, reparation of perforations, direct pulp capping procedures, apexification and regeneration of the pulp (Parirokh and Torabinejad, 2010). MTA is advertised in two structures gray (GMTA) and white (WMTA). It has been accounted for that the GMTA material may prompt tooth staining particularly when its applied over the crestal bone level through its contents of metallic ions including bismuth oxide (Parirokh and Torabinejad, 2010), aluminum oxide, magnesium oxide and iron oxide (Akbari et al., 2012, Ioannidis et al., 2013). Although WMTA has dispensed with this drawback and can be utilized in positive significant effect (Ferris et al., 2004), several clinical studies reported tooth discoloration can be resulted even with this material (Ioannidis et al.,

2013). Therefore, the application of MTA in aesthetic areas is compromised (Glickman and Koch, 2000, Karabucak et al., 2005, Bortoluzzi et al., 2007).

An effort of sealing of the dentinal tubules on the walls of the pulp chamber by using dentin bonding had been reported as a viable approach to prevent discoloration caused by GMTA (Reynolds et al., 2009).

In the last decade, dental laser such as Nd: YAG and Er,Cr:YSGG have become a popular choice for the management of dental hypersensitivity. Various theories are asserted to describe the impact of laser irradiation on dentine, which including the sealing of dentinal tubule by vaporization of the dentinal fluid, melting and re-solidification of dentine (Kimura et al., 2000, Markowitz and Pashley, 2008). This cooperation of lasers with dentin may demonstrate to have the favorable role to inhibit the staining by occluding dentinal tubules. There is only one report that recognized that dentine bonding, dentin desensitizing agent and laser can be used as preventive methods for tooth discoloration caused by TAP with short incubation time (Küçükekenci et al., 2018). However, none of the previous research identify the effect of these preventive procedures on different tooth discoloring materials and on extended period of incubation.

# Aim of the study

The aim of the study is to compare and evaluate the ability of dentin bonding agent and laser application to prevent tooth discoloration introduced by intracanal application of gray mineral trioxide aggregate and triple antibiotic paste.

# *Chapter One*

# *Review of literature*

# Chapter one

# Review of literature

## 1.1 Tooth structure

### 1.1.1 Enamel

Enamel is one of the major tissues that form the tooth structure. The formation of enamel takes place by cells called ameloblast via amelogenesis process. Enamel distributed in different thickness covering the dental coronal portion. Likewise, it is fixed to the dentin by the dentinoenamel intersection (DEJ) (Ritter, 2017).

Enamel shade range from light yellow to dark white. It likewise differs in thickness, from a limit of around 2.5mm over working surfaces to a feathering edge at the cervical line, this variety impacts the shade of enamel due to the basic yellow dentin is seen through the more translucent zones (Ten Cate and Nanci, 2013).

Enamel have a high substance of mineral salts (Fully formulated enamel comprises of nearly 96% mineral, 4% organic constituents in addition to water; the composition of the inorganic substance related to the enamel is calcium phosphate crystals which called hydroxyapatite and their crystalline organization gives the enamel the property of the hardest calcified tissue in human being body (Ten Cate and Nanci, 2013).

Enamel forming a very hard dental cover, which gives ability to resist the forces during mastication action. Although the enamel is hard, but its brittle, for this reason the enamel breaks easily when losing its underlying basic dentin. Permeability is another property of the enamel by which it can allow the connection between the tooth and the external oral environment, this connection

takes place by the passage of the molecules partially or completely (Ten Cate and Nanci, 2013, Kumar, 2014).

The interlacing arrangements of enamel rods gives it the ability to resist the high forces during food grinding. The deposition of the rods in a keyhole fashion carried out by the ameloblaste (Avery and Chiego, 2006). Ameloblasts keep in close relation during its travelling in various orientation and form the adjacent rods. The fashion of rods via the incidental light observation become apparent as bright and black strings of rod groupings called Hwtter-Schregerbands, because of the bending of the rods in an over twisting manner at the tip of the cusp. At that focuses these structures called gnarled enamel (Avery and Chiego, 2006).

## 1.1.2 Dentin

Dentin is considered as the major constituent of the teeth. Dentin acts as a cushion base for the brittle enamel to withstand the applied force during function. It also provide protection to the pulp from bacterial and other injurious irritation (Tjäderhane, 2019).

Dentin composed of Mineral phase70% in wt. and 40–45% by vol., Organic matrix 20% by wt. and 30% vol.. As well as the water compose 10% in wt. and 20–25% in vol. (Goldberg et al., 2011). The organic matrix composed about 90% of collagen type1 in addition to 10% of ground substances, that including specific type of proteins (Hillson, 2005). Dentin considered as a vital tissue, dentin act as a barrier between the oral cavity and the tissue of the pulp but, it share in the preservation of the condition of dental tissues which called as dentine-pulp complex (Tjäderhane, 2019).

Every tooth consists of thousands of microscopical dentinal tubules which are tubular in shape that emanate outside the pulp. The dentinal tubule diameter differ according to their location, their diameter is 2.5 μm close to the

dental pulp, 1.2 μm when located in the middle dentin, as well as 0.9 μm at DEJ. That mean the dentinal tubule diameter increase as they become closer to the pulp (Ten Cate, 1998). These tubules are connected to the pulp by plasma-like liquid (Chu et al., 2011). The tubule density in the dentine of the cusp was not identical to the dentine close to the central fissure as well as there are Three sorts of side channels, major, fine and microbranches, were recognized on the base of estimating, direction and position (Mjör and Nordahl, 1996).

The major one 0.5–1.0 μm diameter, were persist peripherally as a common delta branching. The fine kind, 300–700 nm diameter, radiate at 45 degree and were abounding in regions such as in the root region in which the tubules count were comparatively less, as well as the diameter of the microbranches range from 25 to 200 nm, branched in about 90 degree angles in all dentinal portions (Mjör and Nordahl, 1996).

The odontoblastic process were tubular in shape and nearly totally occupied by the dentinal tubules that present in predentin-dentin region and extend for about 0.2 mm outward the dental pulp. Within the predentine-dentine zone, these odontoblastic processes were enclosed by a fine sheet like structure (Brännström and Garberoglio, 1972). Additionally the tubule wall seemed to consist of a lining of an shapeless material consist of a little at the boundary, and in regions also longitudinal manner, ordered fibers (Brännström and Garberoglio, 1972).

### 1.1.3 Pulp

The pulp of human teeth is referred as a specialized tissue of mesenchymal origin, distinguished with the aid of the existence of odontoblastic cells and by the presence of the surrounding borders of a hard mineralized tissue (Nor, 2006). The pulp is occupy a mesh of blood vessels and nerve bundles entering the tooth from the apical opening (Nakashima and Akamine, 2005).

Harm of the pulp by high force application, irritants, heat and bacterial cause stimulate different sorts of inflammation including complex vascular, lymphatic and nearby tissue response (Bjorndal and Mjor, 2001). The chance of dental pulp reformation is controlled by a number of considerations (Smith et al., 2008). Because of the design of pulp chamber, the pulp has very little corroboratory blood distribution, compromise the function of the immunity to resist the bacterial attacks (Huang, 2009). By the way, the odontoblastic cells have little no potential to multiply because they are a post-mitotic cells (Arana-Chavez and Massa, 2004).

The dental repairing capability is revealed when caries on the external surface activate odontoblasts to increase its secretory activity (Smith et al., 2008). Dentin present as a tubular system that keep it in an secure connection with the tissue of the pulp by means of the processes of the odontoblasts (Smith et al., 2008). Nevertheless, when teeth undergoes harming effects, like sudden application of high force, very exaggerated preparation for the formation of the cavity or very large carious defect (Mjor, 2009), the odontoblastic cells can succumb and the dental pulp may undergo irreversible pulpitis which lead to necrosis (Smith et al., 2008). Ordinarily, in such conditions the conventional root canal treatment is needed (Huang, 2009).

#### 1.1.3.1 Revascularization:

The Traditional treatment of pulp necrosis of premature dentition were considered as a problem in root canal treatment. This problem is not faced with the adults and this may be related to the root open apex and thin walls of dentin of premature dentition. According to the pulpal tissue condition, the management options for such premature dentition are decided. When pulpal diagnosis was necrotic, the conventional management step for premature dentition is apexification by the usage of calcium hydroxide (Rafter, 2005). The major drawback of this option is that the treatment receiver complaisance is

inescapable because of the need for a number of visits with requirement for long time to complete the management. There is another management option that lacking the previous disadvantages is the procedure of apexification by using MTA for sealing the canal apically (Roberts et al., 2008). In spite of that, both of the mentioned options not permit the continuance of the root development and this resulting in fragile roots with thin walls. In irreversible pulpitis condition, the pain still even the external stimulus, is eliminated (Asgary and Ahmadyar, 2013).

There is other treatment option has been recommended which called regenerative endodontics (RE) as a treatment option for dental management of premature teeth undergo irreversible pulpitis with not closed apex (Kim et al., 2018). The most important step during RE is the procedure of revascularization, it occurs by the induction of bleeding and followed by the formation of blood clot to providing scaffold for stem cells travelling, attachment, multiplication and specialization to produce a vital constituents of dentine-pulp complex (Chen et al., 2015). In proportion to the technique of RE, the revascularization procedure has been revealed to be successful option for pulpal regenerative treatment of the premature dentition undergo necrosis of the pulp in addition to inductance of the root development (Ding et al., 2009, Wigler et al., 2013, Chen et al., 2015).

In irreversible pulpitis condition, the completion of the development of the root has been showed in the premature dentition via vital pulp management (Peng et al., 2015, Sabbagh et al., 2016). During the procedure of revascularization, the dentist utilize the method of engineering of the tissue for the reformation of pulpal tissue and root apex maturation. The previously mentioned scaffold, which is a biologically degradable acts as a temporarily as a matrix for proliferating cells attachment, proliferation, and differentiation to be functional mature cells which can reform pulp and dentin (Itoh et al., 2018, Ataie et al., 2019). The stem cells of mesenchymal origin that migrating from

various regions such as periodontal ligament, apical papilla, in addition to the bone marrow region, have a very important role in the regeneration of dentine-pulp complex (Jung et al., 2019). The stem cell ability to differentiate to be odontoblast-like cell were showed by number of studies previously in vivo and in vitro conditions (Huang et al., 2017, Liu et al., 2018, Zhu et al., 2018).

There were many types of endodontic medicaments which used in the procedures of revascularization comprise $Ca(OH)_2$, formocresol and the creamy mixtures of 3mix of antibiotic medicaments (Moreno-Hidalgo et al., 2014). TAP has been made by mixing 3 antibiotics (tetracycline, metronidazole, ciprofloxacine) and proposed as one intracanal medication for the disinfection of root canal and reformation treatment options (Hoshino et al., 1996, Iwaya et al., 2001, Banchs and Trope, 2004, Jung et al., 2008, Ding et al., 2009, Petrino et al., 2010).

The formation of TAP is by means of admixture of equivalent amounts of 3 antibacterial medicaments in powdery formula with normal saline or distilled water. The main negative effect of TAP is the dental staining in the coronal part particularly leading to an unacceptable appearance of dentition (Kim et al., 2010, Dabbagh et al., 2012, Lenherr et al., 2012). Discoloration induced by TAP due to its binding to the calcium ions of dentin matrix by process of chelation and result in a variation of the dental color (Kim et al., 2010).

## 1.1.4 Origin of normal color of human teeth

The human tooth shade is a result of multiple optical properties of enamel and underlying dentin. When light directed on a tooth surface, 4 phenomena combined with tooth and light interactions which including (Jahangiri et al., 2002): (I) light transmission via the tooth, (II) outward reflection, (III) diffusion of the exterior light reflection and (IV) absorbing and scattering of the light within the dental structure. Teeth shade has been explained as a result of the

scattering of light, i.e. illumination by light following high unsteady path through the teeth earlier to its radiation at the incidence the exterior layer and attains the eyes of the observer (O'Brien et al., 1985, Jahangiri et al., 2002).

Dental colors are consist of combination of effects which includes internal and external colorations (Watts and Addy, 2001). Internal teeth color is combined with light scatter and light absorbing abilities of both enamel in addition to the underlying dentin (ten Bosch and Coops, 1995).

External colors are connected to the adsorption of materials for example: tea, coffee, chlorhexidine onto the exterior layer of the enamel, and specially the covering with pellicle, that essentially result external stain(Joiner et al., 1995). Other color causing factors such as staining due to tetracycline, fluorosis related discoloration and endodontic treatment also affect tooth color (Heymann and Ritter, 2019).

## 1.2 Tooth discoloration

Dental discoloration consist of many types which include internal discoloration which takes place as a result of the compositional changes and thickness variation of dental hard tissue, as well as many of metabolic disorders and systemic factors have a role and affect the development of human teeth and causing discoloration as an end result, in addition to the local causes such as injury are also lead to color changes (Watts and Addy, 2001). Examples of internal discolorations include: Alkaptonuria, Congenital erythropoietic porphyria and congenital hyperbilirubinemia, Amelogenesis imperfecta and dentinogenesis imperfect, discoloration due to tetracycline, hypoplasia of enamel, fluorosis., contamination by the hemorrhage product of the pulp, resorption of roots and color change that associated with the increase of age.

Other type of dental color change referred as the external discoloration which is exterior to the dental structure and located on the dental external region or in the

dental plague. Different factors that causing such type of discoloration which including: Metallic (i.e. amalgam), Non-metallic (i.e. chlorehexidine, tobacco.. etc.) and colored diet and beverages (Watts and Addy, 2001, Barber and King, 2014).

