AN INVITRO STUDY TO EVALUATE AND COMPARE TOOTH DISCOLORATION AFTER THE APPLICATION OF CALCIUM SILICATE BASED CEMENTS IN THE PRESENCE OR ABSENCE OF BLOOD USING SPECTROPHOTOMETER

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation titled **"AN INVITRO STUDY TO EVALUATE AND COMPARE TOOTH DISCOLORATION AFTER THE APPLICATION OF CALCIUM SILICATE BASED CEMENTS IN THE PRESENCE OR ABSENCE OF BLOOD USING SPECTROPHOTOMETER"**is a bonafide and genuine research work carried out by me under the guidance of **Dr. P. SHANKAR, M.D.S.,** Professor Department of Conservative Dentistry and Endodontics, Ragas Dental College and Hospital, Chennai.

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This dissertation is submitted to **THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY**, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY – CONSERVATIVE DENTISTRY AND ENDODONTICS, BRANCH IV**. It has not been submitted (partial or full) for the award of any other degree or diploma.

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LIST OF ABBREVIATIONS

SL.NO	**ABBREVIATIONS**	**DESCRIPTION**
1	wPMTA	White proroot MTA
2	BD	Biodentine
3	ERRM	Endosequence root repair material
4	EDTA	Ethylenediamine-tetraacetic acid
5	CB	Control blood
6	CS	Control saline
7	NaOCl	Sodium hypochlorite
8	ANOVA	Analysis of variance

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Introduction

INTRODUCTION

Tooth discoloration is the change in color of a tooth surface, either internally or externally, that presents a major aesthetic problem, especially if it involves the anterior teeth. Tooth discoloration caused by dental materials in endodontically treated teeth impairs the aesthetic outcome, as well as patient satisfaction.

Mineral trioxide aggregate (MTA) is a dental material that was first introduced in 1993 by Mohmoud Torabinajad et al of Loma Linda university for the purpose of root-end filling[34]. In 1998 Mineral trioxide aggregate (MTA) as a root canal repair material was approved by the US Food and Drug administration[25]. It is a mechanical mixture of three powder ingredients: Portland cement (75%), bismuth oxide (20%), and gypsum. The major component, Portland cement, is a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, and tetracalcium aluminoferrite [36,10].

This MTA is clinically prepared as a mixture of powder and water and the resultant is in a slurry form. This slurry form is placed in the tooth or teeth to be treated which then gradually hardens in the oral environment. Hydration of MTA material forms a hydrate gel of colloidal silica which sets in about 3-4 hours.

MTA is best for its high sealing ability, excellent biocompatibility and handling characteristic. It also has low cytotoxicity, antimicrobial properties, less

microleakage. MTA has one additional advantage that it has the ability to set in the presence of blood or moisture.[29] MTA was recommended for its use in vital pulp therapy, pulpotomy, apexification perforation repair and root-end fillings.

All dental materials will have their own drawback and one of the main drawbacks of MTA is its discoloration potential. It is the gray material which led to tooth color changes or discoloration on the tooth surface. White MTA was introduced to overcome this type of obstacle. The difference between the white and gray MTA is the lack of iron ions in the white MTA. But, even then, White MTA has also been found to cause discoloration. Gray and White MTA have Bismuth oxide, which was added to enhance the radio opacity was believed to be the cause of the discoloration. In addition, studies have shown that the interaction of MTA slurry with blood during its hydration may cause discoloration.[19,24] This may be because of hemolysis of erythrocytes and the accumulation of hemoglobin and hematin molecules within the dentin tubules. The hydration of MTA is slow and during the hydration process, MTA may permit the absorption and subsequent hemolysis of erythrocytes from the adjacent pulpal tissue, thus resulting in both material and tooth discoloration.

The potential tooth discoloration which is associated with the use of MTA has led to the search for a material similar to MTA that will not cause tooth discoloration. Consequently, manufacturers have competed in designing alternative new endodontic reparative material that have all the properties like

MTA without causing tooth discoloration. One such introduction is newer calcium silicate based cements. Here instead of bismuth oxide, manufacturers have added zirconium oxide as the radiopacifying agent. They are indicated in use of direct and indirect pulp capping, apexification, as root canal sealer, for perforation repair, and as a retrograde canal filling material. These biomaterials provide a tight barrier against the migration of microorganisms, and they stimulate tissue healing without causing inflammation. Biodentine and Endosequence Root Repair paste material are those calcium silicate based materials included in this study.

Biodentine (Septodont, Saint Maur des Foss_es, France), a newly developed Calcium silicate based material, has endodontic indications and properties similar to MTA. Manufacturers claims to exhibit dentin like mechanical properties and can be used as a dentin substitute on crowns and roots. Biodentine is dispensed in a powder liquid form in a single-dose capsule to be triturated in an amalgamator for 30 seconds . The powder is composed of tricalcium silicate, dicalcium silicate which are the main core material of biodentine. Calcium carbonate acts as a filler,zinc oxide provides the shade and zirconium oxide as the radiopacifier, whereas liquid contains calcium chloride as the setting accelerator, a modified polycarboxylate (a super-plasticizing agent). and water-reducing agent. Compared with MTA cements, Biodentine received good ratings for material handling and performance after restoration placement

and has significantly short setting time of 12 minutes. It is a tricalcium silicate cement that promotes pulp healing and remineralization by the production of reactionary dentin and dentin bridges. On the biological level, it appears to be biocompatible and capable of inducing dentin apposition by stimulating odontoblast activity. [42]

Another product is EndoSequence Root Repair Material (ERRM) (Brasseler USA, Savannah, GA), which is dispensed in a premixed, ready-to-use, regular-set putty form and as an injectable fast-set paste form. They contain calcium silicates, zirconium oxide, tantalum pentoxide, calcium phosphate monobasic, and filler agents. However, the particle size in the injectable paste is significantly smaller, which accelerates the setting reaction. The manufacturer describes Endosequence as having antibacterial properties during its setting reaction because of a high alkaline pH. These properties would support its use as an alternative to Calcium hydroxide or MTA in the treatment of external inflammatory root resorption. ERRM is a biocompatible material with good sealing ability and had a better tissue healing response than MTA. They are recommended for perforation repair, apical surgery, apical plug, and pulp capping. They are hydrophilic, insoluble, radiopaque, aluminum-free, and of high pH. Presence of moisture is required for the materials to set and harden. The working time is more than 30 minutes, and the setting time is 4 hours in normal conditions.

Color changes are typically evaluated with a spectrophotometer. Visual spectrophotometry is considered the gold standard for the evaluation of color, and it has been successfully used in dentistry. The parameters of colors are represented with the CIELAB color space.

Each color in the CIELAB sytem was described by the 3 parametes: L, or lightness, ranging 0 (black) to 100 (white); a, indicates green to red; and b, indicates blue to yellow. Delta E values describes the final color which represented the difference between the final and baseline values. Spectrophotometer with CIELAB system is the most widely used method of determining color in dentistry; It meets international standards, and it is compliant with ISO standards.[9]All these materials are known to cause discoloration of tooth in the presence of blood when used as pulp capping agent, as a sealer , root repair and perforation material.

The aim of this invitro study was to evaluate and compare the degree of coronal tooth discoloration of extracted human anterior teeth caused after the application of white ProRoot MTA, Biodentine, and ERRM paste in the presence or absence of blood using spectrophotometer over a time period of 24 hours , after 1 month and after 6 months.

Aim and Objectives

AIM AND OBJECTIVES

AIM:

The aim of the present invitro study was to evaluate and compare tooth discoloration after the application of three different cemental barrier material in the presence or absence of blood using spectrophotometer

OBJECTIVES:

Objectives of the present study were as follows :-

1. To compare the degree of tooth discoloration associated with calcium silicate based barrier materials used in vital pulp therapy.
2. To compare the tooth discoloration in the presence and absence of blood.
3. To compare the degree of discoloration on the composite material in the presence and absence of blood.
4. To compare the degree of tooth discoloration following placement of the 3 barrier materials and composite resin in the presence and absence of blood.
5. To design a model for this invitro study simulating vital pulp therapy.
6. To assess the effect of fresh human whole blood on the discoloration of tooth following the placement of composite resin and barrier materials.
7. To use an external light source, spectrophotometer for color analysis.

8. To use the color measurement tool, the spectrophotometer without causing any shadow with in the visible spectrum wavelength and measuring the reflection coefficient of light with in the visible spectrum wavelength.
9. To use the CIELAB system, a 3 dimensional system for quantitative analysis of color change perceivable to the human eye.
10. To use a method for the numeric evaluation of color using the 3 parameters L*, a* and b*.
11. To express numerically the color difference between 2 objects calculated in the present study as ΔE values denoting the perceivable color change.
12. To quantitatively assess the color change for the 3 different material used in the study at 3 different time intervals

    - After completion of 24 hours.
    - After 30 days or 1 month.
    - After 180 days or 6 month.

13. To analyse the effect of 3 different barrier materials based on their composition to cause discoloration in the presence and absence of blood.
14. To analyse the effect of the 3 barrier materials on the discoloration in the presence and absence of blood over the 3 testing periods.

15. To compare quantitatively the changes in the parameters of color due to composition of the 3 tested barrier materials used in the presence and absence of blood.
16. To compare quantitatively the change in color parameters for the 3 materials tested over different selected times.
17. To compare the following parameters of color using numerical value.

    - ΔL* (change in translucency)
    - Δa* (change in green/red channel)
    - Δb* (change in blue/yellow channel)
    - ΔE*(change in tooth discoloration)

18. To statistically analyse the changes in color parameters due to the presence and absence of blood contamination due to the components of 3 different materials tested with the 2 control groups.
19. To statistically analyse the changes in the parameters of color in the presence and absence of blood for the 3 tested materials and 2 controls at 3 different time periods (24 hours, 1 month and 6 months)
20. To compare and discuss the behavior of the tested barrier materials in the presence and absence of blood and reason out the probable cause for the discoloration due to blood contamination during vital pulp therapy.

Review of Literature

REVIEW OF LITERATURE

Thomas R. Pitt Ford et al (1995) investigated the histologic response to intentional perforation in the furcations of mandibular premolars in seven dogs. In half the teeth, the perforations were repaired immediately with either amalgam or mineral trioxide aggregate; in the rest the perforations were left open to salivary contamination before repair. Results showed that the mineral trioxide aggregate is a far more suitable material than amalgam for perforation repair, particularly when used immediately after perforation.

Marin et al (1997) investigated tooth staining following pulpal haemorrhage. Samples of whole blood, erythrocytes, plasma and platelet concentrate and saline were individually placed in the pulp chambers of groups of five teeth and centrifuged twice daily for 25 min over a period of 3 consecutive days. He confirmed that the blood pigment responsible for the staining was found only in those samples containing erythrocytes. The tests done by Marin et al showed that, following haemolysis of erythrocytes within dentine, haemoglobin was found either intact or as one of the haematin molecules with no further breakdown ofmthe haem structure and no evidence of any free ferric ions or haemosiderin.

Mahmoud Torabinejad et al (1999) investigated mineral trioxide aggregate (MTA), as a potential alternative restorative material to the presently used materials in endodontics. Several in vitro and in vivo studies have shown that MTA prevents microleakage, is biocompatible, and promotes

regeneration of the original tissues when it is placed in contact with the dental pulp or periradicular tissues. He described the clinical procedures for application of MTA in capping of pulps with reversible pulpitis, apexification, repair of root perforations nonsurgically and surgically, as well as its use as a root-end filling material.

Craig Main et al (2004) evaluated the success rate of root perforation repairs using MTA and the results showed that all 16 cases demonstrated normal tissue architecture adjacent to the repair site at the recall visit. Teeth with existing lesions showed resolution of the lesion, and teeth without preoperative lesions continued to demonstrate absence of lesion formation at the follow-up visit. He then concluded MTA provides an effective seal of root perforations and shows promise in improving the prognosis of perforated teeth that would otherwise be compromised.

Ahmed Al-Kahtani et al (2005) evaluated the seal created by varying depths of mineral trioxide aggregate (MTA) plugs placed in an orthograde fashion in five groups of 10 teeth. One group received a 2 mm thick orthograde apical plug of MTA, the second group a 5 mm apical MTA plug, and the third group a 2 mm apical MTA plug with a second 2 mm increment, 24 h later. The remaining portion of the canal in these groups was left unfilled. Group four received a 2 mm MTA plug that set for 24 h and the canal was then back-filled with guttapercha and eugenol based sealer. Group five was a positive control without an MTA plug. The apical seal was tested using a bacterial leakage model of Actinomyces viscosus. Results showed a

statistically significant difference in only the 5 mm apical plug, which completely prevented bacterial leakage.

W. T. Felippe et al (2006) evaluated the influence of mineral trioxide aggregate (MTA) on apexification and periapical healing of teeth in dogs with incomplete root formation and previously contaminated canals and to verify the necessity of employing calcium hydroxide paste before using MTA. He concluded that Mineral trioxide aggregate used after root canal preparation favoured the occurrence of the apexification and periapical healing. The initial use of calcium hydroxide paste was not necessary for apexification to occur, and has shown to be strongly related to the extrusion of MTA and formation of barriers beyond the limits of the root canal walls.

Claudio E. Iwamoto et al (2006) evaluated teeth clinically and histologically using white proroot mta in direct pulp capping. white MTA were diagnosed as clinically successful, i.e. an absence of clinical symptoms and did not show evidence of periapical pathosis. Histologically dentin bridge had developed. Iwamoto et al concluded that white ProRoot MTA was equally successful as calcium hydroxide when used for direct pulp capping in mechanically exposed teeth.

Jin-Seon Song et al (2006) observed that difference between white and gray MTA was the lack of iron ions in white MTA. the principal components of the gray-colored formula are tricalcium silicate, bismuth oxide, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, and calcium sulfate dehydrate, and the white-colored formula lacks the

tetracalcium aluminoferrite. The fluxing agent is used for production of the white version to remove the ferrite phase during the clinkering process. Portland cement differed from MTA by the absence of bismuth ions and presence of potassium ions. In addition, ProRoot MTA appeared to have more homogeneous composition than Portland cement and gray MTA-Angelus.

Ilya Belobrov et al (2011) describes the treatment of tooth discoloration caused by white MTA used for the management of a complicated crown fracture. A partial pulpotomy was performed with the use of WMTA after a complicated crown fracture of the upper right central incisor. Seventeen months later, upon access, the WMTA was completely discolored. The WMTA was removed because of tooth discoloration, and internal bleaching was performed. The tooth remained vital, and a dentin bridge was confirmed clinically and radiographically. He concluded that WMTA used for vital pulp therapy in the esthetic zone may need to be reconsidered.

Stephen W. Hansen (2011) Intracanal mineral trioxide aggregate may provide an alternative to calcium hydroxide in the treatment of external inflammatory root resorption. This in vitro study using human matched pairs of teeth compared white ProRoot MTA and EndoSequence Root Repair Material, by measuring pH in simulated root surface resorptive defects after intracanal placement and concluded, in matched pairs of teeth, intracanal placement of WMTA compared with ES resulted in a higher pH in simulated root resorption defects that was time and root level dependent.

Karen F. Lovato et al (2011) has done an in vitro study to determine whether proroot mta, endosequence root repair material putty and endosequence root repair syringeable paste possess antibacterial properties against a collection of Enterococcus faecalis strains recovered from root canal infections and concluded that ESP, ESS, and MTA had similar antibacterial efficacy against clinical strains of E. faecalis. Clinical strains varied in their susceptibility to the root repair materials.

Daniel Felman et al (2013) characterized discoloration when white MTA was placed in the coronal aspect of the root canal ex vivo and the influence of red blood cells on this discoloration. Color was assessed using standardized digital photographs. All teeth discolored when restored with wMTA, which was most prominent in the cervical third of the crown. The presence of blood within the canal adjacent to the setting wMTA exacerbated the discoloration.

Konstantinos Ioannidis et al (2013) studied chromatic alterations in human tooth crowns induced by a Mineral Trioxide Aggregate-based sealer and a commonly used ZnOE-based sealer . Mandibular third molars were selected and sectioned 1 mm below the cemento-enamel junction and the pulp chambers were chemomechanically debrided via the cervical access. The specimens were randomly assigned into three groups Group 1: MTA Fillapex, Group 2: Roth 811, Group 3: control and evaluated with UV-VIS spectrophotometer. He observed MTA Fillapex resulted in minimal color

alterations than ZnOE-based sealer and suggested that, in terms of aesthetics, the use of MTA Fillapex appears to be favorable.

Ji-Hyun Jang et al (2013) evaluated tooth discoloration after the use of mineral trioxide aggregate (MTA) and to examine the effect of internal bleaching on discoloration associated with MTA. He obnserved that the ProRoot and Angelus groups displayed increasing discoloration during a period of 12 weeks. The discoloration associated with ProRoot and Angelus was observed at the MTA-dentin interface and on the interior surface of the dentin. He concluded then ProRoot and Angelus caused tooth discoloration. However, Endocem did not affect the contacting dentin surface. Removing the discolored MTA materials contributed more to resolving the tooth discoloration than post-treatment internal bleaching.

Marina Angélica Marciano et al (2013) studied if the increase in radiopacity provided by bismuth oxide is related to the color alteration of calcium silicate-based cement. Calcium silicate cement was mixed with 0%, 15%, 20%, 30% and 50% of bismuth oxide (BO), determined by weight and Mineral trioxide aggregate was the control group. The assessments were performed using a spectrophotometer to obtain the ΔE, Δa, Δb and ΔL values. He concluded that increase in radiopacity provided by bismuth oxide has no relation to the color alteration of calcium silicate-based cements.

Marta Vall'es et al (2013) used five different calcium silicate cements namely ProRoot WMTA, Angelus WMTA, White Portland Cement [PC], PC

with bismuth oxide, and Biodentine. They exposed each group of cements to combined environment of light and anaerobic condition and evaluated the color change in each specimens in different time period through spectrophotometer. From the results he concluded Biodentine and Portland cement demonstrated color stability than the other cements such as ProRoot WMTA, Angelus WMTA, White Portland with bismuth oxide.

Todd Berger et al (2014) investigated the role of bismuth oxide, a constituent of contemporary mineral trioxide aggregate (MTA) materials, and its response to various solutions that may contribute to the potential discoloration. He observed that all forms of ProRoot MTA showed discoloration and concluded that exposing MTA in various forms to a variety of liquids has determined that bismuth oxide in combination with other chemical moieties is the prime cause of staining.

Josette Camilleri et al (2014) viewed that Immersion of white MTA and bismuth oxide in sodium hypochlorite resulted in the formation of a dark brown discoloration. This change was not observed in Portland cement. He concluded that Contact of white MTA and other bismuth-containing materials with sodium hypochlorite solution should be avoided.

Hannah Beatty et al (2015) compared tooth discoloration between ProRootMTA, Biodentine, and EndoSequence Root Repair Material. He used bovine mandibular incisors and prepared them from the apical aspect after root resection. Canals were prepared with sequentially larger ParaPost drills coronal to the cementoenamel junction. Experimental materials were

condensed into the crowns and the access sealed. Color was assessed at various times up to 2 months according to the CIE L*a*b* color space system and concluded that BioDentine and EndoSequence root repair material discolor bovine tooth structure to a perceptible degree. At 8 weeks, this was significantly more than ProRootMTA .

Josette Camilleri et al (2015) evaluated three materials namely Neo MTA Plus (Avalon Biomed Inc, Bradenton, FL), MTA Plus(Avalon Biomed Inc), and Biodentine (Septodont, Saint-Maur-des-Foss_es, France) that are used for pulpotomy procedures in immature permanent teeth to view their color stability in the presence of sodium hypochlorite.he compared the color stability using photography, spectrophotometry, and X-ray diffraction analysis. He concluded all materials used in the study are suitable to be used pulpotomy procedure of immature teeth as all the material produced calcium hydroxide as their byproduct in their early stage.but when comparing color stability Neo MTA Plus and Biodentine are suitable alternatives to MTA, and they do not exhibit discoloration.

Behnaz Esmaeili et al(2015) compared the discoloration potential of calcium enriched mixture cement, white mineral trioxide aggregate and calcium hydroxide, after placement in pulp chamber. The highest ΔE value belonged to WMTA group. They concluded that CEM cement may be the material of choice in the esthetic region, specifically pertaining to its lower color changing potential compared to WMTA.

Shin-Hong Kang et al (2015) compared the discolorationof these various MTA-based materials and concluded less discoloration was observed with ENDOCEM Zr and RetroMTA which contain zirconium oxide than with ProRoot MTA and MTA Angelus which contain bismuth oxide

Zohreh Khalilak et al (2015) The aim of this *in vitro* study was to compare discoloration induced by tooth colored mineral trioxide aggregate (MTA) and calcium-enriched mixture (CEM) cement in extracted human teeth. Color measurement was carried out by spectrophotometry and observed that tooth discoloration was similarly detectable with both of the two experimental materials.

Marta Vall_Es et al (2015) assessed the color stability of teeth restored coronally with WMTA or Biodentine under artificial light. In this invitro study he prepared cavities on coronal tooth and restored with WMTA + composite, Biodentine + composite, or composite alone. Color was assessed spectrophotometrically at 6 time points (initial, 1 week, 2 weeks, 1 month, 3 months, and 6 months), and color difference values were calculated. He concluded teeth treated with WMTA exhibited discoloration, whereas those treated with Biodentine maintained color stability throughout the study.

S. Alsubait et al (2016) compared the discoloration potential of Endosequence Root Repair Material putty and ProRootMTA when placed coronally in human extracted teeth over a 4-month period. Premolars were selected for this study and sectioned 2mm below the cementoenamel junction. The pulp chambers were cleaned chemo-mechanically. The specimens were

randomly assigned to three groups: Endosequence Root Repair Material putty and ProRootMTA and control group. Tooth color was measured spectrophotometrically at six time points: after material placement, after 2,4,8,12,and16 weeks. Results showed ProRootMTA group exhibited a significantly higher discoloration when compared with the Endosequence Root Repair Material putty and control groups. He concluded teeth restored using PMTA exhibited a visually progressive dark discoloration. The control and ERRMF group specimens exhibited color stability.

Christian A. Dettwiler et al (2016) viewed to investigate the discoloration potential of different endodontic cements, dressings, and irrigants used in dental traumatology. The specimens were selected and the cavities were filled with a range of endodontic materials, sealed with composite and stored in physiological saline. The color of the labial enamel surface was measured with a spectrophotometer at 7 time intervals. After 12 months, significant staining was observed among the endodontic cements only in the Portland cement group with additional bismuth oxide .other commercially available calcium silicate cements containing bismuth oxide were not significantly discolored. He concluded that the presence of bismuth oxide in calcium silicate cements was not shown to be a reliable predictor for tooth discoloration.

JOAO CARLOS RAMOS et al (2016) compared tooth discoloration that occurs in teeth filled with ProRoot MTA or Biodentine over the course of 1 year and found that all groups revealed perceptible color changes between

immediately after material filling and after 6 weeks and after 6 weeks and 1 year. After 1 year, no differences could be detected between Biodentine and WMTA. He concluded then delayed tooth discoloration was detected for the 2 materials at the 1-year evaluation, but it was more evident for ProRoot MTA than Biodentine. Luminance was the most affected parameter, with a higher decrease for ProRoot MTA

Noushin Shokouhinejad et al (2016) compared the discoloration potential of mta namely ProRoot MTA (Dentsply Tulsa Dental Products, Tulsa, OK) and calcium silicate based material such as Biodentine (Septodont, Saint Maur des Foss_es, France), OrthoMTA (BioMTA, Seoul, Korea), and EndoSequence Root Repair Material (ERRM; Brasseler, Savannah, GA) in the presence or absence of blood. He concluded all materials showed discoloration in the presence of blood but biodentine and errm showed significantly less tooth discoloration in the absence of blood.

SCHEMBRI WISMAYER et al (2016) compared the setting of MTA *in vitro* and *in vivo* in contact with blood by subcutaneous implantation in rats. The tissue reaction to the material was also investigated. He impanted ProRoot MTA in the subcutaneous tissues of Sprague- Dawley rats in opposite flanks and left *in situ* for 3 months. Furthermore the material was also stored in physiological solution *in vitro*. At the end of the incubation time, tissue histology and material characterization were performed. He observed that the tissue histology showed a chronic inflammatory cell infiltrate associated with the MTA.

Selen Esin Yoldas et al (2016) evaluated and compared the discoloration potential of 3 different tricalcium cements using a bovine tooth model. He used 4 groups namely BioAggregate, Biodentine, mineral trioxide aggregate Angelus, and only blood.Crowns separated from the roots and materials have been placed to the standardized cavities on the lingual surfaces of the crowns, and their contact with blood has been provided. The color values of the samples were measured with a digital tooth shade before the placement of the materials, after the placement of the materials, in the 24th hour, in the first week, in the first month, in the third month, and in the first year. Results showed all groups displayed increasing discoloration during a period of the first year. The ‘‘only blood group’’ showed the highest color change values, and it was followed as Bio-Aggregate, mineral trioxide aggregate Angelus, and Biodentine, respectively. He finally concluded that Biodentine is found to have the least discoloration potential among the tested materials.

Rashmi Keshav Chahande et al (2017) evaluated the coronal discoloration effect of Apexit Plus and Resino Seal in mandibular premolars using spectrophotometer and concluded that Resino seal sealer has greater potential to cause discoloration of crown as compared to apexit plus sealer over a period of time.

C. Farrugia et al (2017) used proroot mta material disc to view the antimicrobial activity of Portland cement based material. He used material disc to evaluate the antimicrobial activity of proroot mta following contact

with water, or heparinized blood after 1 day and 7 days aging. Before doing evaluation he observed severe discoloration of the material disc when in contact with blood.

Marina Angelica Marciano et al (2017) investigated the addition of variable amounts of zinc oxide to inhibit dental discoloration caused by mineral trioxide aggregate.

He used MTA Angelus and MTA with additions of 5%, 15%, and 45% zinc oxide in weight were tested the set cements using a combination of scanning electron microscopy, energy dispersive spectroscopy, and x-ray diffraction. The pH and calcium ion release were measured after 3 hours, 24 hours, and 28 days.Dental discoloration in contact with the cements was measured after 24 hours, 28 days, and 90 days. The results showed addition of ZnO did not alter significantly the radiopacity, setting time, volume change, pH, and biocompatibility compared with MTA Angelus. He concluded the addition of 5%, 15%, or 45% zinc oxide to MTA Angelus inhibits dental discoloration without modifying the radiopacity, setting time, volume change, pH, and biocompatibility.

Da-A Yun et al (2018) evaluated tooth discoloration induced by contact with various calcium silicate based pulp capping materials in the presence or absence of blood *in vitro* using ProRoot, Endocem, or EndocemZr and concluded that ECZ which contains zirconium oxide as a radiopacifier showed less discoloration irrespective of blood contamination compared to PR and EC.

Materials and Methods

MATERIALS AND METHODS

Armamentarium

- 66 maxillary anterior teeth
- White Proroot MTA (MTA; Dentsply Tulsa Dental, Johnson City, TN)
- Biodentine (Septodont, Saint Maur des Fosses, France)
- Endosequence root repair material (Brasseler USA, Savannah, GA).
- 5.25% sodium hypochlorite solution(Chemdent)
- Normal saline (Eurolife)
- cotton
- Ultrasonic scaler and tip (Acteon satelec P5 booster)
- Water
- Pumice powder
- High speed diamond fissure bur (Mani size SF-11)
- Gates Glidden drills (Mani size #1 to #6)
- 17% EDTA(Prime Dental)
- Plastic white foam
- MD Temp (meta biomed)
- Self etch bonding agent (G bond GC)
- A2 SHADE Composite materials (G aenial anterior GC)
- Light curing unit
- Self cure Acrylic resin (DPI)
- Spectrophotometer

METHODOLOGY

Sample collection

A freshly extracted sixty six single rooted permanent human anterior teeth that was extracted for periodontal reason were selected for the study. For disinfection,the teeth were immersed in 5.25% sodium hypochlorite solution for 1 hour and then stored in normal saline solution until use.