Additionally there are a discoloration phenom which is referred as internalized discoloration, this type of discoloration is resulted from the fusion of extraneous stain inside the tooth substance following dental maturation. It happens in the anomalies of enamel and in the permeable surface of uncovered dentin (Watts and Addy, 2001).

### 1.2.1 Internal discoloration related to endodontic problem

Dental internal discoloration perhaps resulted from dental injury, dental necrosis, managements of teeth endodontically, and restoration work beside the factors that produce effects locally systematically (Ten Bosch and Coops, 1995, Wray and Welbury, 2001, Torabinejad and Walton, 2002).

Discoloration of crown after treating it endodontically is respected as an ordinary aesthetic obstacle bothering both the treatment preserver and the endodontist, especially in the dentition within the esthetic zone (Cohen et al., 1998). The primary reasons that resulting in the internal coronal color change are caused by managements of root canals including: necrotic products of the pulp and disintegration, blood in the pulp chamber, and intracanal medications and filling substances (Van der Burgt et al., 1986, Cohen et al., 1998, Torabinejad and Walton, 2002).

Discoloration of tooth due to endodontic substances is respected as a usual outcome and negatively affect the dental appearance that endodontically managed (Van der Burgt et al., 1986). The gradual ingressing of the material in to the dentinal tubules result in progressive discoloration (Van der Burgt et al., 1986). However, visible crown color change may not related to the tubule

penetration only, but it could be due to the material remnants that persist in the pulp chamber, which increase in darkness with time and transmitted via the dental tissue layers (Davis et al., 2002). The previous studies showed that the discoloration that induced by endodontic material depending on the location of the material applied, wither it applied in the pulp chamber or limited to specific area below the CEJ, as well as the immature teeth exhibit more discoloration in comparison to the mature teeth after the application of the endodontic material, that may be related to the difference in the anatomy of the dentinal tubules which resulting in increase of the rate of the diffusion through these tubules (Kim et al., 2000).

Nearly all the studies that aim to evaluate the staining ability of endodontic sealers concluded that they result in teeth discoloration. Lab researchs of AH26, Kerr Pulp Canal Sealer, Roth 801, Sealapex, Endofill, Tubliseal, zinc oxide eugenol, Apatite Root Canal Sealer, Cavizol, AH Plus and EndoREZ (Parsons et al., 2001, Davis et al., 2002, Partovi et al., 2006, Elkhazin, 2011). Assessment of the possibility of discoloration caused by Ledermix (Kim et al., 2000) and by (Day et al., 2011). According to these 3 studies, the pastes of calcium hydroxide has been applied as standards. It has been revealed that, in comparison with Ledermix, calcium hydroxide had been resulted in only slight but noticeable dental discoloration.

**1.2.1.1 Mineral trioxide aggregate:**

MTA formulated as a calcium silicate-based cement, it is formulated by bismuth oxide and Portland cement (Torabinejad et al., 1995). Bismuth oxide which present as a radiopacifier within the material composition, has been referred to be the main cause of discoloration occurred by MTA (Vallés et al., 2013). As well as increased amounts of aluminum oxide, magnesium oxide and iron oxide in GMTA, result in increased possibility of color changes (Akbari et al., 2012, Ioannidis et al., 2013).

MTA applications include repairing of root perforations, apical surgeries, direct pulp capping and apexifications (Torabinejad et al., 1995, Bortoluzzi et al., 2007). These operations require MTA implementation in immediate associate with the dental treated region. After the anterior dentition are involved, the MTA color keeping ability is an essential point to be taken in to considerateness. Lately, dental color changes has been revealed with MTA implementation in direct relation to the dental treated region (Jacobovitz and De Pontes Lima, 2009, Felman and Parashos, 2013). The first marketed MTA formulation was grey. MTA grey color was concerned with obvious discoloration of teeth, thus being not to be applied in the anterior areas (Bortoluzzi et al., 2007). WMTA formula was developed to resolve the dental discoloration problem (Primus, 2011). However, white MTA has been reported to develop greyish discoloration in both laboratory and clinical studies (Boutsioukis et al., 2008, Jacobovitz and De Pontes Lima, 2009, Felman and Parashos, 2013, Ioannidis et al., 2013). A noticeable discoloration was shown with the application of MTA in vital pulp management (Belobrov and Parashos, 2011) and to internal resorptions repairing (Jacobovitz and De Pontes Lima, 2009).

Dental managements of pulpal necrosis and teeth with not closed apex were takes place by the usage of Ca $(OH)_2$ paste or MTA to act as a closing bar apically which is known as apexification procedure. Apexification procedure achieve disinfection beside filling of the root canal, but it not permit further root development (Raldi et al., 2009).

**1.2.1.2 Triple antibiotic paste**

According to Chuensombat et al. (2013) who showed that the efficacy and cytotoxicity of TAP against bacteria in vitro condition, it shows when using one type of antibiotic is with less cytotoxicity than the usage of a multiple antibiotic types. There is no antibiotic type that have a wide spectrum to be effective against all bacterial types that present in the canals and apical parts; a

mixture of antibiotics should be used to provide the highest range effect. These pastes of antibiotics should be applied in suitable dose for balancing between the cytotoxicity for the stem cells and highest bacterial killing. According to the previous studies it shown that the concentration TAP of 39 μg/mL were the best for application in disinfection root canal(Chuensombat et al., 2013) .

Hoshino (1998) reported that the application of a mixture of 3 pastes of 3 antibiotic types into the canals with a conc. of 20 μg/mL reduce the present bacterial colonies number for about 99% (Hoshino, 1998). By another study reported by Hoshino et al. conclude that every antibiotic applied in a single manner is not active against the bacterial colonies that present in the pulp, dentinal tubules, and apical abscess, while the mixture of 3 antibiotic types sterilize the canals completely from the germs (Hoshino et al., 1996, Hoshino, 1998). Sato et al. (1996) produce TAP for the coverage of the various types of germs, the 3 antibiotics including minocycline ($G^+$ and $G^-$), ciprofloxacin ($G^+$ and $G^-$), and metronidazole (anaerobic bacteria and protozoa) (Chuensombat et al., 2013). But these types of antibiotics have adverse effect on the stem cells, since the acidity of the pH of minocycline is not suitable to the stem cells survival; because it increase the permeability of the cell for the antibiotics, which resulting in stem cell cytotoxicity for long term. Ciprofloxacin ph is also acidic. While metronidazole pH is neutral and it is not cytotoxic for the cells (Chuensombat et al., 2013).

Minocycline is one of tetracycline derivatives producing the same action. It replaced by cefaclor to eliminate it is discoloration effect (Thibodeau and Trope, 2007) because of the chelation of minocycline with $Ca^{2+}$ and produce insoluble complex (Tanase et al., 1998). Cefaclor having less activity against enterococci type of bacteria. Tetracycline have the ability of inhibition of collagenase and metalloproteinases; tetracycline have no toxicity and have the ability to increase interleukin-10 level (anti-inflammatory cytokine). The replacement of minocycline instead of cefaclor due to discoloration side effect,

will deprive of the positive benefits of tetracycline derivatives, for this reason we should choose the option to seal the dentinal tubules to prevent this coronal discoloration (Reynolds et al., 2009).Ciprofloxacin and Metronidazole could stimulate the fibroblasts formation(Reynolds et al., 2009).Bose et al. (2009) reported that TAP usage resulting in the increasing the canal wall thickness in highest percentage in comparison to formocresol and calcium dihydroxide (Bose et al., 2009).

## 1.2.2. Methods of treatment and prevention of internal discoloration

Insufficient removal of endodontic sealer remnants on the pulpal chamber is one of the major leading causes for dental discoloration (van der Burgt et al., 1986).

The coronal discoloration risk caused by the residue of endodontic sealer can be prevented either by its removal completely or by dentinal tubules closing procedure to prevent the discoloring agents from penetration, therefore different strategies have been used to eliminate the remnants of sealers from the pulp chamber.

Many types of solvents were used for the removal of the gutta percha such as chloroform, eucalyptol, xylol, or halothane. Chloroform have been suggested by many studies as a very effective solvent for most endodontic filling substances (Tamse et al., 1986, Görduysus et al., 1997, Whitworth and Boursin, 2000, Schäfer et al., 2002, Schuur et al., 2004). It has been revealed to has an excellent effectiveness of dissolution in comparison to the other dissolving agents such as eucalyptol, xylol, or halothane (Görduysus et al., 1997, Schäfer et al., 2002, Whitworth and Boursin, 2000). However, the sealer resistance to the dissolution and debridement completely may differ considerably (Friedman et al., 1992, Moshonov et al., 1994, Wilcox, 1995) .

The clinical efficacy of any solvent has been determined by the physicochemical characteristics endodontic sealers which influence the dissolution ability of the solvent (Moshonov et al., 1994, Erdemir et al., 2003).

In usual endodontic operation, root canal sealer residue can be cleaned from the walls of the pulp chamber walls by rinsing with sterile water and make dryness with dry little piece of cotton. However, total debridement of the pulp chamber from the residue of endodontic sealers is often difficult in clinical practice (Parsons et al., 2001). However, most of the available endodontic sealer have low solubility in water or other solvents such as alcohol which make their cleaning by these methods inefficient procedure (Schäfer and Zandbiglari, 2003). Additionally, the structure of the dentin with wider diameters of the dentinal tubules toward the pulp make this cleaning more complicated (Boutsioukis et al., 2008).

Different methods also suggested to be implemented as prophylactic option prior to applying any endodontic treatment materials which cause tooth discoloration. These methods depend on the dentinal tubule occluding affect that prevent engagement of the endodontic materials within the dentine surface and entrance to dentinal tubules (Küçükekenci et al., 2019). Among these methods the usage sodium fluoride, potassium oxalate, calcium phosphate, which interfere with hypermineralisation of the dentinal tubules to decrease opened lumen diameter (Scherman and Jacobsen, 1992, Tay et al., 2003, Duran and Sengun, 2004, CHOW, 2009).

Shokouhinejad et al. (2018) showed that the application of dentin bonding agent to the walls of the pulp chamber to seal the dentinal tubules before the application of TAP for revascularization procedure can reduce tooth discoloration effectively but not completely.

Other materials and methods such as varnishes, cements, and lasers that can be used to close the dentinal tubules to prevent the protrusion of the

discoloring materials especially within the pulp chamber walls (Miglani et al., 2010).

**1.2.2.1 Dentin Bonding**

The classical conception of conservative department were disagreed at 1980s and 1990s due to the production of advanced adhesion methods, foremost with enamel and later on with dentin. Despite that, sticking with dentin surface stay complicated. Adherent agents can be cooperated with dentin in multiple ways: mechanical adhesion, chemical adhesion, or by the 2 ways (Asmussen and Munksgaard, 1988, Van Meerbeek et al., 1998, Nishitani et al., 2006). Adhering agents are mixture containing monomer of resins that aid in the resin dental material interactions (Perdigao, 2007).

Adhering liquid is formulated by monomers including groups with hydrophilic properties and groups of hydrophobic properties. Prior constituent produce moisture to hard tissues of dentition, whilst the later permits incorporation with the adhered substances (Van Landuyt et al., 2007). Chemical composition of the adhering liquids additionally contain curing initiators, stabilizers or inhibitors, solving constituents and, in number of instances, inorganic fillers (Van Landuyt et al., 2007).

An advanced type which is a modern and new substance, in conservative dentistry, was universal adhesives production, that were in usage from 2011 in office dental procedures. That recent adhering liquids are referred as (multi-purpose) adhering liquid and this name related to its usage mode like self-etch (SE) adhering liquid, etch-and-rinse (ER) adhering liquid, or used as SE adhering technique with dentin, as well as ER adhering technique with enamel (this adhering method is usually referred as selective etching of enamel) (Hanabusa et al., 2012, Perdigao et al., 2012) .

Universal adhering liquids were produced to allow of the usage of that adhering agent accompanied by etching with phosphoric acid in the etching totally or selectively to increase durability of adhesion to the surface of enamel,

that has been agreed by revealing positive outcomes in vitro researches (Munoz et al., 2015) as well as *in vivo* studies (Erickson et al., 2009, Peumans et al., 2010).

In spite of resemblance among the adhering agents, the chemical constituents that contained in the universal adhering liquid vary from SE adhering system because of monomers internalization that have the ability of achievement of adhesion chemically and micromechanically to the tooth surface (Hanabusa et al., 2012, Perdigao et al., 2012). Its chemical composition is a critical aspect to be taken consideration, for the reason that most of these adhering liquids consist of particular monomers of carboxylate and/or phosphate that linked in ionic manner with calcium that persist in the crystals of hydroxyapatite (Ca10[PO4]6[OH]2) (Fukegawa et al., 2006, Yoshida et al., 2012), and this could affect its bonding affectivity (Muñoz et al., 2013). A good example is MDP (Methacryloyloxydecyl Dihydrogen phosphate) is an effective monomer that contained in the modern adhering agents, but not contained in the elderly produced adhering liquids. This monomer is hydrophilic in nature with low-etch properties. MDP a monomer that contained in the universal adhering liquids and give the property of the type and make it applicable with the various etching methods. MDP-calcium salts are proceed throughout the chemical interaction and resulted in the deposition of self-assembled nano-layers by various degrees and properties based on the type of adhering liquid in usage (Yoshida et al., 2012, Yoshihara et al., 2014). Additionally, it produce the effect of strong adherences with the surface of the tooth due to the creation of non-soluble $Ca^{+2}$ salts. Moreover, it contain of biphenyl dimethacrylate (BPDM), dipentaerythritol pentaacrylate phosphoric acid ester (PEN-TA)(Yoshihara et al., 2014) and polymers of polyalkenoic acid that may improve adhering properties to surfaces of dentition .