Inclusion criteria

Maxillary anterior teeth with fully formed apex having one separate straight canal were selected for the experimental procedures.

Exclusion criteria

Teeth that is free of caries, cracks, restorations,and discolorations are excluded.

Sample preparation

Sixty six maxillary anterior teeth were selected and all external surfaces of each tooth were visually inspected. Extrinsic debris and stains were removed using an ultrasonic scaler followed by polishing with pumice paste and water.

Each tooth sample were kept into a transparent cover and numbered from 1 to 66 with permanent marker for preoperative color assessment before the application of material.

For creating standardized specimens, the apical part of each root was removed perpendicular to its long axis with a high-speed diamond fissure bur

(#010) under a continuous water spray until 5 mm of root remained. Endodontic access cavities were prepared using endo access bur and the root which is shortened to 5mm was cleaned and shaped with gates glidden drills size #1 to 6. The canal was then irrigated with 5.25% sodium hypochlorite solutions which is then followed by 17% EDTA for 1 minute. Finally the tooth was rinsed with normal saline solution. A white plastic cylindrical foam which is 3 mm in height and 2mm in breadth was made. Then in all the experimental teeth this cylindrical piece of plastic foam was inserted into the root canal through the apical opening up to the cementoenamel junction of the labial surface. The apical opening of the root canals and the total surrounding dentin of the cross sectional surface were applied with 3M ESPE ADPER easy bond self-etch adhesive and light cured for 20 seconds. After that, composite resin material was placed incrementally two times and this two increments were cured using a light emitting diode curing light, for 40 seconds.

The experimental teeth filled with composite apically and with the white cylindrical plastic foam were kept mounted in acrylic block measuring 10mm×10mm. Each block is numbered individually beneath the block.

Blood Collection

Whole fresh human blood was collected from a volunteer by venipuncture by a specialist general surgeon. 5ml blood was collected in a collection tube that were sterile and spray coated with the anticoagulant EDTA to prevent clotting in order to facilitate the experiment.

Experimental Setup

The experimental teeth were randomly divided into 2 groups. The inserted foam in each specimen was then saturated with normal saline solution (group 1) and fresh human blood (group2), respectively, using an insulin syringe to prevent contamination within the access cavity. The specimens of each experimental group were then randomly assigned A, B, AND C of experimental subgroups (n = 10) and labeled according to the following applied materials:

Group 1A - ProRoot MTA in the presence of normal saline

Group 1B - Biodentine in the presence of normal saline

Group 1C - ERRM in the presence of normal saline

Group 2A - ProRoot MTA in the presence of blood

Group 2B- Biodentine in the presence of blood

Group 2C- ERRM in the presence of blood

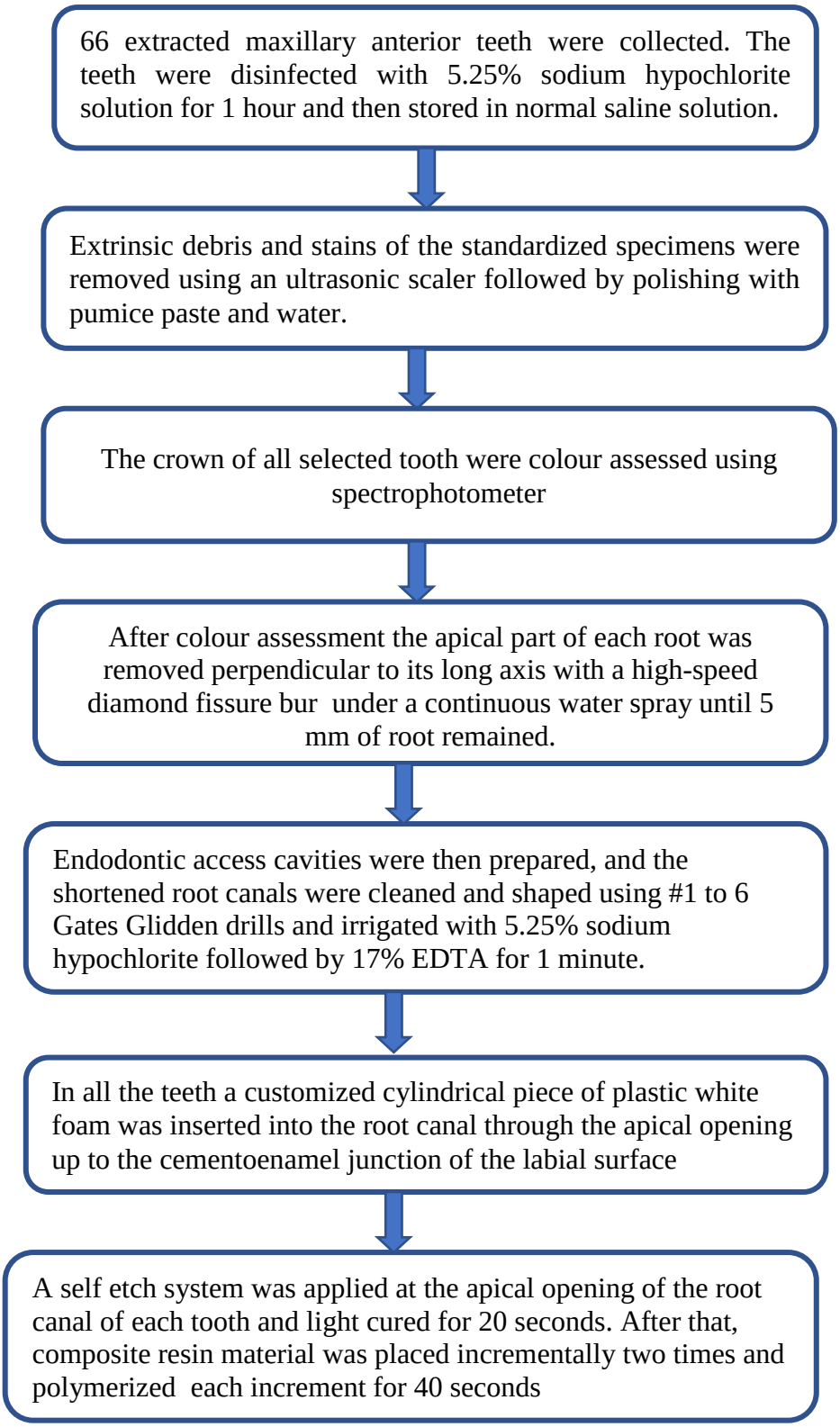
Each material was prepared according to the manufacturers' instructions and a 3-mm increment of each material was then placed inside the access cavities using a flat plastic instrument used with minimal pressure to ensure contact with the coronal surface of the plastic foam insert.

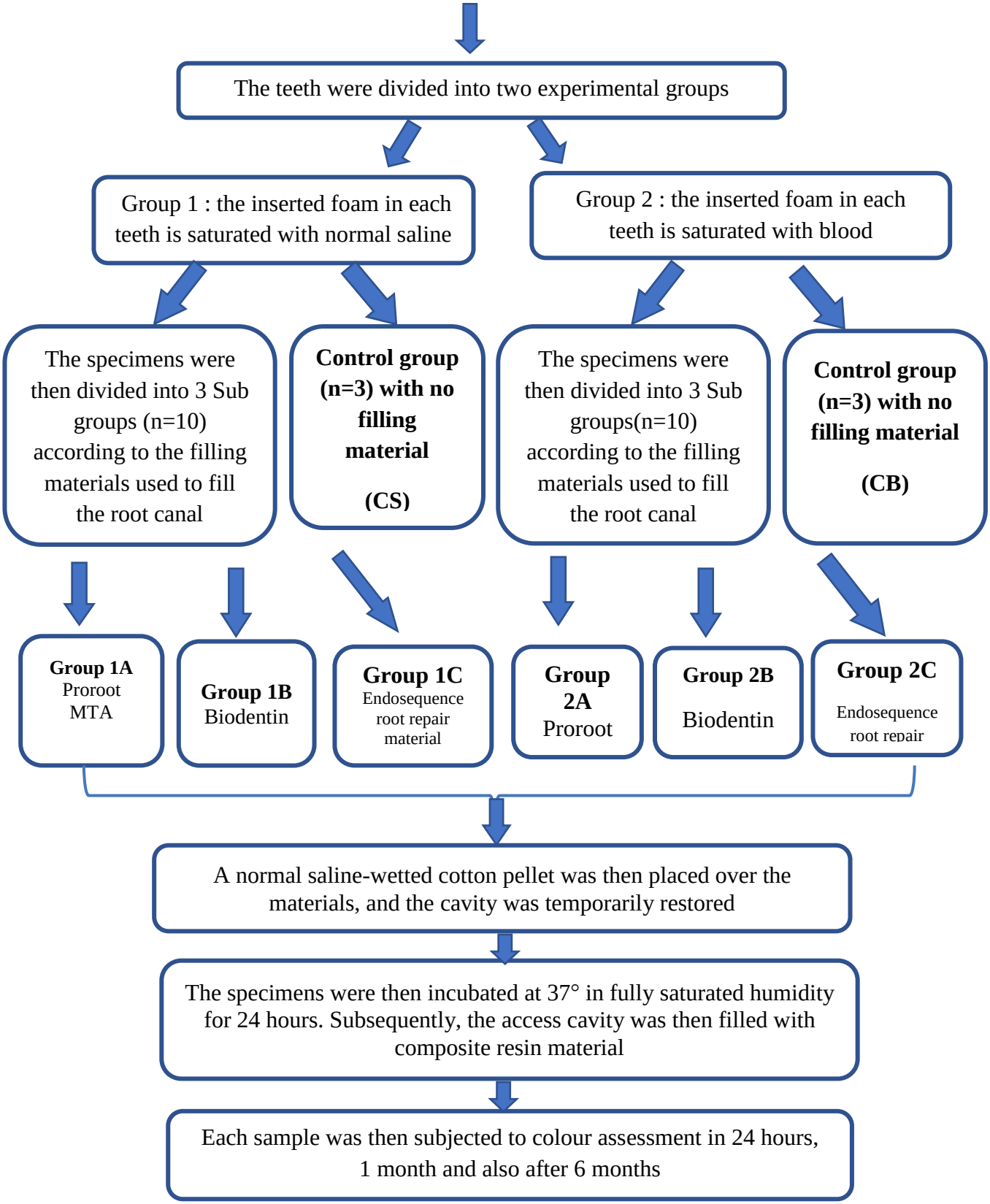
A normal saline-wetted cotton pellet was then placed loosely over the materials of the prepared canal from the apical access, and the cavity was temporarily restored with MD-Temp. The specimens were then incubated at 37□ in fully saturated humidity for 24 hours. Subsequently, after 24 hours the

MD-Temp was removed with a slow speed handpiece and the access cavity was then filled coronally with 2 increment , 2mm, thick composite resin material, A2 shade as described for sealing the apical opening.

In the remaining 6 teeth, the customized plastic foam was placed up to the level of the cementoenamel junction of the labial surface and then saturated with normal saline (n = 3) or blood (n = 3), and the access cavity was filled with composite resin material. The specimens were kept ready for further evaluation with spectrophotometer after 24 hours, 1month and 6^{th} month.

FLOWCHART ILLUSTRATING THE METHODOLOGY OF THE STUDY




The teeth were divided into two experimental groups
Group 1 : the inserted foam in each teeth is saturated with normal saline
Group 2 : the inserted foam in each teeth is saturated with blood
The specimens were then divided into 3 Sub groups (n=10) according to the filling materials used to fill the root canal
Control group (n=3) with no filling material
(CS)
The specimens were then divided into 3 Sub groups(n=10) according to the filling materials used to fill the root canal
Control group (n=3) with no filling material
(CB)
Group 1A Proroot MTA
Group 1B Biodentin
Group 1C Endosequence root repair material
Group 2A Proroot
Group 2B Biodentin
Group 2C Endosequence root repair
A normal saline-wetted cotton pellet was then placed over the materials, and the cavity was temporarily restored
The specimens were then incubated at 37° in fully saturated humidity for 24 hours. Subsequently, the access cavity was then filled with composite resin material
Each sample was then subjected to colour assessment in 24 hours, 1 month and also after 6 months