The universal adhering agent matrix is established on containing of 2 types of monomers which have 2 properties including hydrophilic

(hydroxyethul methacrylate /HEMA) and hydrophobic (decandiol dimethacrylite /D3MA) and transitional characterstics (bis-GMA). These variable properties related to universal adhesives permit it to form a fixed connecting bar that passing the presented space that separating the dental hydrophilic surface and resin hydrophobic material.

**1.2.2.2 Laser**

There are numerous laser kinds were applied for multiple dental treatments, which consist of 2 laser sorts including hard lasers along with soft cold lasers. The types of lasers that included under the term of hard lasers including Carbon dioxide laser (CO2), Nd: YAG laser in addition to Er: YAG laser. The mentioned laser sorts were applicable for the treatment of both hard tissues and soft tissue conditions, but these sorts produce a risk of pulp harming in addition to the increased price. whilst, Soft lasers were established by a semiconductor diode aids which transport laser energy by non-thermal means termed as biostimulation or low level laser therapy (LLLT) (Verma et al., 2012). Lasers implications in dental treatments of hard and soft tissues. Laser type which called Erbium. Chromium: Yttrium-scandiumgallium-garnet (Waterlase®) (©BIOLASE, Inc., USA) had been defined as one of laser sorts that used for dental treatments and used in this study.

The photons that emitted by Er,Cr:YSGG sort of laser have a wavelength about 2.78mm, this wavelength is within the major band of $H_2O$ absorption. Energy absorption by water is result in to cause quick vaporization with micro-explosions in the treated dense tissues, therefore result in ablation of dentition in addition of bones (Rizoiu et al., 1998, Cobb, 2006). Additionally an effect in a thermo-mechanical manner were produced by the ablation process (Visuri et al., 1996, Rizoiu et al., 1998, Yamazaki et al., 2001).

Lately Er,Cr:YSGG sort of laser was recommended for the ablation procedure of soft and hard tissue (Cobb, 2006, Botta et al., 2009). The elevated Er,Cr:YSGG absorption in an emission about (2,78 μm) can be highly absorbed

in water. Thus dentinal fluid vaporizes from the exposed tubules leaving the insoluble salts behind. Hence, it had been asserted that this process of deposition is the leading factor for the dentinal tubule closing (Addy, 2000).

Er.Cr:YSSG laser kind is pain free and laser management was applicable with no need anesthetize the area to be managed. Besides that, this sort of laser is suitable, harmless and accurate (Verma et al., 2012, Asnaashari and Moeini, 2013). Indication of the application of Er.Cr:YSGG (Waterlase®) were numerous including in hard tissues management such as its usage in the debridement of caries, enameloplasty procedures, etching of the surface of hard tissues. Additionally Er.Cr:YSGG laser managed for the liquefaction of peritubular dentin, and lead to dentinal tubule blockage in a partial manner or in whole which achieve the reduction of patient's hypersensitivity symptoms (Gholami et al., 2011) .Whilst with in soft tissue type, its applicable in operations established for pulpal managements, 2 types of biopsies which include excisional and incisional kinds, surgical exposure of unerupted dentition, frenectomy procedures, gingival and periodontal procedures (Verma et al., 2012).

Lasers application in dental managements produce a positive results, but in some dental applications such as endodontic or periodontics managements, it is necessary to control the forward emitted radiation, which elevate the possibility of perforations occurrence or transportation danger when placing the tip of the fiber near to the apical region (Jahan et al., 2006). Additionally the forward emitting radiation result in elevated risk of damage due to the very high temperature in the tissues that located in the apical region.

Er,Cr:YSGG laser have the ability to deliver waves of shock within the fluid at a velocity of about 100 km/hr. (Blanken and Verdaasdonk, 2007), When utilizing fibers with plain ends, a production of shock waves that move in a forward orientation will be produced and cause extrusion of fluid with remnants beyond the root apex (George and Walsh, 2008).

The available tips of laser are not effective in lateral production of energy (Anić et al., 1998, Altundasar et al., 2006). Therefore, a modified design of fiber tip which produce even distribution of energy along the canal and also prevent the risk of apical extrusions. Recently Side Firing Tip were produced which is a modified type of laser tips that produce the laser beam laterally with diminished output of laser energy in the straightforward orientation in about half of percentage (George and Walsh, 2011). The Side Firing Tip is a premium sapphire tip which is a reusable tip with a diameter about 800µm and length: 18mm and consist of a directional handle that direct the dentist to orient the energy laterally opposite to the facing of the handle (Waterlase®) (©BIOLASE, Inc., USA).

## 1.3 Color measurement

### 1.3.1 Color phenomenon

Color defined as an entangled psychobiophysical wonder that came about because of light provocation of eyesight. Thus, shade perception related to the properties of light which entering the human eye and not the properties of the viewed things (Volpato et al., 2010). For color perception, light reflection occur by the viewed thing and this light reflection effect will make stimulation for the sensors that presents in the eye retina by which a signal will be sent for the vision part in the brain cortex (Sikri, 2010). Color perception is the responsibility of the light which is considered as the main element of color viewing phenomenon. Composition of light is consisting of waves that electromagnetic in type with variable wavelength. Visible light is the light portion that has been viewed with the eyes of human ,which lie within the range of wavelength ( 400nm - 700nm), beside that the entire viewed colors were lie within this range confines (Sproull, 2001) .

## 1.3.2. Colorimetric determinants or three dimensions of color

Color illustration is routinely accomplished with the usage of color Munsell's 3 dimension which including: hue, chroma and value (see figure1-1). These dimensions come to be familiar internationally, for this reason it is essential to understanding them at the time of doing analysis of color (Volpato et al., 2010) .

(I) Hue: is referred as the term of shade which include (red, yellow, blue), as well as hue is firstly attributed for color recognition. It represents the wavelength that undergo reflection from the viewed thing (Volpato et al., 2010). It is the parameter that utilized for distinguishing between the color families. In Vita Classic shade guide the hue is represented by means of A, B, C and D (Sikri, 2010) .

(II) Chroma: referred as the color intensity or saturation. Chroma illustrates the color purity. By increasing chroma the viewed object appears darker, as well as when there is decreasing of chroma the viewed item shade become brighter (Volpato et al., 2010) .

(III) Value (brightness): is defined as the intensity of light too, which represents the light quantum that undergo reflection from the viewed item. Value description on Munsell is within a scale of white tob lack gray shades, as the color that observed clearly having elevated value and diminished quantum of grayish color and darker shades having reduced value and high level grayish color. There is an opposite relationship between value and chroma, since when there is elevated value level, this will be associated with decrease of chroma level and vice versa (Boksman, 2007, Sikri, 2010).

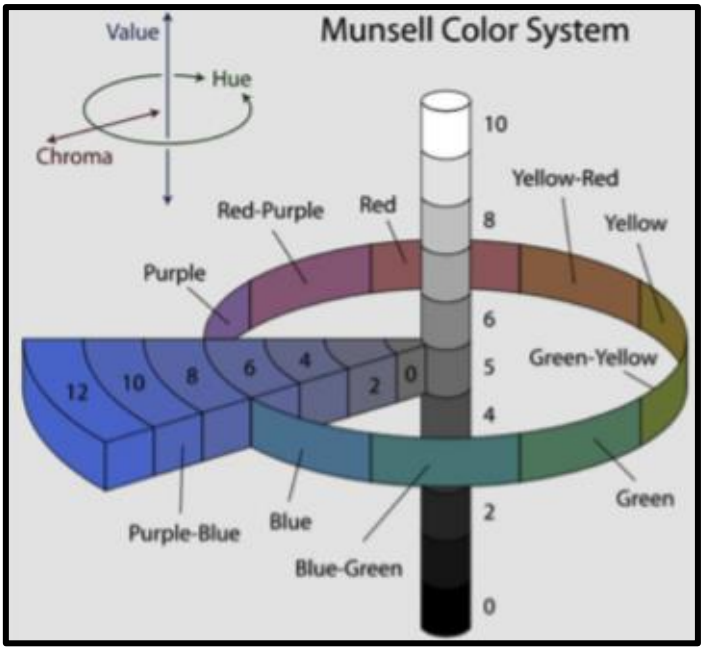


**Figure (1-1): Munsell's color system (Rus, 2007) .**

## 1.3.3 CIE Lab system

Recognizing the variation of shades by the usage of CIE L*a*b* system which illustrated by the Commission International de l'Eclairage (CIE), the L*a*b* color system was designed after a theory of shade opposition in which revealed that 2 shades are not to be red and green simultaneously or yellow and blue simultaneously. As illustrated in figure (1-2), L* refers to lightness, a* is illustrating red/green shade parameters, as well as b* is referring to yellow/blue shade. Difference for L* ( L2-L1=ΔL*), a* (a2-a1=Δa*) and b* (b2-b1=Δb*) could be with (+) positive mark or (-) negative mark, while the total shade variation (ΔE*) is only associated with (+) positive mark depending on the following equation(Yuan et al., 2007) :

$\Delta E = [(L1 - L0)^2 + (a1 - a0)^2 + (b1 - b0)2]^{1/2}$.

L* demonstrate the lightness and darkness (100= white, 0= black), a* is referring to red and green shade (+ = red, - = green ), b* is referring to shade and blue color (+ =yellow, - = blue) (Sikri, 2010). In the review that related to Akbari et al. (2012), dental shade variation was clarified by the term of CIE

total shade variation (ΔE*). The term of ΔE* representing the quantum of color changing, but this item does not clarify the position of color variation within the CIE L* a* b* system. Evaluation of CIE L* a* b* chromatic coordinates which permits quantifying the variations that occur in the shade which were expressed in terms of the visual human perceptions (Ioannidis et al., 2013).

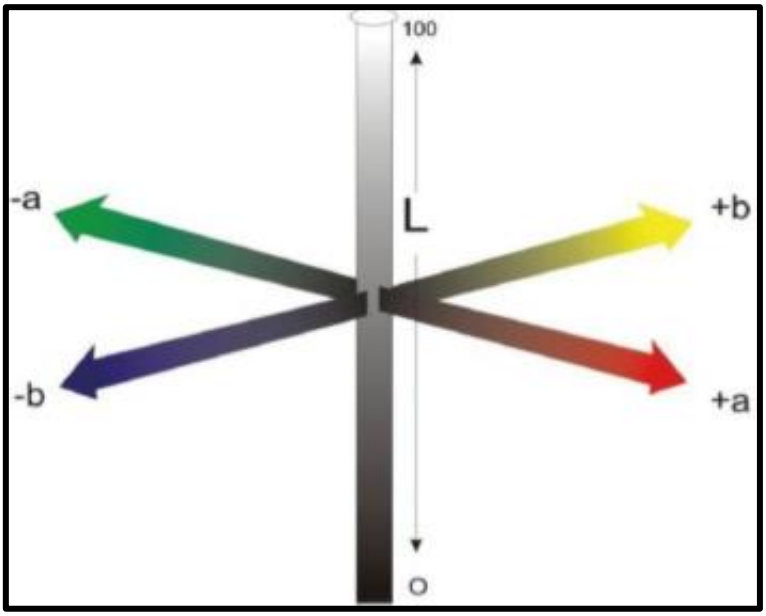


**Figure (1-2): CIE L* a* b* system (Sikri, 2010)**

## 1.3.4 Methods of shade selection in dentistry

There are 2 methodologies for the determination of dental shade and divided in to 2 kinds depending on the method of determination that including visual determination of the shade (such as shade guides) or an instrumental determination of shade for example shade determination by spectrophotometers and colorimeters devices (Volpato et al., 2010).

### 1.3.4.1 Shade guides

Determination of the color of dentition is ordinarily established during routine dental work, however it is a subjective method of color detection. There are many types of shade guide that introduced for shade selection such as

(Vitapan classical, chromoscop, VITA 3D master shade guide). Shade selection using these guides require many principles which include (Sikri, 2010):

(I) During shade selection the patient should be sitting at the same level of the dentist eyes. This helps to view patient's teeth by the portion of the retina which is very reactive region in the eye of the dentist.

(II) Different lighting conditions should be used during shade comparison. This will be done by the dentition examination under natural daylight after the determination with the presence of fluorescent and incandescent lightening types.

(III) Cleaning of dentition should be bone before color matching.

(IV) Determination of dentition shade should be done at the starting of the visit.

(V) Clothes that have bright color should be covered and removal of any lipstick should be done, if it is presented .

(VI) The selection of colors should be done in quick manner, with attention should be paid that samples of shades were situated immediately beneath the lip adjacent to the dentition surface being examined.

(VII) Resting for the eyes of the operator necessarily done by making focus on a surface that gray-blue in color directly before the color determination procedure, because this lead for balancing of the whole retina sensors by the way making the dentist eye very reactive to the yellowish shade of the dentition.

**1.3.4.2 Devices of Shade determination**

The introduction of these measuring devices were for the reduction of the troubles and wrong color selection for the restorative work. Many sorts of determination devices are designed, as well as these devices were classified in to spectrophotometers and colorimeters (Corciolani, 2009).

(I) Colorimeters: this device is comparatively simple device with less price. By this device the color measurement were takes place depending on the 3 axis of CIE L*a*b* diagram by the usage of filters which have a resemblance for the eye of human (Sakai, 2012).

The measurement of the reflected light from an item surface is done by the colorimeters usage following its traveling throughout red, green and blue filtering layers (Zenthöfer et al., 2014). Colorimeter considered as the second class when compared to spectrophotometer in manner of precision of measurement, this due to the absence of the registration ability for the reflection of spectrum by wavelengths. Colorimeters types which were marketed include: ShadeVision (X-Rite, Grandville, MI), Shade Eye (Shofu Dental, Menlo Park, CA), and ShadeScan (Cynovad, Montreal, Canada) (Corciolani, 2009).

A few detriments of colorimeters have been delineated, colometers shade measurement can be done only on the flattened aspects. Teeth are regularly not flattened and can have surface abnormalities. teeth are translucent which can prompt light disappearance at the edge of the dental surface that being estimated and this ending with inaccurate shading esteems, and poor correlation between measuring aids (Joiner and Luo, 2017).

(II) Spectrophotometers: Spectrophotometers are comparatively with great complex design than designs of colorimeters and spectrophotometers were fabricated for the determination of shades that lie within the confines of the scale that ranging from 350 nmg to 800 nm, in addition to that it is used for calculation of the factors of reflection and transmission of an item for the total curve of spectrum (Sakai, 2012). This spectrophotometer is consist of one photodiode detector, optical radiation generator and monochromator for the light conversion into signals for analysis. Assessments which acquired from the determination by the usage of spectrophotometer device are reformed to be comparable to the shade guide for comparing of colors (Apratim et al., 2015). Spectrophotometer has the ability to determine the essential constituents of a spectrum sequence, and this property is considered as the main positive property of these devices. Additionally it’s have the ability of data translation in to variants of systems for shade determination (Vichi et al., 2011).

Spectrophotometer is a very accurate device, but its usage initially was restricted for research purposes, because of its bulkiness and design complexity and the need for hard and precise manipulation with an increased price. Late electronics progress is lead to the evolution of a spectrophotometer which is used in a simple manner in the dental clinic. Spectroshade micro (MHT, Niederhasli, Switzerland) and VITA Easyshade Advance (VITA Zahnfabrik, Bad Sackingen, Germany) are examples of spectrophotometers (Sakai, 2012). Gerhke et al (2009) found that determination of the shade using spectrophotometer is exceptionally reproducible when contrasted to customary visual shade appraisal, upgrading shade investigation, correspondence and dental restoration manufacturing (Gehrke et al., 2009).

The first presented VITA Easyshade devices was intended to quantify the CIE L*a*b* shading esteems for common teeth as it were, and estimated just the distinctions in values at the point when utilized with fabrication of ceramic. After that Easyshade concise was the second generation which regarded as a development because of the use of LED innovation (Corciolani, 2009).

The last generation is VITA Easyshade advance which is small, cordless, hand held gadget, using LED light, similarly to Easyshade compact, but with more software sophistication. Easyshade Advance give the allowance of the assessment of tooth color in a very quick manner in 3D master and VITA classical. It can save 30 colors and as claimed by the manufacture it can quantify the shade autonomous from surrounding effects(Apratim et al., 2015).

Zenthofer et al (2014) made a comparative study between Easyshade Advance and Easyshade compact in which they evaluate the repeatability and reliability of these two devices. Although both devices were found to be reliable, the Easyshade Advance was more accurate in color measurements(Zenthöfer et al., 2014).

VITA Easyshade advance measure the color by different modes including: (I) Single tooth measure the color of tooth measured by the placement of the probe of device at the middle third of the tooth, (II) measurements at multiple points by measuring the color of the teeth at 3 point cervical, middle and incisal, (III) restoration mode, which measure the color of dental ceramics) (Vichi et al., 2011).

# Chapter Two

# Materials and Method

# Chapter Two

# Materials and Method

## 2.1 Materials

The materials, Instruments and Equipment's that used in the study include:

### 2.1.1 Materials:

-Ciprofloxacin tablets (ciproneer, pioneer Co., Iraq, expiry date:03/2021).

- Composite light cure (DX Universal, sino-dentex co., ltd., China, expiry date: 27/11/2020).

-C-silicon heavy body and catalyst (Zetaplus, Zhermack, Italy, expiry date:06/2020).

- Cotton (Yazan, AL Rawan medical supplier, Turkey, expiry date: 01/ 2022).

- Cotton disk (Golden Rose, Turkey, expiry date: 01/2021).

- Disposable syringe (PROVI, med inject, Switzerland).

-EDTA (modern medical equipment llc, uae, expiry date: 05/2021).

-Gray MTA (angelus, Brasil , expiry date: 09/2021 ).

-Glass Ionomer Cement (Shanghai Rongxiang Dental Material Company, China, expiry date: 01/2021).

-Metronidazole tablets (metrosule, India, expiry date: 02/2021).

-Non-vacum tubes (EDTA K3, China, expiry date: 01/11/2022)

-Normal saline (Bioneer Company, Iraq, expiry date 12/2021).

-Paperpoint F3(DiaDent, China, expiry date :09/2021).

-Self- etch flowable Composite (Olident, Christo Botewa, Expiry date: 03/2020).

-Sodium hypochloride (CANOX, commercial house hold, Iraq, expiry date: 08/2021).

-3M ESPE bond Universal adhesive (3M, Germany, expiry date: 08/2020).

-Temporary filling (cavimed, modern medical equipment llc, expiry date: 05/2021).

- Tetracycline HCI Capsules (samacycline, sdi-Iraq, expiry date: 05/2021).

## 2.1.2 Instruments:

- Bond micro applicator (TPC, China).
- Carver (China).
- Diamond cutting Disk (China).
- Glass slab(China).
- Magnifying eye lens (10x, China).
- Measuring Digital caliper(China).
-$MTA^{+}$ applicator (cerkamed medical company, poland).
-pessoreamer #2(China).
-plugger(China).
-protaper gold Sx (Densply, Maillefer, Switzerland).
-side firing tip (Biolase, USA).
-Spatula (China).
- Sieve (China).

## 2.1.3 Equipments:

- Easyshade advance (vivadent, Germany).
-Electronic scale (AUY FH-200).
-Endomotor (D-smart I, China).
- Er:Cr:YSSG laser (Waterlase, Biolase, usa).
-Portable unit (LINGCHEN, China).
- Incubator (jrad, Germany).
- Light cure (lingchen, China).
- Low speed angled handpiece (NSK, Japan).
- Straight hand piece (NSK, Japan).
- Turbine (lingchen, China).


**Figure (2-1): some of the materials and devices that used during the study.**

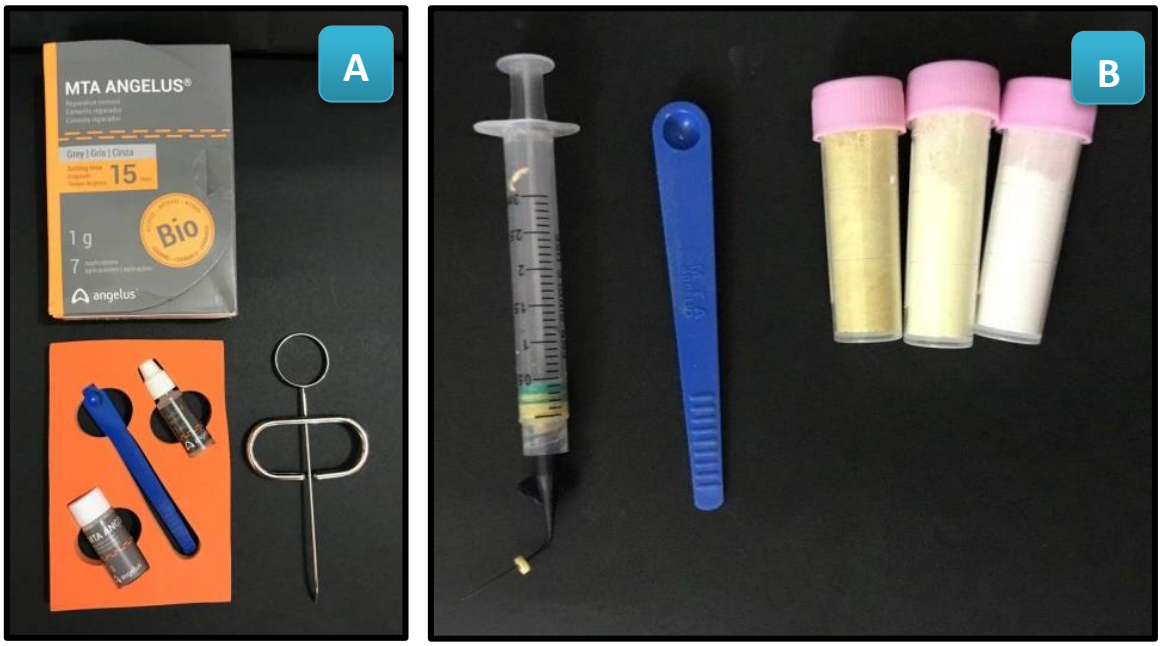


**Figure (2-2): the two materials that used for the two main groups of the study. A-GMTA kit with the applicator, B- triple antibiotic paste materials .**

# 2.2. Methodology

## 2.2.1. Sample selection

Human maxillary first premolar teeth were used in this study. Sixty caries-free teeth, which were extracted for orthodontic reason, were collected from different dental practice in the center of Basrah city in Iraq. The Teeth were disinfected by immersion in Thymol 1g/100ml of Distilled Water for 48 hours. The teeth were then cleaned by scalar and polished with brush and pumice. Teeth were carefully size calibrated by the digital caliper to have buccopalatal and mesiodistal dimension at range 8-10mm for size standardization and examined by magnifying lens to ensure that all the selected teeth were free from any defect such as caries, external staining, cracks or any abnormality that my affect teeth color. If any of the above defect was present the tooth was excluded. All selected teeth were stored in normal saline until the start of the experiment.

## 2.2.2. Pilot study of cavity dimensions

This pilot study were made on four samples to determine the suitable cavity dimensions to get good access for both canals and for standardization to eliminate the difference of dentine thickness between the samples after preparation of the cavity, after cavity preparation in the four teeth the cavity dimensions (buccoligual and mesiodistal) were calibrated and found that the suitable cavity to get good access for both canals were a round cavity with a diameter of 2.8mm see table (2-1).

**Table (2-1): Table of the Pilot study for the standardization of the dimensions of the cavity preparation.**

| NO. | buccolingual | mesiodistal |
|---|---|---|
| **1 St.** | 2.5 | 2.6 |
| **2nd.** | 2.4 | 2.6 |
| **3rd** | 2.8 | 2.8 |
| **4 th.** | 2.8 | 2.6 |

## 2.2.3. Preparation of the samples

The teeth apical root parts were first amputated at 10 mm apical to the cementoenamel junction (CEJ) see figure (2-3) by using diamond disk in a straight handpiece with water cooling. The apical part of the root were closed by GIC (Eslami et al., 2019). The teeth were then embedded in a silicon mold with dimensions (width= 21 mm, length= 32 mm, height= 32mm) in order to facilitated sample handling in the next procedures. An access cavity was then prepared in each tooth by using a diamond fissure bur (size 2mm) with high speed handpiece under constant water cooling. To standardize the access cavities, a pilot study was conducted on four teeth to identify the size of the cavity that much suitable for the access opening of the selected upper premolar teeth. According to this pilot study, a round cavity with a diameter about 2.8 mm in the center of the tooth was the most appropriate size to have an accessible opening for the 2 root canals with complete removal of the roof of the pulp chamber. The dimensions of the standard cavity were measured by digital caliber and marked by a permanent marker on the occlusal surface of the tooth by 4 points (buccal, palatal, mesial and distal) and then the distance between these points divided in to equal parts by ruler to mark the center of the cavity. then the drilling of the occlusal surface of the tooth were done by holding the bur perpendicular to the tooth and drill till feeling the absence of resistance to the drilling. After that the cavity dimensions finished according to

the marks that were done previously. If the canals were not found and there were need for increase the cavity dimensions more than the standard one, the tooth should be excluded. All remaining teeth were prepared according to these dimensions. This is followed by root canal preparation by using the SX Protaper gold rotary file by D-Smart endomotor before enlarging the canals by peaso reamer #2 by using angled handpiece (Turk et al., 2015), to get larger canal to have good access for the intracanal instruments and condensation. Irrigation with 3ml. NaOCl was used between each step of the previous procedures to facilitated debris removal, in addition to the final irrigation protocol of 3ml NaOCl, 3ml EDTA (17%), let them act for about 2 minutes and then rinse by 5ml normal saline (Küçükekenci et al., 2019). The NaOCl used in previous irrigation was prepared from house hold bleaching solution after dilution by the ratio of 1ml NaOCL/ 5ml normal saline with a care to use freshly prepared solution in each time before doing canal instrumentation. The prepared canals were then dried by using at least 3 paper point size F2, before closing the palatal canal with flowable composite exclude these roots from the experiment (Oh et al., 2016). A composite ring frame was also made on the labial surface of each sample to identify the region for shade measurement. A ring of 7 mm in diameter was marked on the buccal surface, about 1 mm above the CEJ, by the digital caliper 4 points (2 horizontally and 2 vertically) were done by the marker on the buccal surface to demarcate the limits of the ring. Composite were used according to these marked points for making a frame to demarcate the region of color measurement (Küçükekenci et al., 2019).


**Figure (2-3): the calibration of root length.**

## 2.2.4. Sample grouping

The samples were contained in one container and then selected randomly for each group. The samples divided into 2 main groups (n=30) (MTA and triple antibiotic groups). Each group were then subdivided into 3 subgroups (n=10) consisted of one control and two treated groups (Laser and bonding treatment).