Figures

FIGURE 1: Extracted maxillary anterior teeth used in this study

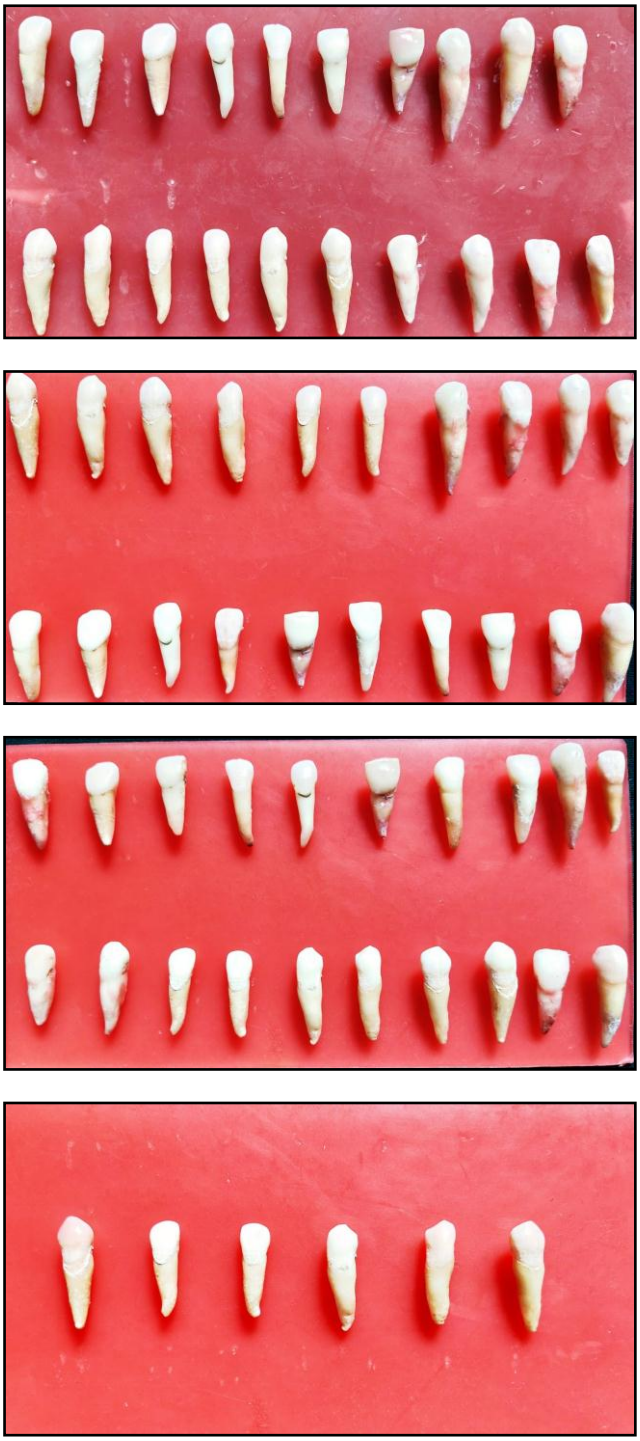


FIGURE 2: Armamentarium

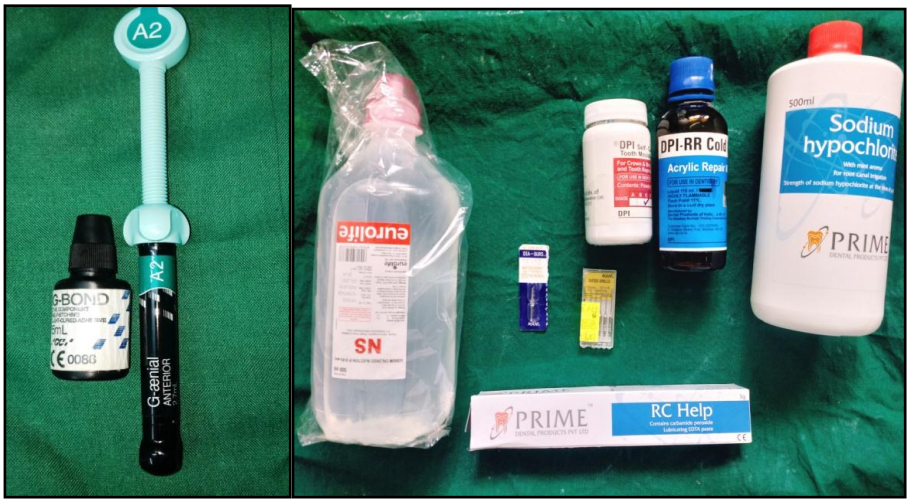


FIGURE 3: Calcium silicate based cements used in this study

FIGURE 4: The prepared specimens which kept mounted in acrylic block

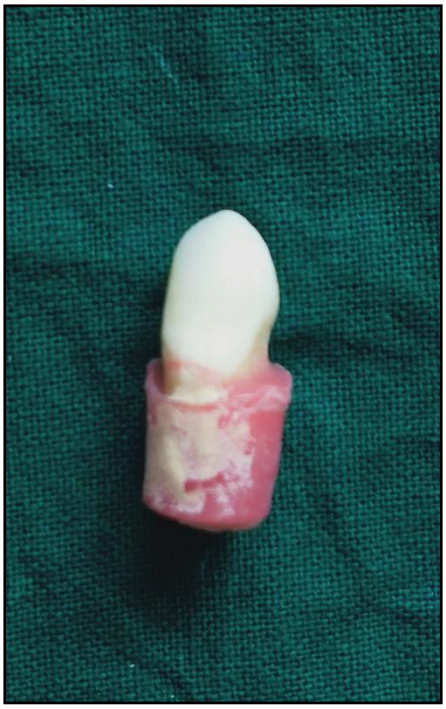


FIGURE 5: The entire labial coronal surface of the specimen to be visualized for spectrophotometer examination with white back ground

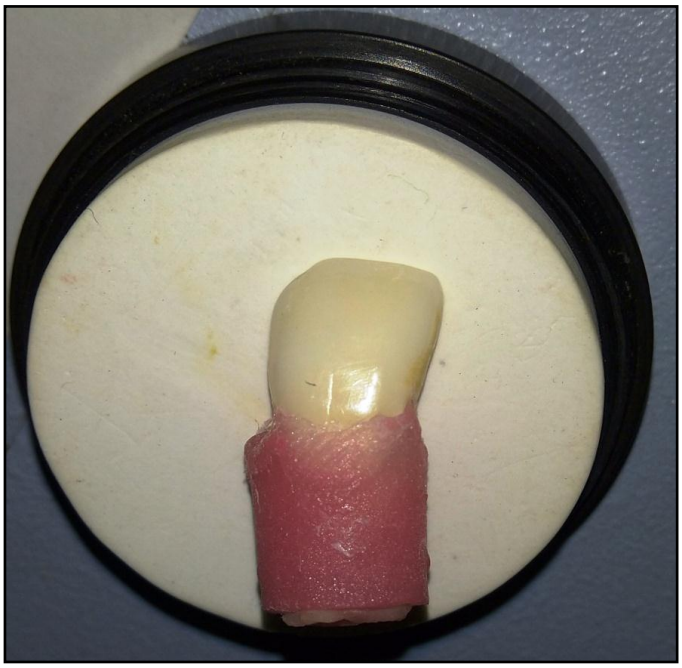
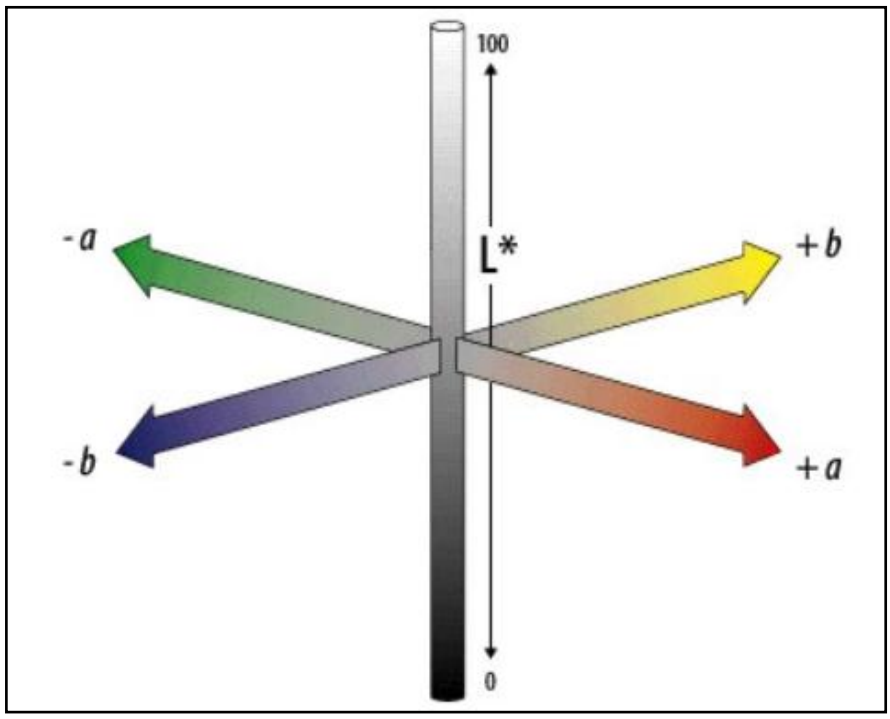


FIGURE 6: Spectrophotometer

FIGURE 7: CIELAB parameters



The L* parameter corresponds to the range of lightness to darkness, the a* parameters corresponds to the range in green/red channel and the b* parameter corresponds to the range in blue/yellow channel

Results

RESULTS

Results of the present study was subjected to statistical analysis to interpret the discoloration of tooth ΔE on the labial coronal part of the tooth after measuring 3 color parameters namely translucency denoted by ΔL, Δa (green/red channel) and Δb (blue/yellow channel) after placement of three calcium silicate based cement white proroot MTA, Biodentine and Endosequence root repair material to correlate with control saline and control blood group. Repeated measures ANOVA was used to interpret comparison of materials with saline and blood group

Bonferroni Post Hoc test was used for multiple comparison of materials within the group and between the groups at different time period. Paired T-Test was used to find significance among each groups

Bonferroni post-hoc test is needed after repeated measures ANOVA in order to determine which groups of materials differs from each other. For the Bonferroni post-hoc test the mean difference between all the groups of materials were found. Mean Difference is compared to a critical value so as to see whether the difference is significant.

The paired sample t-test is used to compare mean of two groups where two samples are present in which observation in one sample can be paired with observations in other samples. In a paired sample t-test each subject or entity is measured twice, resulting in pairs of observation ΔE* Blood contamination significantly increased the ΔE* value of all the materials tested

in accordance with time duration and type of materials used. Irrespective of the type of material in the presence of blood ΔE* was significant (P< .05) in correlation with time. The materials tested after 24 hours, Biodentine showed significantly less color change than proroot MTA followed by errm in the presence of blood. After 1st month and 6th month proroot MTA and errm showed significantly increased ΔE* than biodentine .

In the absence of blood ΔE* of Biodentine and ERRM paste was significantly less than prorootMTA after 1st and 6th month.

ΔL*

ΔL* significantly decreased in all the experimental groups over time (P< .05). Irrespective of the type of material and time ΔL* significantly decreased in the presence of blood contaminated group. ΔL* of biodentin showed significant lightness than proroot MTA and ERRM paste. Proroot MTA exhibited significantly decreased ΔL* value than biodentine and ERRM after 1st month where as after 6 months no significant difference was observed between ProrootMTA and ERRM.

In the absence of blood no significant difference was noted among the tested experimental groups

<u>Δa*</u>

Δa* denotes the change in color observed in the green /red channel and denoted by a numerical value. The change in a* denoted as Δa, is dependent on the value of a*, before and after the comparison of change in color in the green/red axis of the CIELAB numerical system

Δa* significantly was seen to be in the red range of the green/red channel for all the three materials tested at all time periods except in the control saline group in the first 24 hours where it was significantly closer to the baseline value. Maximum significance towards the red axis was present in the control blood group at the end of 24 hours.

Δb*

Δb*denotes the change in color observed in the blue/yellow channel and denoted by a numerical value. The change in b* denoted as Δb, is dependent on the value of b*, before and after the comparison of change in color in the blue/yellow axis of the CIELAB numerical system.

Δb showed significant change towards the yellow axis of the blue /yellow channel for all the three materials tested at all time periods. Maximum significance towards the yellow axis was present in the control blood group after 24 hours. Both wPMTA and ERRM. Δb* was significantly high with wPMTA at the end of 6 months.

Tables and Graphs

Table 1

CONTROL SALINE												
	L*	24 HRS	IST MONTH	6TH MONTH	A*	24 HRS	IST MONTH	6TH MONTH	B*	24 HRS	IST MONTH	6TH MONTH
1	76.8	76.4	74.6	75.5	2.4	3.1	5.2	5.1	19.53	18.3	23	22.7
2	71.4	71.1	69.1	70.3	2.31	2.31	4.3	4.2	18.76	18.6	22.3	21.6
3	68.8	68.5	67.1	67.9	2.235	2.2	4.7	4.5	18.96	18.9	22.7	21.9
	217	216	210.8	213.7	6.945	7.61	14.2	13.8	57.26	55.8	68	66.2
mean	72.33	72	70.26	71.23	2.315	2.53	4.73	4.6	19.08	18.6	22.66	22.06

The table 1 shows L*, a* and b* parameters for control saline group showing values preoperatively and after 3 time intervals

Table 2

	ΔL* 24 HRS	ΔL*1 MONTH	ΔL*6 MONTH	ΔE* 24 HRS	ΔE*1 MONTH	ΔE*6 MONTH
N valid	3	3	3	3	3	3
Missing	0	0	0	0	0	0
Mean	-.333	-2.067	-1.100	.667	4.73	3.86
Std.deviation	.0577	.3215	.2000	.6351	.1528	.4041

The following table shows mean and standard deviation of ΔL (change in translucency) and ΔE(change in discoloration of control saline group

Table 3

CONTROL BLOOD												
	L*	24 HRS	IST MONTH	6TH MONTH	A*	24 HRS	IST MONTH	6TH MONTH	B*	24 HRS	IST MONTH	6TH MONTH
1	75	60	61.3	61.1	2.36	5.5	5.6	5.5	18.66	25.3	25.6	25.3
2	68.4	53.2	55.2	55.2	2.25	5.1	5.3	5.1	17.86	24.3	25	24.3
3	74.2	59.7	61.3	61.1	2.41	5.2	5.7	5.2	19.56	23.2	23.8	23.2
	217.6	172.9	177.8	177.4	7.03	15.8	16.6	15.8	56.1	72.8	74.4	72.8
mean	72.53	57.63	59.26	59.13	2.34	5.26	5.53	5.26	18.7	24.26	24.8	24.26

The table 3 shows L*, a* and b* parameters for control blood group showing values preoperatively and after 3 time intervals

Table 4

	ΔL* 24 HRS	ΔL*1 MONTH	ΔL*6 MONTH	ΔE* 24 HRS	ΔE*1 MONTH	ΔE*6 MONTH
N valid	3	3	3	3	3	3
Missing	0	0	0	0	0	0
Mean	-14.90	-13.26	-13.40	16.67	14.93	14.8
Std.deviation	.361	.4041	.4359	.8386	.9074	.9539

The following table shows mean and standard deviation of ΔL (change in translucency) and ΔE(change in discoloration of control blood group

Table 5

PROROOT SALINE												
	L*	24 HRS	1ST MONH	6TH MONTH	a*	24 HRS	1st MONTH	6th MONTH	b*	24 HRS	1st MONTH	6TH MONTH
1	73.6	73.2	70.1	70	2.43	2.4	3.8	4.6	18.7	18.5	20.6	21.9
2	75	73.9	72.6	72.5	2.38	2.37	4.7	5.1	18.66	18.5	23.2	24.2
3	75.2	73.9	72.6	72.1	2.37	2.33	4.6	4.9	18.56	18.5	22.8	23.5
4	72.8	71.5	70.1	69.9	2.28	2.31	5.3	5.5	18.93	18.83	22.9	23.5
5	74.4	72.3	71.1	70	2.34	2.34	4.7	5	19.26	19.1	21.8	22.4
6	73.8	72.2	70.2	70.1	2.32	2.3	5.4	5.7	18.63	18.52	20.6	21.8
7	73	72.7	70	69.8	2.29	2.29	4.3	4.8	18.46	18.3	22.3	22.9
8	69.2	68.6	67.5	67.2	2.31	2.31	5.1	5.6	18.93	18.5	22.5	23.6
9	71.8	70.6	69.2	68.9	2.37	2.36	4.4	5	19.23	19.1	23.2	23.9
10	73.4	72.1	72.1	71.8	2.32	2.31	4.3	4.6	18.7	18.63	22.9	24
	732.2	721	705.5	702.3	23.43	23.32	46.6	50.8	188.1	186.48	222.8	231.7
mean	73.22	72.1	70.55	70.23	2.34	2.332	4.66	5.08	18.81	18.648	22.28	23.17

The table 5 shows L*, a* and b* parameters for wPMTA saline group(1A) showing values preoperatively and after application of material at 3 time

Table 6

	ΔL* 24 HRS	ΔL*1 MONTH	ΔL*6 MONTH	ΔE* 24 HRS	ΔE*1 MONTH	ΔE*6 MONTH
N valid	10	10	10	10	10	10
Missing	0	0	0	0	0	0
Mean	-1.120	-2.670	-2.990	1.130	5.050	6.010
Std.deviation	.5534	.7424	.8225	.5438	.4378	.3573

The following table shows mean and standard deviation of ΔL (change in translucency) and ΔE(change in discoloration) of white Proroot MTA saline group

Table 7

PROROOT BLOOD												
	L*	24 HRS	IST MONTH	6TH MONTH	a*	24 HRS	IST MONTH	6TH MONTH	b*	24 HRS	IST MONTH	6TH MONTH
1	75.2	70.1	66.3	67.3	2.4	2.48	2.7	5.7	18.66	18.6	19	25.6
2	75.8	71.2	66.5	67.5	2.37	2.5	2.6	5.1	18.66	18.7	18.8	24.6
3	76	70.8	67.8	68.2	2.255	2.5	3	4.9	18.96	18.7	19.4	24.8
4	63.2	57.6	55.4	54.5	2.37	2.4	2.6	5.3	18.36	18.25	20.6	24.1
5	72.4	67.5	63.8	64.8	2.455	2.6	2.7	5.6	18.7	18.6	18.9	24.3
6	68.8	63.7	60.5	60.6	2.31	2.33	2.51	4.6	18.63	18.5	19.1	26.3
7	73.4	68.3	63.9	65.1	2.475	2.66	3.1	5.2	18.86	18.69	19.15	24.3
8	74.2	68.6	66.1	65.7	2.4	2.7	2.8	5.9	20.6	20.7	20.9	26.6
9	71.6	67.3	62.5	64.3	2.38	2.55	2.7	4.8	18.73	18.6	19	26.7
10	74.8	69.4	65.5	66.3	2.38	2.3	2.5	4.9	18.43	18.47	19.6	26.5
	725.4	674.5	638.3	644.3	23.795	25.02	27.21	52	188.63	187.81	194.45	253.8
mean	72.54	67.45	63.83	64.43	2.3795	2.502	2.721	5.2	18.86	18.78	19.45	25.38

The table 7 shows L*, a* and b* parameters for wPMTA blood group(2A) showing values preoperatively and after application of material at 3 time intervals

Table 8

	ΔL* 24 HRS	ΔL*1 MONTH	ΔL*6 MONTH	ΔE* 24 HRS	ΔE*1 MONTH	ΔE*6 MONTH
N valid	10	10	10	10	10	10
Missing	0	0	0	0	0	0
Mean	-5.090	-8.710	-8.110	5.090	8.740	10.7710
Std.deviation	.4122	.5915	.4458	.4122	.5461	.6272

The following table shows mean and standard deviation of ΔL (change in translucency) and ΔE(change in discoloration) of white Proroot MTA blood group

Table 9

BIODENTINE SALINE												
	L*	24 HRS	IST MONTH	6TH MONTH	a*	24 HRS	IST MONTH	6TH MONTH	b*	24 HRS	IST MONTH	6TH MONTH
1	75.2	74.9	73.8	72.7	2.32	2.6	3.4	3.6	18.63	19.55	22.5	23
2	70.2	69.7	68.6	67.9	2.32	2.5	3.6	4	18.73	19.7	21.6	22.8
3	74.4	74.3	72.5	72.1	2.43	2.6	3.3	3.9	19.3	20.2	22.4	22.8
4	71.6	71.2	70.1	69.5	2.28	2.4	3.6	4	18.8	18.8	21.1	22.6
5	73	72.9	70.9	69.8	2.30	2.5	4.3	4.6	18.96	19.8	21.8	22.6
6	69.8	69.7	67.9	66.7	2.19	2.7	4.2	4.7	18.26	19.3	20.3	22.3
7	69.2	68.7	67.5	66.5	2.24	2.6	4.3	4.7	18.4	19.2	20.4	22
8	76.8	76.2	74.7	74.5	2.32	2.4	4.6	4.9	19.26	20	21.3	23.1
9	71.2	70.7	69.6	68.3	2.21	2.3	4.1	4.5	18.53	19.4	21.4	23.4
10	69.6	69.5	67.6	66.8	2.34	2.6	4.7	5	19.03	19.8	22.1	22.9
	721	717.8	703.2	694.8	22.98	25.2	40.1	43.9	187.9	195.75	214.9	227.5
mean	72.1	71.78	70.32	69.48	2.298	2.52	4.01	4.39	18.79	19.57	21.49	22.75

The table 9 shows L*, a* and b* parameters for Biodentine saline group (1B) showing values preoperatively and after application of material at 3 time intervals

Table 10

	ΔL* 24 HRS	ΔL*1 MONTH	ΔL*6 MONTH	ΔE* 24 HRS	ΔE*1 MONTH	ΔE*6 MONTH
N valid	10	10	10	10	10	10
Missing	0	0	0	0	0	0
Mean	-0.320	-1.780	-0.90	0.90	3.680	5.160
Std.deviation	.2044	.2530	.205	.205	.4050	.4858

The following table shows mean and standard deviation of ΔL (change in translucency) and ΔE(change in discoloration) of Biodentin saline group

Table 11

BIODENTINE BLOOD												
	L*	24 HRS	IST MONTH	6TH MONTH	a*	24 HRS	IST MONTH	6TH MONTH	b*	24 HRS	IST MONTH	6TH MONTH
1	75.2	73.2	70.3	70.1	2.4	2.41	3.1	3.7	18.76	18.6	19.6	21.2
2	70.4	68.6	65.9	65.2	2.26	2.24	4.6	4.9	18.36	18.2	19.1	21.3
3	65.2	62.9	61.1	60.5	2.205	2.3	4.9	5.2	18.26	18.25	19.3	20.6
4	66.2	64.1	61	61.1	2.26	2.26	3.8	4.2	18.03	18	19.6	21.6
5	69.4	66.8	64.9	64.2	2.24	2.24	4.3	4.6	18.6	18.5	19.8	21.7
6	73	70.3	68.7	67.6	2.365	2.3	4.6	5.2	18.8	18.7	20.1	22.3
7	69.8	67.8	65.2	64.2	2.255	2.2	3.7	4.5	18.93	18.6	19.7	21.5
8	75.4	73	70.5	70.3	2.405	2.35	3.9	4.2	18.56	18.3	20.3	22.6
9	75	72.3	70.9	70.1	2.425	2.37	4.6	5.1	18.7	18.63	19.8	23.8
10	67.2	64.8	62.9	62.4	2.34	2.31	4.5	5.6	18.3	18.3	19.6	22.5
	706.8	683.8	661.4	655.7	23.155	22.98	42	47.2	185.33	184.08	196.9	219.1
mean	70.68	68.38	66.14	65.57	2.3155	2.298	4.2	4.72	18.53	18.408	19.69	21.91

The table 11 shows L*, a* and b* parameters for Biodentine blood group (2B) showing values preoperatively and after application of material at 3 time intervals

Table 12

	ΔL* 24 HRS	ΔL*1 MONTH	ΔL*6 MONTH	ΔE* 24 HRS	ΔE*1 MONTH	ΔE*6 MONTH
N valid	10	10	10	10	10	10
Missing	0	0	0	0	0	0
Mean	-2.30	-4.540	-5.110	2.30	5.0620	6.597
Std.deviation	.316	.3658	.2685	.314	.26666	.5216

The following table shows mean and standard deviation of ΔL (change in translucency) and ΔE(change in discoloration) of Biodentin blood group

Table 13

ERRM SALINE												
	L*	24 HRS	IST MONTH	6TH MONTH	a*	24 HRS	IST MONTH	6TH MONTH	b*	24 HRS	IST MONTH	6TH MONTH
1	69	67.8	67.6	67.3	2.37	2.3	4.5	4.6	19.4	19.41	22.3	22.5
2	71.2	70.5	69.5	68.5	2.39	2.33	5.2	5.3	19.1	19.1	22.9	23.2
3	70.8	68.7	68.5	68.3	2.32	2.34	4.9	5.3	18.8	18.8	22.6	22.7
4	71	69.6	68.2	68.1	2.42	2.5	4.3	4.5	19.53	19.5	22.6	22.9
5	71.6	70.5	69.5	69.2	2.39	2.3	4.7	5	18.9	18.91	21.3	21.6
6	68.2	67.3	67.1	66.7	2.22	2.2	4.3	4.6	18.4	18.42	22.2	22.8
7	73.4	72.2	72.1	71.8	2.34	2.31	3.9	4.4	19.16	19.2	22.8	23.2
8	70.4	68.5	68.3	67.6	2.35	2.33	4.8	5.1	19.13	19.11	22.1	22.6
9	68.4	67.5	67.1	66.4	2.24	2.23	4.6	4.9	18.8	18.8	22.6	22.7
10	72.2	70.9	70.1	69.9	2.29	2.22	5.1	5.5	18.8	18.8	22.7	22.9
	706.2	693.5	688	683.8	23.365	23.06	46.3	49.2	190.03	190.05	224.1	227.1
mean	70.62	69.35	68.8	68.38	2.3365	2.306	4.63	4.92	19.00	19.005	22.41	22.71

The table 13 shows L*, a* and b* parameters for ERRM saline group(1C) showing values preoperatively and after application of material at 3 time intervals

Table 14

	ΔL* 24 HRS	ΔL*1 MONTH	ΔL*6 MONTH		ΔE* 24 HRS	ΔE*1 MONTH	ΔE*6 MONTH
N valid	10	10	10	N valid	10	10	10
Missing	0	0	0	Missing	0	0	0
Mean	-1.270	-1.820	-2.240	Mean	1.270	4.490	5.050
Std.deviation	.4398	.5453	.5125	Std.deviation	.4398	.4909	.5148

The following table shows mean and standard deviation of ΔL (change in translucency) and ΔE(change in discoloration) of ERRM saline group

TABLE 15

ERRM BLOOD												
	L*	24 HRS	IST MONTH	6TH MONTH	a*	24 HRS	IST MONTH	6TH MONTH	b*	24 HRS	IST MONTH	6TH MONTH
1	72.2	66.4	66.3	64.5	2.3	2.34	5.8	6	18.66	18.6	23	23.5
2	72.8	66.2	66.4	64.9	2.23	2.21	5.2	5.5	17.4	17.6	20.6	21.2
3	71.6	64.9	65.9	64.1	2.41	2.44	5.4	5.7	19.53	19.31	24.3	24.8
4	71.4	65.2	65.2	63.3	2.325	2.4	6.1	6.4	18.66	18.7	23.6	24.3
5	69	62.2	62.8	61.4	2.29	2.24	6.4	6.6	19.06	19.5	24.3	25.2
6	72.4	65.7	66.3	64.3	2.145	2.12	5.6	6.1	18.2	18.3	23.4	24.1
7	77	71.6	71.5	68.6	2.205	2.3	5.7	5.9	19.26	19.3	25.1	26.3
8	73.8	68.6	67.3	65.1	2.375	2.31	5.8	6.4	19.33	19.32	24.7	25.6
9	69.6	63.8	63.5	61.3	2.28	2.26	5.5	5.8	18.6	18.5	24.1	25.4
10	71.6	66.3	65.8	63.9	2.345	2.33	2.33	4.5	19.03	19.1	24.5	25.1
	721.4	660.9	661	641.4	22.905	22.95	53.83	58.9	187.76	188.23	237.6	245.5
MEAN	72.14	66.09	66.1	64.14	2.2905	2.295	5.383	5.89	18.77	18.823	23.76	24.55

The table 15 shows L*, a* and b* parameters for ERRM blood group (2C) showing values preoperatively and after application of material at 3 time intervals

TABLE 16

	ΔL* 24 HRS	ΔL*1 MONTH	ΔL*6 MONTH	ΔE* 24 HRS	ΔE*1 MONTH	ΔE*6 MONTH
N valid	10	10	10	10	10	10
Missing	0	0	0	0	0	0
Mean	-6.050	-6.040	-8.000	6.050	8.460	10.480
Std.deviation	.6294	.3134	.3887	.6294	.4835	.7584

The following table shows mean and standard deviation of ΔL (change in translucency) and ΔE(change in discoloration) of ERRM blood group

Table 17 comparison of ΔL (change in translucency) and ΔE (change in discoloration) with time in the presence of blood