### 2.2.4.1. Control groups

***Gray MTA***

After finishing Samples preparation as mentioned in (2.2.3). A freshly mixed gray MTA was used to fill the buccal canal in each sample of this group until the Cementoenamel Junction. The required quantity of gray MTA was prepared according to the manufacturer instruction. This was done by mixing one scope of gray MTA with one drop of distilled water that associated with the material until the desired consistency was obtained (wet sand), then the mixture was carried into the canal by MTA applicator to be packed by endodontic plugger, to fill the canal completely till the CEJ see figure (2-4). This level has

been verified by using the periodontal probe to measure the level of cementoenamel junction on the external tooth surface and then this measurement was used to determine the end of the intracanal MTA filling for each sample. The Cavity was then filled by small piece of Cotton about 2mm thickness and temporary restoration. The space of the cotton piece within the pulp chamber by the usage of a graduated probe and marked using permanent marker (Jagdale et al., 2018).

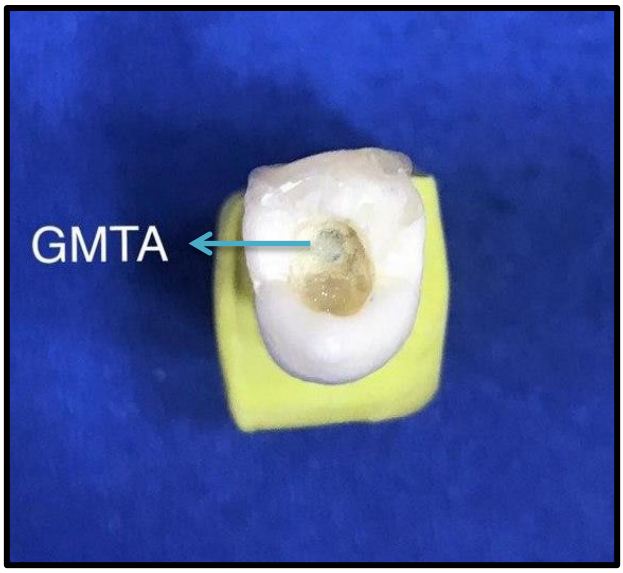


**Figure (2-4): GMTA applied inside the buccal canal.**

***Triple Antibiotic Paste Group***

The Samples were prepared as previously mentioned in (2.2.3). The triple the triple antibiotic paste was prepared by grinding equal quantities of ciprofloxacine tablets, metronidazole, and tetracycline. by coffee grinder before filtering the ground powder through suitable sieve. This powder was mixed with normal saline, (3:1) powder/ liquid ratio, on a glass slab until homogenous mixture was obtained (Jagdale et al., 2018). Afresh mixture should be used during the canal filling step and discarded after that. This fresh mixture was then filled in a disposable syringe with a dispenser tip to be used to deliver the triple antibiotic paste into the prepared canal to the level of cementoenamel junction see figure (2-5), the level of CEJ were measured from the external

surface of the tooth by the dispenser tip with stopper and take the buccal cusp tip as a reference point see figure (2-6) and the paste applied by forceful pressure on the syringe piston with gradual movement coronally to fill all the canal space till reaching the reference point by the stopper and then the cavity filled with cotton piece and temporary restoration (as mentioned in *Gray MTA* group).

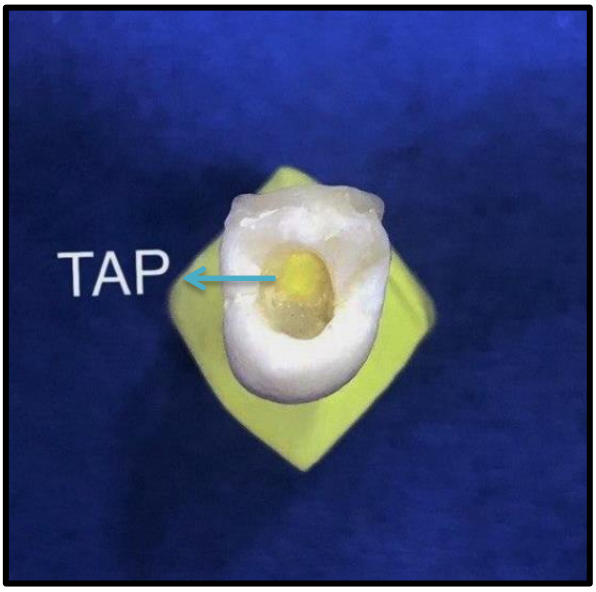


**Figure (2-5):TAP applied inside the buccal canal.**

**Figure (2-6): the measurements of the level of the CEJ.**

### 2.2.4.2. Bonding groups

In these groups (n=20), two layers of universal bond was applied on the walls of the pulp chamber by disposable micro applicators (TPC, regular type, orange) before spreading by gentle air 5s and light cured with light-emitting diode photopolymerization unite perpendicular to the cavity at 1200mW/cm$^2$ for 20 seconds (Küçükekenci et al., 2019). After the application of bonding 2 layers, ten of these samples were filled with gray MTA and other ten with triple antibiotic past as mentioned previously.

### 2.2.4.3. Laser Groups

Another 20 prepared samples were treated by laser before applying gray MTA or triple antibiotic past. The Laser used in this study is Er:Cr:YSSG Laser and waterlase side firing tip with a diameter about 800µm and length 18mm (Waterlase®) (©BIOLASE, Inc., USA), at a Power of 0.5 w, Frequency 20Hz, (Gholami et al., 2011, Yilmaz and Bayindir, 2014), for 30 sec for each wall with scanning movement from1mm distance (Ozlem et al., 2018),with water 1% and air 1% to avoid carbonization effect of laser (Aranha and de Paula Eduardo, 2012), The distance between the Laser tip and the pulp chamber wall had been standardized by using of autoclavable endodontic stopper which has been cut into square piece to be 1 mm in dimension and glued to the Laser tip on the side of the firing see figure (2-7). After laser treatment to the walls of pulp chamber, ten of these samples were filled with gray MTA and other ten with triple antibiotic past as mentioned previously.

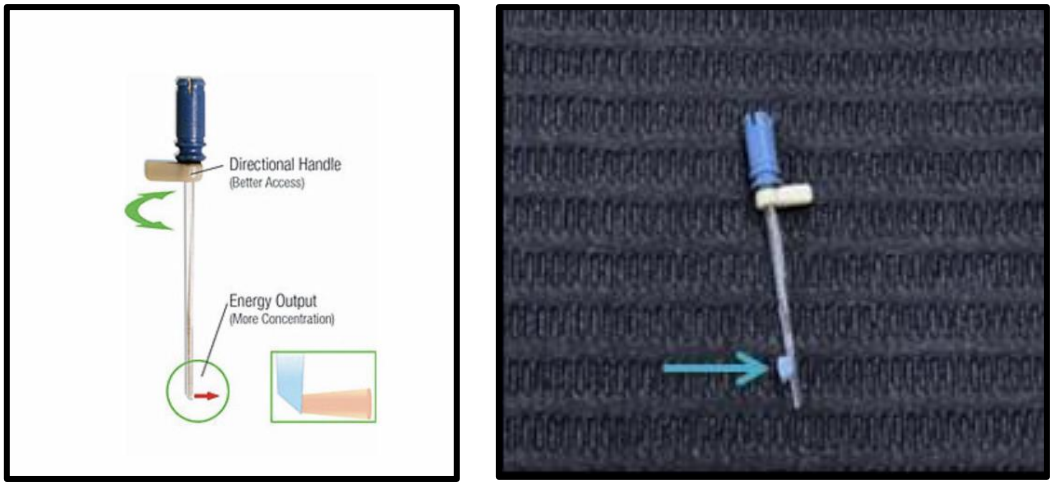


**Figure (2-7): (A) side firing tip (www.scivisionmedical.com/sft)**
**(B) the modified side firing tip.**

## 2.2.5. Shade measurements and sample storage

After samples preparation and filling were finished, The intial Shade were taken using Easyshade advance. The VITA Easyshade head had been positiond in the composite frame that had been done on the buccal surface as mentioned previously see figure (2-8), the measurment done at 10:00pm on white background. The prepared Tooth put in moist Cotton disk and inserted inside non-vacum Tube. The prepared Samples were put in Incubator for 3 weeks and 4 months. After 3weeks the final Shade has been taken as mentiond previously and at the same illumination environment and the samples incubated for 4 months and also the final color measured. The intial and final results after three weeks and 4 months were calculated by the following equation to have $\Delta E^*$ (Yuan et al., 2007): $\Delta E^*=[(L1-L0)^2+(a1-a0)^2+(b1-b0)^2]^{1/2}$

$\Delta E^*$: is total color difference.

L0: baseline color lightness. L1:final color lightness.

a0: baseline red-geen color. a1: final red-geen color.

b0: baseline red-geen color. b1: final yellow-blue color.


**Figure (2-8): the color measurment of the samples.**

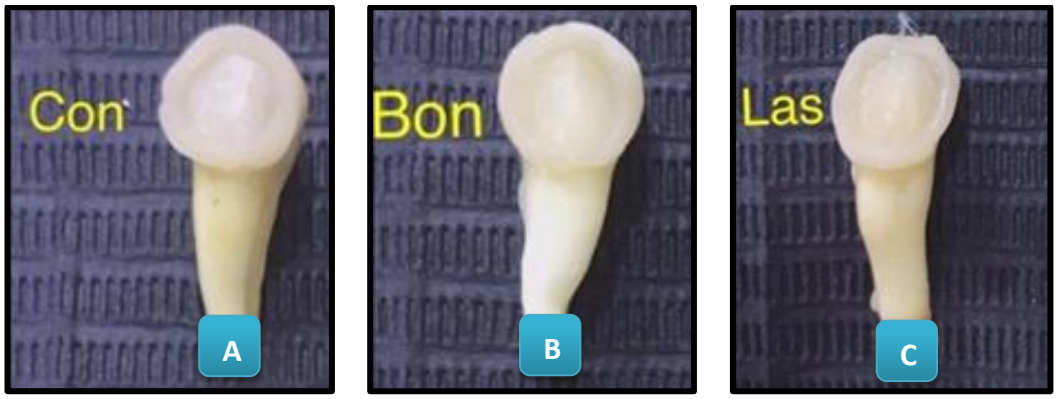


**Figure (2-9): Three samples of GMTA undergo discoloration after 4 months incubation period. (A): the control group sample. (B): the bond group sample . (C): the laser group sample .**

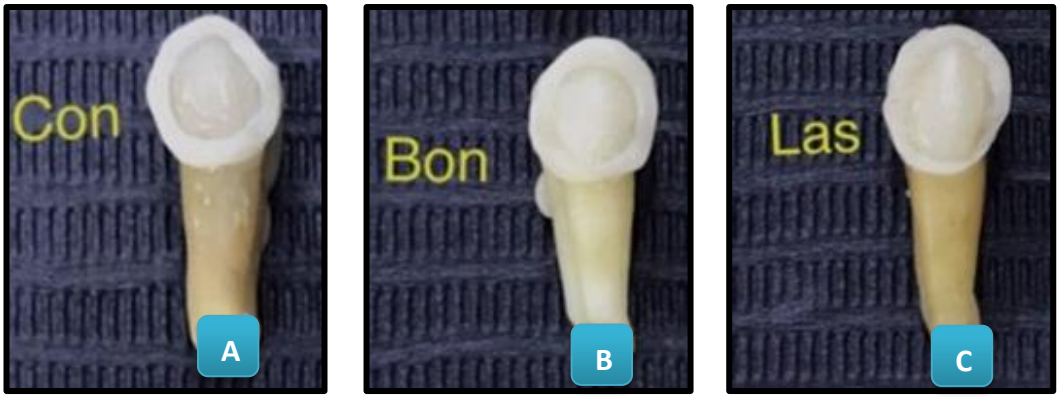


**Figure (2-10): three samples of TAP group undergo discoloration after 4 months incubation period. (A): the control group sample. (B): the bond group sample. (C): the laser group sample.**

# Chapter Three

# Results

# Chapter three

# Results

## 3.1. Descriptive statistics

Maximum, minimum, mean and standard deviation values of color lightness and color changes for all groups are shown in table (3-1) and figure (3-1).