		Sum of Squares	df	Mean Square	F	Sig.
ΔL* 24 hrsPB	Between Groups	372.011	3	124.004 .216	575.010	.000
	Within Groups	6.254	29			
	Total	378.265	32			
ΔL* 1month PB	Between Groups	216.699	3	72.233 .192	376.506	.000
	Within Groups	5.564	29			
	Total	222.262	32			
ΔL* 6 month PB	Between Groups	167.044	3	55.681 .144	386.492	.000
	Within Groups	4.178	29			
	Total	171.222	32			
ΔE* 24 hrsPB	Between Groups	448.250	3	149.417 .255	586.422	.000
	Within Groups	7.389	29			
	Total	455.639	32			
ΔE* 1 month PB	Between Groups	237.769	3	79.256 .244	324.885	.000
	Within Groups	7.075	29			
	Total	244.844	32			
ΔE* 6 month PB	Between Groups	191.636	3	63.879 .448	142.564	.000
	Within Groups	12.986	29			
	Total	204.622	32			

Table 18 comparison of ΔL (change in translucency) and ΔE (change in discoloration) with time in the presence of saline

		Sum of Squares	df	Mean Square	F	Sig.
ΔL* 24 hrsPSL	Between Groups	6.103	3	2.034 .168	12.090	.000
	Within Groups	4.880	29			
	Total	10.982	32			
ΔL* 1month PSL	Between Groups	5.055	3	1.685 .290	5.804	.003
	Within Groups	8.420	29			
	Total	13.475	32			
ΔL* 6 month PSL	Between Groups	9.086	3	3.029 .338	8.954	.000
	Within Groups	9.809	29			
	Total	18.895	32			
ΔE* 24 hrs PSL	Between Groups	1.210	3	.403 .193	2.093	.123
	Within Groups	5.589	29			
	Total	6.799	32			
ΔE* 1 month PSL	Between Groups	9.780	3	3.260 .187	17.453	.000
	Within Groups	5.417	29			
	Total	15.196	32			
ΔE* 6 month PSL	Between Groups	11.989	3	3.996 .206	19.365	.000
	Within Groups	5.985	29			
	Total	17.973	32			

Table 19 Multiple comparison -Bonferroni Post hoc test

Dependent Variable	(I) GROUP	(J) GROUP	Mean Difference (I- J)	Std. Error	Sig.	95% ... Lower Bound
ΔL* 24 hrsPB	1	2	-2.7900¨	.2077	.000	**-3.378**
		3	.9600*	.2077	.000	**.372**
		4	9.8100*	.3057	.000	**8.944**
	2	1	2.7900¨	.2077	.000	**2.202**
		3	3.7500*	.2077	.000	**3.162**
		4	12.6000*	.3057	.000	**11.734**
	3	1	-.9600ˆ	.2077	.000	**-1.548**
		2	-3.7500*	.2077	.000	**-4.338**
		4	8.8500*	.3057	.000	**7.984**
	4	1	-9.8100ˆ	.3057	.000	**-10.676**
		2	-12.6000*	.3057	.000	**-13.466**
		3	-8.8500*	.3057	.000	**-9.716**
1 monthPB	1	2	-4.1700ˆ	.1959	.000	**-4.725**
		3	-2.6700*	.1959	.000	**-3.225**
		4	4.5567*	.2883	.000	**3.740**
	2	1	4.1700ˆ	.1959	.000	**3.615**
		3	1.5000*	.1959	.000	**.945**

		4	8.7267*	.2883	.000	**7.910**
	3	1	2.6700^	.1959	.000	**2.115**
		2	-1.5000*	.1959	.000	**-2.055**
		4	7.2267*	.2883	.000	**6.410**
	4	1	-4.5567^	.2883	.000	**-5.373**
		2	-8.7267*	.2883	.000	**-9.543**
		3	-7.2267*	.2883	.000	**-8.043**
6 monthPB	1	2	-3.0000^	.1697	.000	**-3.481**
		3	-.1100	.1697	1.000	**-.591**
		4	5.2900*	.2499	.000	**4.583**
	2	1	3.0000^	.1697	.000	**2.519**
		3	2.8900*	.1697	.000	**2.409**
		4	8.2900*	.2499	.000	**7.583**
	3	1	.1100	.1697	1.000	**-.371**
		2	-2.8900*	.1697	.000	**-3.371**
		4	5.4000*	.2499	.000	**4.693**
	4	1	-5.2900^	.2499	.000	**-5.997**
		2	-8.2900*	.2499	.000	**-8.997**
		3	**-5.4000***	**.2499**	**.000**	**-6.107**

Table 20 Multiple comparison -Bonferroni Post hoc test

Dependent Variable	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% ... Lower Bound
ΔE* 24 hrsPB	1	2	2.7880^	.2257	.000	**2.149**
		3	-.9600*	.2257	.001	**-1.599**
		4	-11.0767*	.3323	.000	**-12.018**
	2	1	-2.7880^	.2257	.000	**-3.427**
		3	-3.7480*	.2257	.000	**-4.387**
		4	-13.8647*	.3323	.000	**-14.806**
	3	1	.9600^	.2257	.001	**.321**
		2	3.7480*	.2257	.000	**3.109**
		4	-10.1167*	.3323	.000	**-11.058**
	4	1	11.0767^	.3323	.000	**10.136**
		2	13.8647*	.3323	.000	**12.924**
		3	10.1167*	.3323	.000	**9.176**
1 monthPB	1	2	3.6780^	.2209	.000	**3.053**
		3	.2800	.2209	1.000	**-.345**
		4	-6.1933*	.3251	.000	**-7.114**
	2	1	-3.6780^	.2209	.000	**-4.303**
		3	-3.3980*	.2209	.000	**-4.023**
		4	-9.8713*	.3251	.000	**-10.792**
	3	1	-.2800	.2209	1.000	**-.905**
		2	3.3980*	.2209	.000	**2.773**

		4	-6.4733*	.3251	.000	**-7.394**
	4	1	6.1933*	.3251	.000	**5.273**
		2	9.8713*	.3251	.000	**8.951**
		3	6.4733*	.3251	.000	**5.553**
6 monthPB	1	2	4.17400*	.29926	.000	**3.3266**
		3	.29100	.29926	1.000	**-.5564**
		4	-4.02900*	.44050	.000	**-5.2763**
	2	1	-4.17400*	.29926	.000	**-5.0214**
		3	-3.88300*	.29926	.000	**-4.7304**
		4	-8.20300*	.44050	.000	**-9.4503**
	3	1	-.29100	.29926	1.000	**-1.1384**
		2	3.88300*	.29926	.000	**3.0356**
		4	-4.32000*	.44050	.000	**-5.5673**
	4	1	4.02900*	.44050	.000	**2.7817**
		2	8.20300*	.44050	.000	**6.9557**
		3	**4.32000***	**.44050**	**.000**	**3.0727**

Table21 Multiple comparison -Bonferroni Post hoc test

Dependent Variable	(I) GROUP		(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% ... Lower Bound
ΔL* 24 hrs PSL	1		2	-.8000^	.1834	.001	**-1.319**
			3	.1500	.1834	1.000	**-.369**
			4	-.7867*	.2700	.041	**-1.551**
	2		1	.8000^	.1834	.001	**.281**
			3	.9500*	.1834	.000	**.431**
			4	.0133	.2700	1.000	**-.751**
	3		1	-.1500	.1834	1.000	**-.669**
			2	-.9500*	.1834	.000	**-1.469**
			4	-.9367*	.2700	.010	**-1.701**
	4		1	.7867^	.2700	.041	**.022**
			2	-.0133	.2700	1.000	**-.778**
			3	.9367*	.2700	.010	**.172**
1 monthPSL	1		2	-.8900^	.2410	.005	**-1.572**
			3	-.8500*	.2410	.009	**-1.532**
			4	-.6033	.3547	.598	**-1.608**
	2		1	.8900^	.2410	.005	**.208**
			3	.0400	.2410	1.000	**-.642**

		4	.2867	.3547	1.000	**-.718**
	3	1	.8500^	.2410	.009	**.168**
		2	-.0400	.2410	1.000	**-.722**
		4	.2467	.3547	1.000	**-.758**
	4	1	.6033	.3547	.598	**-.401**
		2	-.2867	.3547	1.000	**-1.291**
		3	-.2467	.3547	1.000	**-1.251**
6 monthPSL	1	2	-.3700	.2601	.993	**-1.106**
		3	-.7500*	.2601	.044	**-1.486**
		4	-1.8900*	.3828	.000	**-2.974**
	2	1	.3700	.2601	.993	**-.366**
		3	-.3800	.2601	.929	**-1.116**
		4	-1.5200*	.3828	.003	**-2.604**
	3	1	.7500^	.2601	.044	**.014**
		2	.3800	.2601	.929	**-.356**
		4	-1.1400*	.3828	.035	**-2.224**
	4	1	1.8900^	.3828	.000	**.806**
		2	1.5200*	.3828	.003	**.436**
		3	**1.1400***	**.3828**	**.035**	**.056**

Table 22 Multiple comparison -Bonferroni Post hoc test

Dependent Variable	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% ... Lower Bound
ΔE* 24 hrsPSL	1	2	.2300	.1963	1.000	**-.326**
		3	-.1400	.1963	1.000	**-.696**
		4	.4633	.2890	.718	**-.355**
	2	1	-.2300	.1963	1.000	**-.786**
		3	-.3700	.1963	.417	**-.926**
		4	.2333	.2890	1.000	**-.585**
	3	1	.1400	.1963	1.000	**-.416**
		2	.3700	.1963	.417	**-.186**
		4	.6033	.2890	.274	**-.215**
	4	1	-.4633	.2890	.718	**-1.282**
		2	-.2333	.2890	1.000	**-1.052**
		3	-.6033	.2890	.274	**-1.422**
1 monthPSL	1	2	1.3700^	.1933	.000	**.823**
		3	.5600*	.1933	.043	**.013**
		4	.3167	.2845	1.000	**-.489**
	2	1	-1.3700^	.1933	.000	**-1.917**
		3	-.8100*	.1933	.001	**-1.357**
		4	-1.0533*	.2845	.005	**-1.859**
	3	1	-.5600^	.1933	.043	**-1.107**

		2	.8100*	.1933	.001	**.263**
		4	-.2433	.2845	1.000	**-1.049**
	4	1	-.3167	.2845	1.000	**-1.122**
		2	1.0533*	.2845	.005	**.248**
		3	.2433	.2845	1.000	**-.562**
6 monthPSL	1	2	.8500^	.2032	.001	**.275**
		3	.9600*	.2032	.000	**.385**
		4	2.1433*	.2990	.000	**1.297**
	2	1	-.8500^	.2032	.001	**-1.425**
		3	.1100	.2032	1.000	**-.465**
		4	1.2933*	.2990	.001	**.447**
	3	1	-.9600^	.2032	.000	**-1.535**
		2	-.1100	.2032	1.000	**-.685**
		4	1.1833*	.2990	.003	**.337**
	4	1	-2.1433^	.2990	.000	**-2.990**
		2	-1.2933*	.2990	.001	**-2.140**
		3	**-1.1833***	**.2990**	**.003**	**-2.030**

Table23 **Group Statistics**

GROUP		1	Mean	Std. Deviation	Std. Error Mean
∆L* 24 hrsP	1	10	-5.090	.4122	**.1303**
	2	10	-1.120	.5534	**.1750**
1 monthP	1	10	-8.710	.5915	**.1871**
	2	10	-2.670	.7424	**.2348**
6 monthP	1	10	-8.110	.4458	**.1410**
	2	10	-2.990	.8225	**.2601**
∆E* 24 hrsP	1	10	5.090	.4122	**.1303**
	2	10	1.130	.5438	**.1719**
1 monthP	1	10	8.740	.5461	**.1727**
	2	10	5.050	.4378	**.1384**
6 monthP	1	10	10.7710	.62729	**.19837**
	2	**10**	**6.0100**	**.35730**	**.11299**

Table 24 Independent samples test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	**df**
L* 24 hrsP	Equal variances assumed	.793	.385	-18.194	**18**
	Equal variances not assumed			-18.194	**16.636**
1 monthP	Equal variances assumed	.058	.812	-20.121	**18**
	Equal variances not assumed			-20.121	**17.144**
6 monthP	Equal variances assumed	1.881	.187	-17.305	**18**
	Equal variances not assumed			-17.305	**13.868**
∆E* 24 hrsP	Equal variances assumed	.643	.433	18.353	**18**
	Equal variances not assumed			18.353	**16.776**
1 monthP	Equal variances assumed	1.859	.190	16.672	**18**
	Equal variances not assumed			16.672	**17.187**
6 monthP	Equal variances assumed	3.781	.068	20.855	**18**
	Equal variances not assumed			**20.855**	**14.284**

Table 25 Independent samples test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	**Std. Error Difference**
ΔL* 24 hrsP	Equal variances assumed	.000	-3.9700	**.2182**
	Equal variances not assumed	.000	-3.9700	**.2182**
1 monthP	Equal variances assumed	.000	-6.0400	**.3002**
	Equal variances not assumed	.000	-6.0400	**.3002**
6 monthP	Equal variances assumed	.000	-5.1200	**.2959**
	Equal variances not assumed	.000	-5.1200	**.2959**
ΔE* 24 hrsP	Equal variances assumed	.000	3.9600	**.2158**
	Equal variances not assumed	.000	3.9600	**.2158**
1 monthP	Equal variances assumed	.000	3.6900	**.2213**
	Equal variances not assumed	.000	3.6900	**.2213**
6 monthP	Equal variances assumed	.000	4.76100	**.22829**
	Equal variances not assumed	**.000**	**4.76100**	**.22829**

Table 26 Independent samples test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	**Upper**
ΔL* 24 hrsP	Equal variances assumed	-4.4284	**-3.5116**
	Equal variances not assumed	-4.4311	**-3.5089**
1 monthP	Equal variances assumed	-6.6707	**-5.4093**
	Equal variances not assumed	-6.6729	**-5.4071**
6 monthP	Equal variances assumed	-5.7416	**-4.4984**
	Equal variances not assumed	-5.7551	**-4.4849**
ΔE* 24 hrsP	Equal variances assumed	3.5067	**4.4133**
	Equal variances not assumed	3.5043	**4.4157**
1 monthP	Equal variances assumed	3.2250	**4.1550**
	Equal variances not assumed	3.2234	**4.1566**
6 monthP	Equal variances assumed	4.28138	**5.24062**
	Equal variances not assumed	**4.27228**	**5.24972**