**Table (3-1): Descriptive statistics of color lightness values (L) at different stages of the study for both gray mineral trioxide aggregate (GMTA) and triple antibiotic paste (TAP).**

| Groups | Stages | Subgroups | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Con | | | | Bon | | | | Las | | | |
| | | Mean | ±SD | Max | Min | Mean | ±SD | Max | Min | Mean | ±SD | Max | Min |
| GMTA | L0 | 83.7 | 2.2 | 87.7 | 80.3 | 85.9 | 4.4 | 90.5 | 77.8 | 88.9 | 3.6 | 96.3 | 84.6 |
| | L1 | 83.2 | 4.8 | 91.7 | 76.2 | 88.6 | 2.6 | 93.2 | 84.5 | 83.6 | 2.5 | 86.0 | 77.5 |
| | L2 | 80.1 | 4.2 | 84.9 | 71.8 | 84.9 | 2.7 | 89.0 | 79.7 | 82.0 | 5.4 | 87.7 | 69.8 |
| TAP | L0 | 83.2 | 6.0 | 93.0 | 75.7 | 87.4 | 3.1 | 93.7 | 82.4 | 83.3 | 4.0 | 89.3 | 75.4 |
| | L1 | 79.1 | 2.8 | 82.9 | 75.1 | 81.5 | 4.0 | 89.6 | 76.4 | 79.0 | 2.7 | 81.6 | 73.8 |
| | L2 | 74.4 | 4.0 | 81.4 | 69.2 | 76.5 | 4.0 | 81.2 | 66.8 | 77.6 | 2.8 | 81.5 | 73.4 |

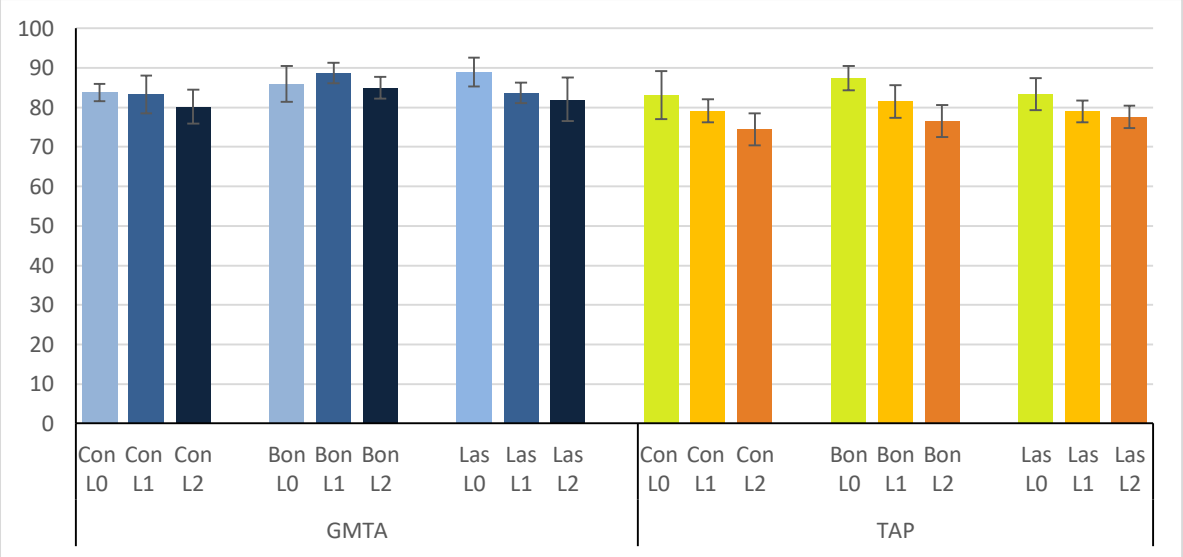


**Figure (3 -1): Color lightness value bar chart. L values (L0= baseline, L1= 3 weeks, and L2= 4 months) for both gray mineral trioxide aggregate (GMTA) (light blue to dark blue bars) and triple antibiotic paste (TAP) (green to orange bars) for means of color lightness at three stages of study.**

From table (3.1) and Fig (3.1), all groups show decrease in color lightness with time. The highest change in color lightness is clearly detected within the second stage measurements (L2) for both GMTA and TAP. For GMTA groups, bonding treatment decrease the effect of GMTA on color lightness. This is clearly apparent in the (L1) measurements, which shows the highest lightness value (88.6 ± 2.6) and become less after 4 months (L2= 84.9 ± 2.7). While, laser treatment shows little effect on maintaining the color lightness for GMTA groups at the different stages of the study. On the other hand, both bonding and laser treatment appear to have undetectable effect on color lightness within TAP groups in comparison to their control group measurements.

**Table (3-2): Descriptive statistics of total color changes (ΔE*) at different stages of the study for both gray mineral trioxide aggregate (GMTA) and triple antibiotic paste (TAP).**

| Groups | Stages | Subgroups | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Con | | | | Bon | | | | Las | | | |
| | | Mean | ±SD | Max | Min | Mean | ±SD | Max | Min | Mean | ±SD | Max | Min |
| GMTA | ΔE1 | 7.0 | 2.9 | 3.7 | 12.3 | 10.3 | 3.0 | 3.9 | 14.5 | 8.5 | 3.4 | 4.2 | 14.8 |
| | ΔE2 | 6.7 | 3.9 | 1.8 | 12.4 | 8.5 | 2.0 | 4.7 | 11.6 | 12.9 | 5.6 | 4.2 | 21.5 |
| TAP | ΔE1 | 6.9 | 3.8 | 2.7 | 16.1 | 7.5 | 2.2 | 4.1 | 11.3 | 6.9 | 3.6 | 2.2 | 14.7 |
| | ΔE2 | 9.7 | 5.3 | 3.4 | 23.2 | 12.8 | 3.2 | 7.6 | 16.6 | 8.3 | 3.9 | 4.2 | 14.7 |

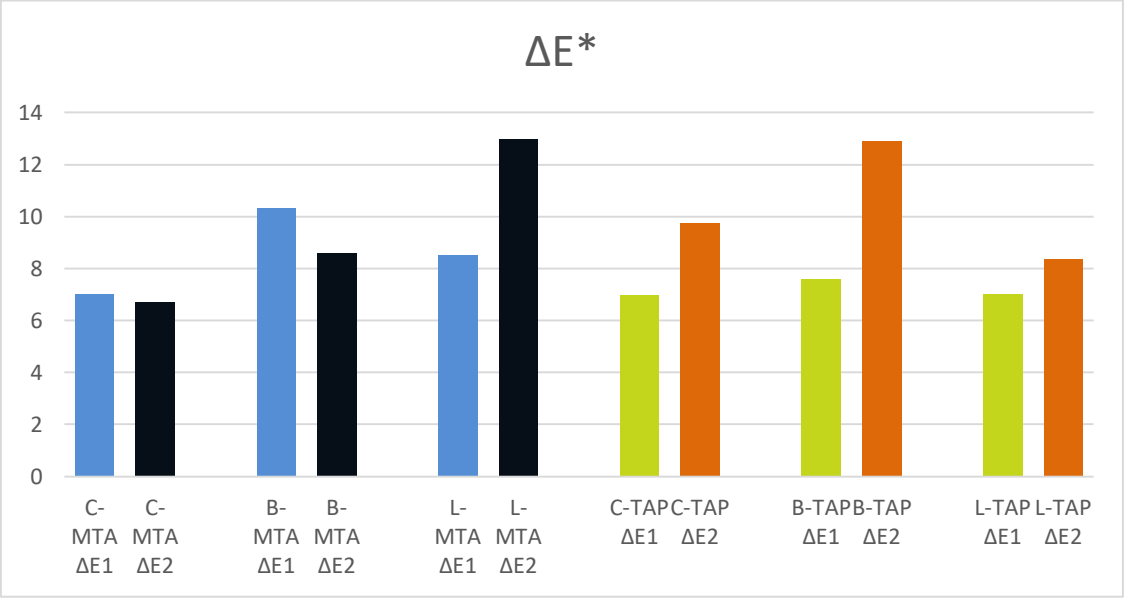


**Figure (3-2): ΔE* bar chart. ΔE values (ΔE1 and ΔE2) for both GMTA (light and dark blue bars) and TAP (green and orange bars).**

About total color change values (ΔE*) which are shown in table (3. 2) and Fig (3.2), these values represented the total changes in CIElab color parameters (L, a, and b values). It is clearly obvious in this study that the different materials used (GMTA and TAP) have different effect on ΔE. In GMTA groups, the highest value for ΔE1 is for bonding group followed by laser and control groups (10.3 ± 3.01, 8.5 ± 3.4 and 7.02 ± 2.9 respectively).

While the ΔE2 shows the highest value in the laser group (12.9 ± 5.6) in comparison to bond (8.5 ± 2.05) and control (6.7 ± 3.9) groups. However, total color changes in the TAP groups show increase in ΔE within time i.e. higher ΔE2 values in comparison to ΔE1. The highest ΔE2 value in TAP was recorded in the bond group (12.8 ± 3.2)

## 3.2. Normality test

In order to detect the normality in the distribution of the values within the groups of this study, the Shapiro-Wilk test was conducted (See table 3-3). All groups of the study show (p> 0.05) in this test, which means that all values are normally distributed. Therefore, only parametric statistical analysis tests were used during the statistical analysis between these groups.

**Table (3-3): Normality test for GMTA and TAP subgroups or lightness (L) and total color change values (ΔE*).**

| Subgroups | | Shapiro-Wilk P-value | | |
|---|---|---|---|---|
| | | Con | Bon | Las |
| **GMTA** | L0 | .945 | .078 | .254 |
| | L1 | .561 | .909 | .032 |
| | L2 | .281 | .875 | .132 |
| **TAP** | L0 | .304 | .014 | .095 |
| | L1 | .304 | .014 | .095 |
| | L2 | .706 | .041 | .499 |
| **GMTA** | ΔE1 | .135 | .612 | .084 |
| | ΔE2 | .224 | .933 | .789 |
| **TAP** | ΔE1 | .045 | .718 | .613 |
| | ΔE2 | .025 | .221 | .124 |

There is no statistical significant when $p > 0.05$

There is statistical significant when $p \leq 0.05$

# 3.3. Comparison between different measurements within the same subgroup

## 3.3.1. Color lightness (L*) measurements

As shown in table (3-4) these comparisons are between L0, L1 and L2 measurements within the same group. Both ANOVA for repetitive measures and Bonferroni corrections were used to find the statistical significance between these groups. ANOVA test shows statistical significant difference ($p< 0.05$) among L0, L1, and L2 of all subgroups for both GMTA and TAP.

To find the statistical significance among these measurements, Bonferroni test was used. This test shows statistical significant difference ($p < 0.05$) among laser GMTA subgroups only with no statistical difference between control and bond subgroups. On the other hand, among TAP subgroups there is higher statistical significant difference ($p < 0.001$) between bond subgroups, and between L0 and L1 for control and laser subgroups ($p < 0.05$).

**Table (3-4): ANOVA for repetitive measures and Bonferroni test to compare among (L0, L1 and L2) within the same group for both GMTA and TAP.**

| Groups | ANOVA p-value | Stages | Bonferroni test p-value Stages | |
|---|---|---|---|---|
| | | | L1 | L2 |
| **Con-GMTA** | .012 | L0 | 1.000 (NS) | .130 (NS) |
| | | L1 | | .017 (S) |
| **Bon-GMTA** | .013 | L0 | .382 (NS) | .733 (NS) |
| | | L1 | | .029 (S) |
| **Las-GMTA** | .005 | L0 | .003 (S) | .005 (S) |
| | | L1 | | .478 (NS) |
| **Con-TAP** | .001 | L0 | .194 (NS) | .002 (S) |
| | | L1 | | .005 (S) |
| **Bon-TAP** | .000 | L0 | .000 (S) | .000 (S) |
| | | L1 | | .041 (S) |
| **Las-TAP** | .037 | L0 | .077 (NS) | .024 (S) |
| | | L1 | | .597 (NS) |

There is no statistical significant when $p > 0.05$

There is statistical significant when $p \leq 0.05$

### 3.3.2. Total color change (ΔE*)

Another analysis was made between the ΔE* values within the same group (ΔE1 and ΔE2) by using paired T-test (see table 3-5). This test shows statistical significant difference ($p < 0.05$) between GMTA Laser subgroup of only with no statistical significant difference ($p > 0.05$) between GMTA control and bond subgroups. On the other hand, there is statistical significant difference ($p \leq 0.05$) between TAP control and bond subgroups and no statistical significant difference ($p > 0.05$) between TAP Laser subgroup values.

**Table (3 -5): Paired T-test comparison between first and second stages for the total color changes (ΔE1 and ΔE2) for all subgroups of GMTA and TAP.**

| Groups | Subgroups | p-value |
|---|---|---|
| **GMTA** | Con- ΔE1 Vs. Con-Δ E2 | .782$^{(NS)}$ |
| | Bon- ΔE1 Vs. Bon- ΔE2 | .081$^{(NS)}$ |
| | Las- ΔE1 Vs. Las- ΔE2 | .013$^{(S)}$ |
| **TAP** | Con- ΔE1 Vs. Con-Δ E2 | .057$^{(NS)}$ |
| | Bon- ΔE1 Vs. Bon-Δ E2 | .006$^{(S)}$ |
| | Las- ΔE1 Vs. Las- ΔE2 | .293$^{(NS)}$ |

There is no statistical significant when $p > 0.05$

There is statistical significant when $p \leq 0.05$

## 3.4. Comparisons between different subgroups of both GMTA and TAP

### 3.4.1. Color lightness (L*) measurements

These comparisons are between control, bond and Laser measurements within the same group of either GMTA of TAP. Both ANOVA and Bonferroni tests (table 3-6) were used to analyze statistically the significant differences between these groups for lightness (L) and total color change (ΔE*) values.

By using ANOVA test, only the L0 and L1 measurements for GMTA show statistical significant differences ($p \leq 0.05$) when comparing between control, bond and laser. Further statistical analysis by Bonferroni test shows the highest significant difference is between the control and bond subgroups for GMTA ($p=.006$). Other color lightness measurement including all TAP subgroups show no statistical significant difference ($p>0.05$).

Furthermore, analysis using ANOVA test shows no statistical significant difference between ΔE* values for control bond and laser subgroups for both GMTA and TAP, except for the GMTA ΔE2 values. These shows highest statistical significant difference between control and laser subgroup.

**Table (3-6): ANOVA and Bonferroni test to compare between (Con, Bon and Las) of both GMTA and TAP.**

| Stages | ANOVA p -value | Subgroups | Bonferroni test Subgroups | |
|---|---|---|---|---|
| | | | Bon | Las |
| **GMTA-L0** | .012(S) | Con | .529 (NS) | .010(S) |
| | | Bon | | .228(NS) |
| **GMTA-L1** | .003(s) | Con | .006(S) | 1.000(NS) |
| | | Bon | | .011(S) |
| **GMTA-L2** | .061(NS) | Con | | |
| | | Bon | | |
| **TAP-L0** | .088(NS) | Con | | |
| | | Bon | | |
| **TAP-L1** | .180(NS) | Con | | |
| | | Bon | | |
| **TAP-L2** | .169(NS) | Con | | |
| | | Bon | | |
| **GMTA- ΔE1** | .086(NS) | Con | | |
| | | Bon | | |
| **GMTA- ΔE2** | .007(S) | Con | .579(NS) | .006(S) |
| | | Bon | | .061(NS) |
| **TAP- ΔE1** | .896(NS) | Con | | |
| | | Bon | | |
| **TAP- ΔE2** | .070(NS) | Con | | |
| | | Bon | | |

There is no statistical significant when $p > 0.05$

There is statistical significant when $p \leq 0.05$

## 3.5. Comparison between different groups (GMTA Vs. TAP)

Another statistical comparisons were made to detect the differences between GMTA and TAP groups by using unpaired T-test for color lightness (L) and total color change (ΔE*) values (see table 3-7).

For lightness values, the baseline measurements (L0) show no statistical significant difference between GMTA and TAP for control and bonding subgroups, while the laser shows statistical significant difference (p=0.005). Larger statistical significant difference between GMTA and TAP are clearly detected in the L1 and L2 measurements with highest significant differences (p<0.001) between bond subgroups.

The total color change (ΔE*) show no statistical significant difference between GMTA and TAP at the two stages (ΔE1 and ΔE2), except between the bonding subgroups which give statistical significant difference at p=0.038.

**Table (3 -7): Unpaired T-test to compare the color lightness values of GMTA Vs. TAP at different stages of study.**

| Stages | Subgroups | P-value |
|---|---|---|
| **L0** | Con-GMTA Vs. Con-TAP | .795$^{(NS)}$ |
| | Bon-GMTA Vs. Bon-TAP | .415$^{(NS)}$ |
| | Las-GMTA Vs. Las-TAP | .005$^{(S)}$ |
| **L1** | Con-GMTA Vs. Con-TAP | .031$^{(S)}$ |
| | Bon-GMTA Vs. Bon-TAP | .000$^{(S)}$ |
| | Las-GMTA Vs. Las-TAP | .001$^{(S)}$ |
| **L2** | Con-GMTA Vs. Con-TAP | .006$^{(S)}$ |
| | Bon-GMTA Vs. Bon-TAP | .000$^{(S)}$ |
| | Las-GMTA Vs. Las-TAP | .039$^{(S)}$ |
| **ΔE1** | Con-GMTA Vs. Con-TAP | .982$^{(NS)}$ |
| | Bon-GMTA Vs. Bon-TAP | .038$^{(S)}$ |
| | Las-GMTA Vs. Las-TAP | .349$^{(NS)}$ |
| **ΔE2** | Con-GMTA Vs. Con-TAP | .169$^{(NS)}$ |
| | Bon-GMTA Vs. Bon-TAP | .002$^{(S)}$ |
| | Las-GMTA Vs. Las-TAP | .047$^{(S)}$ |

There is no statistical significant when $p > 0.05$

There is statistical significant when $p \leq 0.05$

**Table (3 -8): Raw data of color (L*, a* and b*) for all groups.**

| Sample NO. | Parameter | stages | Con | | Bon | | Las | |
|---|---|---|---|---|---|---|---|---|
| | | | GMTA | TAP | GMTA | TAP | GMTA | TAP |
| 1 | L | L0 | 83.5 | 75.7 | 79 | 90.4 | 85.7 | 89.3 |
| | | L1 | 76.2 | 82.9 | 89.7 | 89.6 | 77.5 | 81.6 |
| | | L2 | 71.8 | 73.2 | 81.6 | 77.1 | 69.8 | 81.5 |
| | a | a 0 | 1.4 | 4 | 3.2 | -0.1 | 2 | 0.8 |
| | | a1 | 3 | 0.7 | -1.1 | -1.4 | 3.8 | 1.3 |
| | | a 2 | 3.4 | 4.1 | 0.4 | 3.4 | 2.1 | 1.3 |
| | b | b 0 | 29.8 | 40.2 | 42.9 | 35.1 | 41.9 | 34.8 |
| | | b 1 | 39.6 | 35.2 | 38 | 31.3 | 39.5 | 40.9 |
| | | b 2 | 33.2 | 42.6 | 31.9 | 30.6 | 27.3 | 35 |
| 2 | L | L0 | 81.1 | 88.7 | 85 | 88.1 | 85.8 | 87.3 |
| | | L1 | 86.6 | 81.8 | 87.9 | 80.3 | 82.5 | 73.8 |
| | | L2 | 84.2 | 77.4 | 85.9 | 77.7 | 81.8 | 74.8 |
| | a | a 0 | 1.9 | -0.6 | 3 | 1.4 | 0.2 | 1.2 |
| | | a 1 | -1.4 | -1.8 | 0.1 | 0.9 | 1.3 | 1.1 |
| | | a 2 | 0.4 | 2.3 | 0.7 | 2.7 | 1.3 | 4.8 |
| | b | b 0 | 30.8 | 38.8 | 35.6 | 40.2 | 35.6 | 33.7 |
| | | b 1 | 30.2 | 39.9 | 26.2 | 36.7 | 38.1 | 39.6 |
| | | b 2 | 35.8 | 41.5 | 31.5 | 34.1 | 37.4 | 40.7 |
| 3 | L | L0 | 83.5 | 82.1 | 88.7 | 87.4 | 91.5 | 82 |
| | | L1 | 85.5 | 75.1 | 91.2 | 81.2 | 85.9 | 81.4 |
| | | L2 | 84.9 | 75.9 | 85.7 | 79.4 | 87.7 | 73.4 |
| | a | a 0 | 0.7 | -1.1 | 0.3 | 1.5 | -0.2 | 4 |
| | | a 1 | -0.1 | -1.3 | -2.5 | 0.8 | 1.9 | 2.3 |
| | | a 2 | -0.4 | 0.3 | -1.2 | 2.5 | 8.5 | 6.3 |
| | b | b 0 | 29.9 | 29.1 | 34.1 | 40.2 | 40 | 37.8 |
| | | b 1 | 33.8 | 31 | 27 | 33.8 | 36.9 | 39.1 |
| | | b 2 | 36.4 | 31.8 | 25.8 | 40.8 | 57 | 45.7 |
| 4 | L | L0 | 87.7 | 93 | 89.3 | 93.7 | 87.1 | 81.1 |
| | | L1 | 84.4 | 77.3 | 89.6 | 87.9 | 81.4 | 76.2 |
| | | L2 | 83.7 | 70.8 | 89 | 78.1 | 77.6 | 77.4 |
| | a | a 0 | -1.1 | -0.9 | -0.3 | -1.3 | 2.5 | 2.9 |
| | | a 1 | -1.6 | 0.1 | -3 | -2.2 | -0.2 | 4 |
| | | a 2 | -1.1 | 3.7 | -2.3 | 1.4 | 0.2 | 2.8 |
| | b | b 0 | 26.5 | 32.2 | 32.6 | 33.6 | 43.1 | 40.9 |
| | | b 1 | 28.1 | 35.9 | 29.8 | 31.1 | 29.7 | 42.2 |
| | | b 2 | 31.6 | 37.3 | 25.8 | 30 | 28 | 38.9 |
| 5 | L | L0 | 86.2 | 77.6 | 89.8 | 85.7 | 84.6 | 83.2 |
| | | L1 | 79.2 | 78.1 | 93.2 | 80.1 | 86 | 79.7 |
| | | L2 | 76.9 | 69.2 | 87.2 | 78.4 | 84.8 | 80.1 |
| | a | a 0 | 0.8 | 4.9 | 0.7 | -0.6 | 5.7 | 3.3 |
| | | a 1 | 0 | 3 | -4.5 | -0.1 | 3.1 | 1.3 |
| | | a 2 | 1.5 | 6 | -1.6 | 5.5 | 2.2 | 1.4 |
| | b | b 0 | 27.8 | 40.4 | 39.8 | 31.6 | 52.2 | 40.9 |
| | | b 1 | 26.6 | 43.3 | 26.7 | 33.6 | 46.4 | 37.6 |
| | | b 2 | 31.7 | 39.1 | 29.9 | 35 | 36.3 | 33.8 |

| Sample No. | Parameter | Stages | Con | | Bon | | Las | |
|---|---|---|---|---|---|---|---|---|
| | | | **GMTA** | **TAP** | **GMTA** | **TAP** | **GMTA** | **TAP** |
| **6** | L | L0 | 84.4 | 80.6 | 88.3 | 88.5 | 96.3 | 87.5 |
| | | L1 | 91.7 | 79.4 | 84.5 | 80.6 | 84.4 | 79.8 |
| | | L2 | 82.4 | 74.6 | 87.5 | 73.8 | 85.4 | 73.8 |
| | a | a 0 | 0.9 | 2.5 | 0.3 | 2.6 | 0.6 | 1.8 |
| | | a 1 | -2.6 | 0 | -2.9 | 2.5 | 1.6 | 0.7 |
| | | a 2 | 0.8 | 2.3 | -1.6 | 3.5 | 2 | 2.4 |
| | b | b 0 | 33.6 | 36.2 | 38.8 | 37.8 | 44.4 | 34.6 |
| | | b 1 | 27.5 | 36 | 26.9 | 40 | 39.2 | 34.3 |
| | | b 2 | 33.8 | 32 | 30.1 | 35 | 37 | 31.9 |
| **7** | L | L0 | 84.9 | 80.8 | 90.5 | 84.6 | 90.1 | 80.3 |
| | | L1 | 77.7 | 76.3 | 85.2 | 79 | 85.2 | 76.3 |
| | | L2 | 76.6 | 71.7 | 84.6 | 78.2 | 86 | 76.9 |
| | a | a 0 | 0.8 | 2.5 | 0.3 | 2.5 | 0.8 | 5.4 |
| | | a 1 | 0.4 | 2.7 | -1.8 | 0.7 | 0.5 | 4.5 |
| | | a 2 | 1.2 | 4.6 | -0.6 | 1.5 | 1.9 | 3 |
| | b | b 0 | 27.5 | 38.9 | 38.7 | 42.6 | 38.7 | 41.4 |
| | | b 1 | 35.4 | 43 | 29.4 | 36.6 | 34.6 | 46.1 |
| | | b 2 | 36.7 | 42 | 31.8 | 28.9 | 30.5 | 39.6 |
| **8** | L | L0 | 83.6 | 76.5 | 87.1 | 87.3 | 86.2 | 83.2 |
| | | L1 | 85.2 | 76.4 | 88.5 | 76.4 | 83.8 | 81.4 |
| | | L2 | 82.9 | 71.1 | 84.8 | 74.6 | 81.9 | 79.4 |
| | a | a 0 | 1.3 | 0.9 | 0.3 | 2 | -0.9 | 3.9 |
| | | a 1 | -1.7 | 0.6 | -3.4 | 1 | -1.4 | 2.1 |
| | | a 2 | -0.3 | 1.6 | -1.8 | 2.8 | 1.1 | 2.1 |
| | b | b 0 | 31.3 | 38.3 | 37.7 | 35 | 37.6 | 37.9 |
| | | b 1 | 27.2 | 35.1 | 30.7 | 32 | 31.4 | 36.9 |
| | | b 2 | 30.8 | 32 | 31.9 | 30.6 | 27.9 | 37 |
| **9** | L | L0 | 82.2 | 89.8 | 77.8 | 82.4 | 91 | 84.6 |
| | | L1 | 86.5 | 82.6 | 87.4 | 79.8 | 84.9 | 78.3 |
| | | L2 | 80.6 | 79.4 | 79.7 | 66.8 | 86.7 | 78.5 |
| | a | a 0 | 1.1 | -0.2 | 4.8 | 2.3 | 1.1 | 2.2 |
| | | a 1 | -0.3 | -0.6 | -1 | 0.8 | 1 | 1.8 |
| | | a 2 | -0.2 | 1.4 | 2 | 6.4 | -0.5 | 1.6 |
| | b | b 0 | 33.3 | 35.9 | 44.2 | 39 | 32.8 | 39.1 |
| | | b 1 | 35.3 | 35.6 | 38.8 | 35.5 | 34.9 | 42.3 |
| | | b 2 | 31.3 | 40.8 | 37.2 | 34.8 | 29.3 | 40.6 |
| **10** | L | L0 | 80.3 | 87.2 | 84.1 | 85.9 | 90.8 | 75.4 |
| | | L1 | 79.9 | 81.4 | 89.4 | 80.1 | 84.8 | 81.6 |
| | | L2 | 77.8 | 81.4 | 83.6 | 81.2 | 78.3 | 80.5 |
| | a | a 0 | 3.3 | 2.5 | 2.1 | 1.3 | 2.2 | 5.1 |
| | | a 1 | 0 | -0.5 | -1.7 | 0.4 | 0 | 1.2 |
| | | a 2 | -0.5 | 1 | 0.5 | 2.3 | 1.5 | 1.9 |
| | b | b 0 | 37.2 | 40.1 | 40.9 | 41.3 | 43.9 | 39.1 |
| | | b 1 | 33.4 | 37.1 | 33.8 | 33.9 | 34.3 | 34.1 |
| | | b 2 | 32.9 | 39.9 | 30.7 | 35.4 | 31.2 | 34.3 |

# *Chapter Four*

# *Discussion*

# Chapter Four

# Discussion

Tooth discoloration is still one of the biggest aesthetic problem for teeth having pulp treatment. Although, some of the discoloration causes are due to the pulp degenerative products, the pulp treating materials are also big participant (Arman et al., 2015). Among the severest discoloration materials those used in regenerative and vital pulp treatments, such as the gray mineral trioxide aggregate (GMTA) and triple antibiotic paste (TAP). These materials have been reported to bind to the dentin to form dark brown to greyish stain (Kim et al., 2010, Santos et al., 2017). Intracoronal bleaching is one of the treatment procedures that can be used, but it is a dependable method with inevitable complications such as root resorption (Arı and Üngör, 2002). The severity of this stain could critically compromise aesthetic and change treatment mode toward none conservative total tooth crowning.