Table 27 GROUP STATISTICS

GROUP		1	Mean	Std. Deviation	Std. Error Mean
∆L* 24 hrsBIO	1	10	-2.30	.316	**.100**
	2	10	-.32	.204	**.065**
1 monthBIO	1	10	-4.540	.3658	**.1157**
	2	10	-1.780	.2530	**.0800**
6 monthBIO	1	10	-5.110	.2685	**.0849**
	2	10	-2.620	.3765	**.1191**
∆E* 24 hrsBIO	1	10	2.30	.314	**.099**
	2	10	.90	.205	**.065**
1 monthBIO	1	10	5.0620	.26666	**.08432**
	2	10	3.6800	.40497	**.12806**
6 monthBIO	1	10	6.597	.5216	**.1649**
	2	**10**	**5.160**	**.4858**	**.1536**

Table 28 Independent samples test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	**df**
ΔL* 24 hrsBIO	Equal variances assumed	2.087	.166	-16.629	**18**
	Equal variances not assumed			-16.629	**15.403**
1 monthBIO	Equal variances assumed	.892	.357	-19.625	**18**
	Equal variances not assumed			-19.625	**16.007**
6 monthBIO	Equal variances assumed	2.770	.113	-17.026	**18**
	Equal variances not assumed			-17.026	**16.274**
ΔE* 24 hrsBIO	Equal variances assumed	3.081	.096	11.810	**18**
	Equal variances not assumed			11.810	**15.509**
1 monthBIO	Equal variances assumed	1.437	.246	9.013	**18**
	Equal variances not assumed			**9.013**	**15.569**

Table 29 Independent samples test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	**Std. Error Difference**
∆L* 24 hrsBIO	Equal variances assumed	.000	-1.980	**.119**
	Equal variances not assumed	.000	-1.980	**.119**
1 monthBIO	Equal variances assumed	.000	-2.7600	**.1406**
	Equal variances not assumed	.000	-2.7600	**.1406**
6 monthBIO	Equal variances assumed	.000	-2.4900	**.1462**
	Equal variances not assumed	.000	-2.4900	**.1462**
∆E* 24 hrsBIO	Equal variances assumed	.000	1.402	**.119**
	Equal variances not assumed	.000	1.402	**.119**
1 monthBIO	Equal variances assumed	.000	1.38200	**.15333**
	Equal variances not assumed	**.000**	**1.38200**	**.15333**

Table 30 Independent samples test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	**Upper**
∆L* 24 hrsBIO	Equal variances assumed	-2.230	**-1.730**
	Equal variances not assumed	-2.233	**-1.727**
1 monthBIO	Equal variances assumed	-3.0555	**-2.4645**
	Equal variances not assumed	-3.0581	**-2.4619**
6 monthBIO	Equal variances assumed	-2.7973	**-2.1827**
	Equal variances not assumed	-2.7996	**-2.1804**
∆E* 24 hrsBIO	Equal variances assumed	1.153	**1.651**
	Equal variances not assumed	1.150	**1.654**
1 monthBIO	Equal variances assumed	1.05986	**1.70414**
	Equal variances not assumed	**1.05622**	**1.70778**

Table 31 Independent samples test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	**df**
6 monthBIO	**Equal variances assumed**	**.065**	**.801**	**6.375**	**18**
	Equal variances not assumed			**6.375**	**17.910**

Table 32 Independent samples test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	**Std. Error Difference**
6 month BIO	**Equal variances assumed**	**.000**	**1.4370**	**.2254**
	Equal variances not assumed	**.000**	**1.4370**	**.2254**

Table 33 Independent samples test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	**Upper**
6 monthBIO	**Equal variances assumed**	**.9635**	**1.9105**
	Equal variances not assumed	**.9633**	**1.9107**

Table 34 GROUP STATISTICS

GROUP		1	Mean	Std. Deviation	Std. Error Mean
∆L* 24 hrsERRM	1	10	-6.050	.6294	**.1990**
	2	10	-1.270	.4398	**.1391**
1 monthERRM	1	10	-6.040	.3134	**.0991**
	2	10	-1.820	.5453	**.1724**
6 monthERRM	1	10	-8.000	.3887	**.1229**
	2	10	-2.240	.5125	**.1621**
∆E* 24 hrsERRM	1	10	6.050	.6294	**.1990**
	2	10	1.270	.4398	**.1391**
1 monthERRM	1	10	8.460	.4835	**.1529**
	2	10	4.490	.4909	**.1552**
6 monthERRM	1	10	10.480	.7584	**.2398**
	2	**10**	**5.050**	**.5148**	**.1628**

Table 35 Independent samples test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	**df**
ΔL* 24 hrsERRM	1	3.734	.069	-19.686	**18**
	Equal variances not assumed			-19.686	**16.098**
1 monthERRM	Equal variances assumed	4.813	.042	-21.218	**18**
	Equal variances not assumed			-21.218	**14.361**
6 monthERRM	Equal variances assumed	1.354	.260	-28.316	**18**
	Equal variances not assumed			-28.316	**16.780**
ΔL* 24 hrsERRM	1	3.734	.069	19.686	**18**
	Equal variances not assumed			19.686	**16.098**
1 monthERRM	Equal variances assumed	.141	.711	18.220	**18**
	Equal variances not assumed			**18.220**	**17.996**

Table 36 Independent samples test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	**Std. Error Difference**
ΔL* 24 hrsERRM	1	.000	-4.7800	**.2428**
	Equal variances not assumed	.000	-4.7800	**.2428**
1 monthERRM	Equal variances assumed	.000	-4.2200	**.1989**
	Equal variances not assumed	.000	-4.2200	**.1989**
6 monthERRM	Equal variances assumed	.000	-5.7600	**.2034**
	Equal variances not assumed	.000	-5.7600	**.2034**
ΔL* 24 hrsERRM	1	.000	4.7800	**.2428**
	Equal variances not assumed	.000	4.7800	**.2428**
1 monthERRM	Equal variances assumed	.000	3.9700	**.2179**
	Equal variances not assumed	**.000**	**3.9700**	**.2179**

Table 37 Independent samples test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	**Upper**
ΔL* 24 hrsERRM	Equal variances assumed	-5.2901	**-4.2699**
	Equal variances not assumed	-5.2945	**-4.2655**
1 monthERRM	Equal variances assumed	-4.6378	**-3.8022**
	Equal variances not assumed	-4.6456	**-3.7944**
6 monthERRM	Equal variances assumed	-6.1874	**-5.3326**
	Equal variances not assumed	-6.1896	**-5.3304**
ΔE* 24 hrsERRM	Equal variances assumed	4.2699	**5.2901**
	Equal variances not assumed	4.2655	**5.2945**
1 monthERRM	Equal variances assumed	3.5122	**4.4278**
	Equal variances not assumed	**3.5122**	**4.4278**

Table 38 t-test

		Levene's Test for Equality of Variances		t-test for Equality of Means	
		F	Sig.	t	**df**
6 monthERRM	**Equal variances assumed**	**2.184**	**.157**	**18.734**	**18**
	Equal variances not assumed			**18.734**	**15.841**

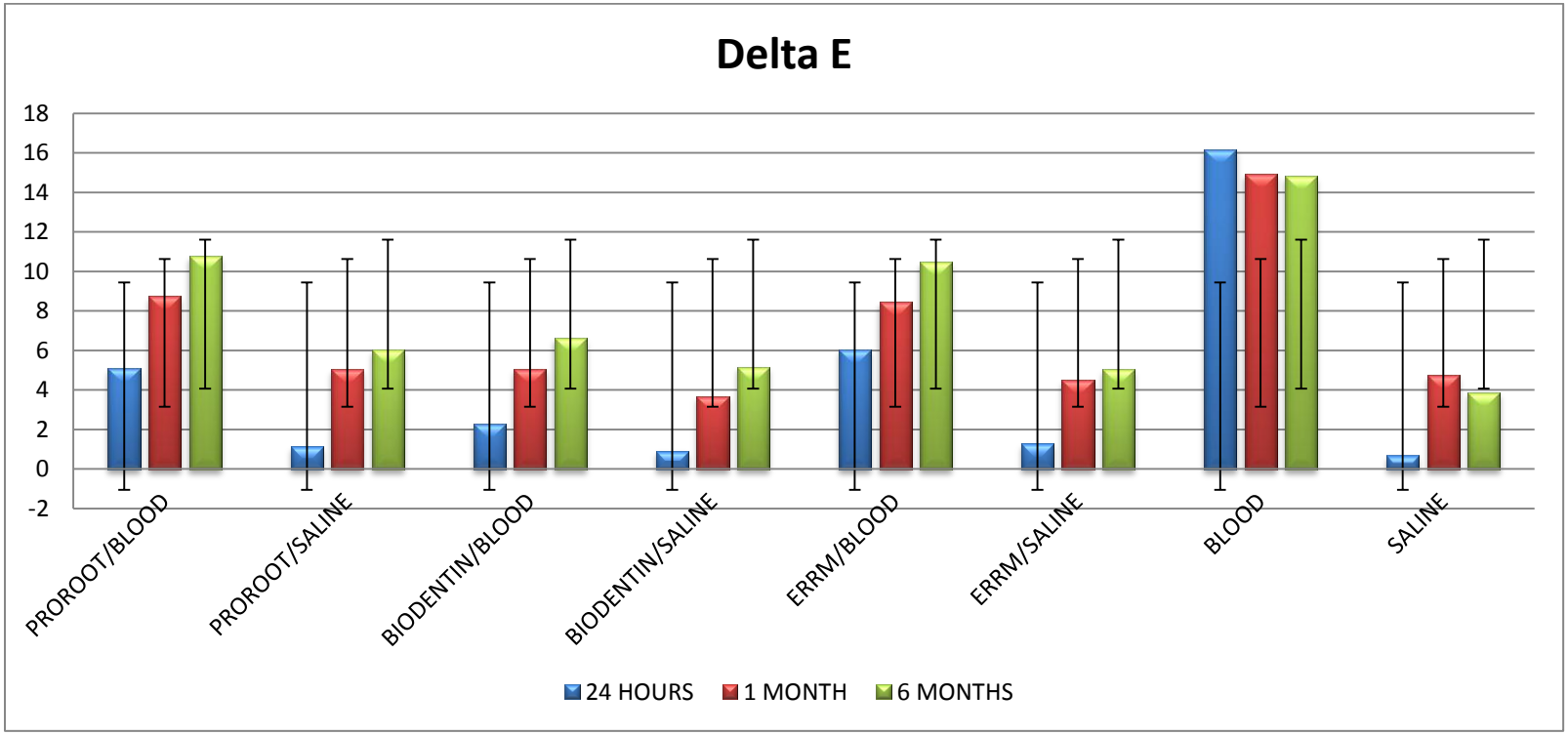
Table 39 t-test

		t-test for Equality of Means		
		Sig. (2-tailed)	Mean Difference	**Std. Error Difference**
6 monthERRM	**Equal variances assumed**	**.000**	**5.4300**	**.2898**
	Equal variances not assumed	**.000**	**5.4300**	**.2898**

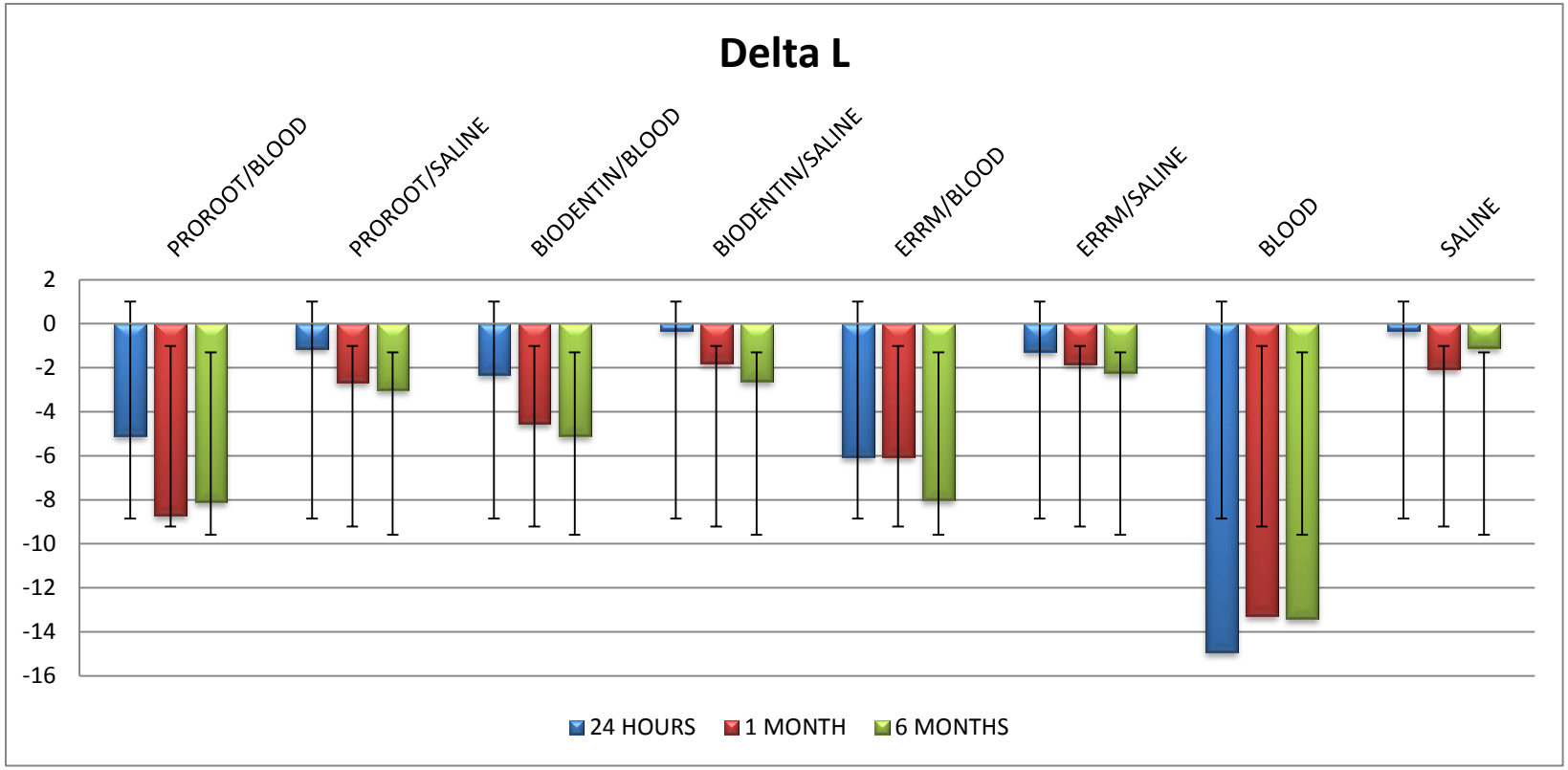
Table 40 t- test

		t-test for Equality of Means	
		95% Confidence Interval of the Difference	
		Lower	Upper
6 monthERRM	Equal variances assumed	4.8211	6.0389
	Equal variances not assumed	4.8151	6.0449