An effort has been developed to avoid unwanted crown staining through the internal coating of the dentinal tubules with dentin bonding prior to applying the treatment materials (Kim et al., 2010, Santos et al., 2017). This is an easy, low cost and minimal invasive method that can decrease the staining by preventing the contact between the dentin and pulp treatment material especially within the pulp chamber. The use of Nd:YAG laser was also used as a color prevention method before the use of TAP (Küçükekenci et al., 2019). Although, laser can occlude the dentinal tubules on the treated surface its effect on decreasing the tooth discoloration still not addressed in comparison to dentin bonding agent. In addition, none of the previous studies used different discoloring materials with different prevention technique and on an extended period of time. Therefore, this study was conducted to evaluate the effect of

dentin bonding and Er:Cr:YSSG laser (Waterlase) as preventive treatment for discoloration caused by GMTA and TAP.

Human maxillary first premolar teeth were used as tooth model for this study. This is because of the availability of these teeth due to extraction for orthodontic purposes. In order to eliminate any factor that may interfere with the tooth color measurements, these teeth were carefully size calibrated and inspected with higher magnification lens and about half of the pool of the gathered teeth were excluded. A randomized method was used during teeth grouping to prevent biasing and minimize the differences between samples within each group. In certain reports, the operations has been done via the apical region in order to keep the crown intact and for inhibition of the microleakage from the coronal region (Akcay et al., 2014, Yasa et al., 2015). In spite of that, this technique not simulate in vivo circumstances. In the present study all the steps of the operation were done coronally to mimic the clinical situation. The size of the cavity was determined according to a pilot study which determine the proper size for endodontic access for both canals. The use of self-etch bond was determined within the current study in order to reduce the multiple steps during bonding procedure which may require further standardization procedure.

The choice of GMTA and TAP in our study due to its dark discoloration that have been previously recognized within researches (Reynolds et al., 2009, Kim et al., 2010, Parirokh and Torabinejad, 2010). TAP have a very important role in disinfection of the canal and also lead to a revascularization the pulp chamber and the canals (Trope, 2011). As well as GMTA have a role in pulp capping, pulpotomy, apexogenesis and apexification, apical sealing, reparation of radical perforation ,resorption management, and used as a root canal filling substance (Schwartz et al., 1999, Parirokh and Torabinejad, 2010), on the other hand both TAP and GMTA have a potential for teeth discoloration as mentioned previously. after cavity and canal preparation the final irrigation protocol EDTA(17%) + NaOCL (1%) is the most recommended by literatures due to

their role in the dissolving inorganic and the organic constituents, removal of dentine and smear layer residues (Zehnder, 2006) and this followed by normal saline flushing. Only the buccal canal was chosen to work on because the surface to be measured was buccal surface which is the visible surface of the teeth. the cavity then closed by standardized size of piece of cotton between the used intracanal material and the temporary filling, this to prevent the interaction between the two materials and to eliminate the difference in the depth and thickness of the temporary filling since it's have an effect on the discoloration of the teeth because of the difference of the number of tubules that were exposed to the effects of intracanal material (Jagdale et al., 2018).

The use of Er,Cr:YSGG type of laser( waterLase) in this study because it is a dental type laser which used for hard tissue treatment and used for desensitization and closure of dentinal tubules. The power of the laser used was (0.5 w/ $cm^2$, Frequency 20Hz, Water 1% and Air 1%, for 2 Minutes), because it is produce dentinal tubules closure (Gholami et al., 2011, Yilmaz and Bayindir, 2014). The laser tip which used were side firing tip because it is produce the laser energy laterally toward the walls of the pulp chamber, while the other types of tips for example saffrine tip produce the energy apically and difficult to orient the energy toward the walls with standardized distance.

Color measurement was done by VITA Easyshade Advance because This device produces not only accurate but also repeatable and quick readings of spectral color space. Moreover, the VITA Easy shade® spectrophotometer has an advantage over the laboratory spectrophotometers and colorimeters which require a flat surface and thus not indicated to measure the spectral color of the teeth. In addition, laboratory spectrophotometers are unable to measure the translucency of the natural teeth(Corciolani and Vichi, 2006, Darling et al., 2006, Darling et al., 2006, Horuztepe and Baseren, 2017, Horuztepe et al., 2017, Joiner and Luo, 2017). Assessment of shade should be done in one area in every single specimen (Ioannidis et al., 2013). Thereby, a circular frame of composite

was fabricated on the specimens buccal surface (Küçükekenci et al., 2019). All recordings of measurements were taken at 10:00 pm to eliminate the effect of day light, also the measurements done on white background since the white back ground have little effect on the shade measurements (Dogheim et al., 2016). The values of L* (lightness) were detected which indicates the darkening and lightening patterns were the ones of the biggest concern (Kirchhoff et al., 2015).

According to the condition of this study, and in comparison to the control groups, the bond and laser samples of GMTA showed higher lightness values during the baseline measurements see table (1-3) and figure (3-1). This was clearly illustrated within laser subgroup see table (3-6), which reflect the whiting action of laser that may occur immediately after use, this may be related to the alteration of the optical properties for the lased surface. This possibly due to the laser thermal effect that can induce alterations in the physical and chemical composition (Bachmann et al., 2005). After the first incubation period the bond subgroup of GMTA gave the highest lightness value among all tested groups see table (1-3),(3-6) and figure (3-1). This can be explained that the application of 2 layers of dentin bonding seal the dentinal tubules and prevent the discoloration effect of GMTA This agreed with Akbari et al. (2012) who concluded that application of two layers of dentin bonding before using gray and white MTA may prevent tooth discoloration. Khim et al. (2018) also recommended the application of dentin bonding as an additional step before endodontic obturation to seal the dentinal tubules and prevent sealer interactions which may reduce tooth discoloration. In addition to the dentinal tubule sealing action of these two layers, they may cause some sort of tooth whiting effect, but the explanation for this is still unknown, it could be due to the effect of these two layers of bonding material on the light reflection and refraction within the measured specimens, which may give this lighter pattern. Whilst, coronal discoloration caused by GMTA and TAP progressed in a time-dependent

manner table (1-3) and figure (3-1), consistent with the previous research (van der Burgt et al., 1986, Parsons et al., 2001, Ioannidis et al., 2013). The reason for this might be due to the inherent composition of GMTA and TAP, which undergo certain chemical changes leading to discoloration. As the time progresses, further chemical reactions and seepages of discoloration products which may lead to more tooth discoloration. This agreed with the findings of Khim et al. study who showed that dentin bonding could not prevent coronal discoloration completely, but it could effectively reduce the risk of coronal discoloration caused by root canal sealer remnants (Khim et al., 2018). Other studies evaluated the efficacy of dentin bonding in preventing coronal discoloration caused by triple antibiotic paste (Kim et al., 2010, Shokouhinejad et al., 2018).

The present study resulted in darker laser specimens when comparing with bond. This may relate to the micro morphological changes reported in the lased dentin surface (Ishizaki et al., 2004, Chou et al., 2009). This possibly produced rough and pitted surface of the lased dentin which may increase in the amount of the GMTA and TAP remnants in contact with the dentin surface.

The present results also found that the prevention methods had less effect on TAP groups in comparison to GMTA. This could be due to the difference in the composition between the two materials. TAP composed of tetracycline which is able to chelate with the dentin surface calcium(Windley III et al., 2005) . This may produce darker and much difficult tooth discoloration products than the main discoloring component of GMTA (bismuth oxide). Also the difference in the mixing consistency and wettability of the fresh mixture between GMTA and TAP could be another cause. Since GMTA workable consistency was a form of wet sand like material, while TAP fresh mixture is more creamy and paste like consistency. This may affect the adaptation and manipulation between the two materials and this may affect their discoloration actions.

The total color variation (ΔE*) between the initial and final color measures after samples aging demonstartes the degree of total color change represented by a numerical value. This means that each paramenter presents within ΔE* equation (L*, a* and b*) is basically part of the color change that totally measured in one number. Therefore, ΔE* could not exactly demonstrate the direction of the color axis on the CIE Lab space (Ioannidis et al., 2013) .

According to the results of this study, the statistical comparisons between ΔE* recorded significant p values only within TAP groups which showed higher changes in all color parameters including L*. While the smaller color differences which recorded statistical significancy wihtin L* values in the GMTA groups were not detected by ΔE*. Additionally, most of the previous studies detecting color changes for external discoloring materials were using the ΔE* values in their statistical comparisons (Evans, 2020). While, other studies that detected color changes caused by internal discoloring materials such as pulp treatement materials were much focused on the L* values in addition to ΔE* values (Kirchhoff et al., 2015). This possibly due to that the external discoloring material could cause faster and severer changes in the CIE Lab parameters detected by the spectrophotometer. On the other hand, the internal dicoloring materials could take longer time to be detected externally by the same device. Therefore, and according to the limitations of this study, the L* values rather than ΔE* has been determined within this study to detect the amout of sample discoloration.

In a clinical situation, the effect of the degenerative products from blood can not be easily excluded from the internal dicoloring materials. Moreover, the use of TAP is normally accompined with GMTA as part of the pulp revasularisation procedure. These can be considered as limitations for the present study, which only used totally blood free samples with only one pulp treatment material.

# *Chapter Five*

# *Conclusion and Suggestion*

# Chapter Five

# Conclusions and Suggestions

## 5.1 Conclusion

According to the results and limitations of the present study, the following conclusions can be drown:

1-Both intracanal materials (GMTA and TAP) produce tooth discoloration within the tested groups.

2- Both intracanal materials (GMTA and TAP) that used in this study cause decrease in color lightness within the tested groups.

3-The use of dentin bonding effectively increased color lightness within the first incubation period of GMTA samples. However, its effect on color lightness deteriorated with time.

4- Laser had less effect on color lightness within GMTA samples, but its efficacy after prolonged incubation was not affected.

5- The preventive measures used in this study (dentin boding and laser) had less effect in maintaining color lightness within TAP groups.

## 5.2. Suggestion

1- Studying the effect of dentin bonding and laser as preventive measures for tooth discoloration in clinical cases.

2- Studying the effect of both laser and dentin bonding together as a preventive procedure for tooth discoloration.

3- Using other materials as a surface treatment with more surface masking characteristics, such as resin cement or flowable composite with an opaquer constituent.

4- Using different bonding agents rather than the universal bonding agent.

5-Using another color measurement device.

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# الخلاصه

لا يزال تلون الأسنان من أكبر المشاكل الجمالية للأسنان التي تتعالج لبياً. هذا التلون يؤثر على النتيجة الجمالية عندما تحدث في المنطقة الجمالية. أجريت هذه الدراسة لتقييم فاعلية تقنيتين لإغلاق الأنابيب العاجيه السنية بواسطه( لاصق العاج والليزر) لتثبيط التلون الناتج عن معجون ثلاثي من المضادات الحيويه (TAP) وتجميع ثلاثي أكسيد الرمادي (GMTA). تم استخدام ستين ضواحك أولية مستخرجة من الفك العلوي في هذه الدراسة. تم إعداد تجاويف الوصول وتم إجراء المعالجه اللبية للقنوات الشدقية. ثم تم تقسيم العينات بشكل عشوائي إلى مجموعتين (ن = 30) كمجموعات TAP أو GMTA. تم تقسيم كل واحدة من هذه المجموعات إلى 3 مجموعات فرعية وفقًا للمعالجة السطحية لجدران غرفة اللب إلى: لاصق العاج ، ليزر ، أو تركت دون علاج كعنصر تحكم. تم تحليل نتائج اللون بأستخدام جهاز ( VITA Easyshade Advance) في الوقت الأساس وبعد 3 أسابيع و بعد 4 أشهر من حضانه العينات. تم حساب اضاءه اللون (L*) وفوارق اللون (Δ E*) خلال المراحل المختلفة من الدراسة وتحليلها. على الرغم من أن كلا من لاصق العاج والمعالجة بالليزر يزيدان من اضاءه اللون عند قياسات الأساس ، فقد انخفض مع مرور الوقت. في 3 أسابيع ، أوضحت مجموعة لاصق العاج الفرعية التابعة لـ GMTA أعلى درجة من اضاءه اللون مقارنةً بالآخرين. بعد فترة حضانة 4 أشهر ، أظهرت جميع المجموعات الفرعية انخفاضًا في اضاءه اللون مع وجود أعلى قيم تم اكتشافها داخل مجموعات TAP الفرعية. كانت القدرة الوقائية للاصق العاج والليزر على تغيير اللون التي تنتجها TAP و GMTA تعتمد على الوقت. لذلك ، لا يزال استخدام هذه المواد غير موصى به في المناطق الجمالية.

**جمهورية العراق**
**وزارة التعليم العالي والبحث العلمي**
**جامعة بغداد**
**كلية طب الأسنان**

# الفعالية الوقائية لاثنين من تقنيات اغلاق الأنابيب العاجيه ضد تلون الأسنان الناتجة عن MTA الرمادي ومعجون ثلاثي من المضادات الحيوية (مقارنة في دراسة مختبريه)

رسالة مقدمة الى مجلس كلية طب الاسنان في جامعة بغداد كجزء من متطلبات نيل شهادة الماجستير في معالجة الاسنان

قدمت من قبل
**فرح عبدالرزاق لازم**
بكالوريوس طب وجراحة الفم والأسنان

بإشراف
**أ.م.د. انس فلاح مهدي**
دكتوراه معالجة الاسنان

ذو الحجة، 1441 هـ  اب، 2020 م