GRAPH 1: MEAN AND STANDARD DEVIATION OF ΔE (CHANGE IN COLOR) OF THE PRESENT STUDY AT DIFFERENT TIME PERIODS



GRAPH 2: MEAN AND STANDARD DEVIATION OF ΔL (CHANGE IN TRANSLUCENCY) OF THE PRESENT STUDY AT DIFFERENT TIME PERIODS



Discussion

DISCUSSION

The science of dental materials has focussed mainly on the function and performance of materials used clinically. Emphasis must be placed on other properties also particularly esthetics.[37] Materials used during endodontic procedures relies solely on biological and functional aspects. However newer materials also have an effect on esthetics. Such materials should have color stability, provide optical properties similar to oral tissues and should not cause staining or discoloration of hard tissues overtime.[35] The goal of endodontic therapy is not only to prevent and treat apical periodontitis but also provide an esthetic result especially for the anterior teeth. Vital pulp therapy procedures such as pulp capping, pulpotomy, regenerative endodontics, and perforation repairs involve placement of materials in the coronal third of the tooth which can produce discoloration of tooth/teeth.

Mineral trioxide aggregate (MTA; Dentsply Tulsa Dental, Johnson City, TN), has been used because of its excellent biocompatible property. However it has disadvantage of causing discoloration to the tooth due to the presence of toxic elements in its material composition, difficult handling properties, long setting time and difficulty in removing after use. This has led to development and improvement of newer materials in this field like Biodentin (Septodont, Saint Maur des Fosses, France) and EndoSequence Root Repair Material (ERRM; Brasseler USA, Savannah, GA). These materials exhibit better physical and chemical properties compared to MTA cements, however there are not many studies concerning the stability and

staining of the dental tissues induced by the newer materials. So the aim of this present invitro study was to compare the discoloration of tooth that occurs in human teeth undergoing vital pulp therapy when used with white Proroot MTA (MTA; Dentsply Tulsa Dental, Johnson City, TN), Biodentin (Septodont, Saint Maur des Fosses, France) and EndoSequence Root Repair Material (ERRM; Brasseler USA, Savannah, GA).

For this purpose 66 human single rooted permanent anterior teeth without any caries, cracks or restorations were selected following extraction due to advanced periodontal disease. This teeth were immersed in 5.25% sodium hypochlorite for one hour and then stored in normal saline solution till they were used for the study. Anterior teeth were preferred as they are commonly involved in traumatic injury and may require vital pulp therapy procedures like pulp capping, pulpotomy, regeneration and apexification.

The collected specimens were cleared of extrinsic debris and stains and apical part of each root was removed with high speed diamond fissure bur under continuous water spray perpendicular to its long axis until 5mm of the root remained. This is similar to the methodology followed by **Shokouhinejad et al** in their study[37]. Access cavities were prepared and the shortened root canals were cleaned, shaped and irrigated similarly as per the study protocol followed .

A customized cylindrical piece of plastic white foam of about 3mm length was inserted into the root canal through the apical end of the access opening prepared. The apical opening of the root canal with the surrounding

dentin was sealed with composite resin material to about 1.5 mm . A self etchant containing bonding agent was used on the dentinal walls and light cured for 10 seconds prior to the placement of composite resin material. A separate etchant was not used as the ing agent may interfere with the discoloration due to blood or the cement used. Care was taken to see the plastic white foam extended only upto the cementoenamel junction of the labial surface. Following this, the specimens were divided divided randomly into two groups (Group 1 and Group 2) of 33 each. The inserted foam in each specimens in group 2 was saturated with normal saline solution, where as the specimens in group 1 were saturated with fresh human blood. Whole fresh human blood collected from a healthy consenting volunteer was used to saturate the inserted foam in each specimen of group 1. This was approved by the ethical committee. Plastic foams were saturated with whole blood to simulate clinical situation following vital pulp therapy where there would be an increased flow of blood in the subjacent layer.

The specimens of both the experimental groups were then divided into 3 experimental subgroups containing 10 specimens each and a control group containing 3 teeth each of blood and saline group. To prevent contamination of the blood an insulin syringe was used within the access cavity. 3 subgroups were labeled as 1A ,1B, 1C and 2A, 2B, 2C respectively and control groups were labeled as control blood(CB) and control saline(CS). In groups 1A and 2A white Proroot MTA (Dentsply Tulsa Dental) was mixed as per manufacturer’s instructions on the paper pad and was placed in the access

cavity just in contact with plastic foam to a height of 1 to 1.5 mm. The access cavity was then sealed with a small pledget of wet cotton followed by md temp for one day. In group 1B and 2B Biodentin (Septodont, Saint Maur des Fosses, France) was mixed as per manufacturer's instructions and was placed and sealed similarly as in group 1A and 2A. In group 1C and 2C EndoSequence Root Repair Material (ERRM; Brasseler USA, Savannah, GA) being a readymade material was injected until the plastic foam was flooded with the material. Following this all the specimens in the two groups were temporized as previously.

In the present study fresh human whole blood was used to assess the effect of blood on the discoloration of tooth following the use of these materials such as white Proroot MTA (Dentsply Tulsa Dental), Biodentin (Septodont, Saint Maur des Fosses, France) and EndoSequence Root Repair Material (ERRM; Brasseler USA, Savannah, GA). This created a design that simulated vital pulp therapy and the blood present there would discolor the rest of the crown of the tooth. After 24 hours the md temp and cotton pledget were removed. The access cavities in all the specimens were sealed with visible light cured composite resin using a self etchant bonding agent. This prevented any discoloration due to the use of a colored etching solution. In the remaining six specimens (control groups), cylindrical plastic foam was placed similiarly to a length of 3mm which was saturated with blood (n=3) or normal saline (n=3). Cotton pledget and md temp were used to seal the cavity for 24

hrs which was then replaced with light cure composite resin material using self etchant containing bonding agent.

All these specimens were mounted on cylindrical acrylic resin blocks such that the apical end of the teeth were embedded till the cervical portion of the labial surface. This facilitated the entire labial coronal surface of these specimens to be visualized for spectrophotometer examination.

The color of the crowns was measured under standard condition in a dark room using 650 lambda spectrophotometer (perkin elmer) with a wavelength range of 190 to 900nm and a wavelength accuracy +/- 0.15nm .The light source which illuminated the surface of the specimens were kept at an angle of 90 degrees from vertical axis and the spectrophotometer was positioned at an angle of 0 degree relative to the vertical axis with a 10-cm distance from the specimen surface.

In the present study a spectrophotometer was used for analysis of color. The spectrophotometer measures colors based on reflectance. It primarily has a stable light source and aperture existing between the detector and the object. Measurement is done by CIELAB SYSTEM calculating the ratio of reflected wavelength of the target object to the wavelength reflected from a white standard reference at intervals of 5, 10 or 20nm of the visible spectrum. They are the instrument of choice for the surface color measurement and are more stable and can be used for evaluation of color difference and also absolute color measurements. The only disadvantage is that there may be loss of edges during calculations.

The main advantage of spectrophotometer over spectroradiometer is that they have a stable light source.[-] Earlier studies by **Browning W.D et al**[7] have shown the measurements using CIELAB system were more accurate and precise during the spectrophotometric analysis. Similar results were observed by **Paul et al** and **Peter et al** earlier.[33] Spectrophotometer measures the full light spectrum unlike colorimeters which measure only the red blue and green points.[7] They measure the space coordinates on any standard illuminant. Because of the following advantages the spectrophotometer was used for this study. In the CIELAB system

The change ΔE* of each specimen was calculated using the following equation

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

Where ΔL* parameter indicates lightness and ranges in value from 0 (black) to 100 (white), Δa* indicates green/red. Here negative value indicates green and positive value indicates red and Δb* indicates blue/yellow where blue value indicates negative value and yellow indicates positive value. The mean of 3 measurements was calculated. Measurements were performed in three axis on the tooth surface at 4 time intervals.

1. Before the application of materials.
2. 24 hours after the application of materials.
3. 1 month after the application of materials.
4. 6 months after the application of materials.

The results of the present study showed that there was statistically significant difference on the whole labial surface of the tooth in all coordinates (L*,a*,b*)for all the 3groups of materials tested at all the 3 time intervals. The amount of change in the value of color shift as measured by color difference (ΔE) was also found to be statistically significant.

At the beginning of the study all the 66 specimens were visualised in the 3 color coordinates by spectrophotometry. The CIELAB measurements L revealed luminescence value of mean 72.5. the value of 'a' showed a mean of 2.3 and the value of 'b' showed a mean of around 19. In the luminescence scale 0 denotes black and 100 denotes whiteness of the tooth surface. 'a' denotes change in green/red scale and specimens with a mean of 2.3 was towards the beginning of red on the scale. Measurement of 'b' on the blue/yellow scale tipped the value towards yellowness with a mean of 19. Further change in the measurements were analysed after 24 hours, end of 1^{st} month and end of 6^{th} month.

The ΔL and ΔE were calculated from the tables, where ΔL denotes the change in luminescence or value between the tested day and the day of start of study. ΔE denotes the change of each specimen between the tested day and starting day. ΔE was calculated using the formula

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

Where as Δa is a mean change in green/red axis and Δb is the mean change in blue/yellow axis. ΔL and ΔE where calculated at the end of 24 hours, 1 month and 6 months.

The data from the tables 1 and 2 which was the positive control group (saline group) suggests the mean of the Value or luminescence (ΔL*) after 24 hours was found to be -0.33 and change in color as measured by ΔE was found to be .67. At the end of 1 month the value of ΔL was -2.06 and ΔE was 4.7. at the end of 6 months the value of ΔL and ΔE were -1.1 and 3.9 respectively. This shows that the luminescence or ΔL decreased significantly over 1 month and slightly increased by the end of 6 months.

ΔE showed significant increase at the end of 1 month as compared with the end of day one and thereafter marginally decreased till the end of 6 months. Since the experiment was done on the extracted teeth outside the oral cavity there is a tendency for the tooth luminescence (ΔL) to decrease overtime. ΔE denotes the color difference value. Values of ΔE from 0-2 are considered as not perceivable, 2-3 just perceivable , 3-8 moderately perceivable and ΔE > 8 as distinctly perceivable [41,43]

From the table 1 and 2 of the positive control group it can be seen the value of ΔE was .67 which is below the perceivable range meaning that there is no perceivable color change in 24 hours and increased to 4.7 by the end of 1 month meaning that the color difference was moderately perceivable and slightly decreased to 3.9 by the end of 6 months suggesting that the color change is still moderately perceivable. Hence it can be seen that there is a

moderate increase in color change overtime starting from day 1 to end of 6 months.

From table 3 and 4 which is negative control group (where in the foam in the specimens were soaked with blood), the value of ΔL showed highly significant decrease to -14.9 at the end of 24 hours and minimally increased to 13.2 at the end of 1 month and again showed a very slight decrease to -13.4. ΔE values changed from 16.17 at the end of 24 hours to 14.9 at the end of 1 month and 14.8 by the end of 6 months. This suggests that the change in color is distinctly perceivable right from the 1st day of the study.

The results of the present study revealed that contamination of blood significantly increased discoloration as the model design for the study simulated the clinical situation of vital pulp therapy where there would be bleeding from the subjacent pulpal tissue . The structure and composition of the tooth and the ability of the blood to penetrate through the dentinal tubules into enamel are the factors determining the change in discoloration. Contamination with saline produced minimal perceivable change in color where as contamination with blood produced a distinctly perceivable discoloration.

The objective of this invitro study was to compare the change in luminescence and degree of coronal tooth discoloration after the application of the currently available vital pulp therapy materials , in the presence and absence of blood, overtime.

The results of the present study from the tables revealed that all the materials when contaminated with blood showed significant change in luminescence (ΔL) and significant discoloration (ΔE) over a period of 6 months.

The following were the changes seen in the values of ΔL and ΔE when white Proroot MTA was used for the simulated vital pulp therapy when there is no contamination. (group 1A) , the values of ΔL were -1.12 , -2.67 and -2.99 at the end of 24 hours , 1 month and 6 months respectively, showing that there was only mild change in luminescence. ΔE values showed a change from 1.13 to 4.95 to 5.96 by the end of 24 hours , 1 month and 6 month respectively. This means that the color change was in the range of not perceivable at the end of 24 hours and changed to moderately perceivable after 1 month and 6 months. Comparing the green/red axis (value of Δa) increased to 5.46 from 0 to 7.49 at the end of one month and 6months suggesting the change in color towards the red axis. Comparing the blue/yellow axis (Δb) the change increased from 0.03 to 12 to 19 from day 1 to end of 1 month and 6 months respectively, suggesting that there is a tendency to discolor towards the yellow axis. Overall there is a tendency towards darkening of the tooth as time progresses as the tooth becomes progressively non vital.

When contaminated with blood the specimens filled with white Proroot MTA(group 2A) showed greater change in luminescence and discoloration as seen from the table 5 6 7 and 8. It can be seen that the value of ΔL changed from -5.09 to 8.71 to -8.11 suggesting that there is change in luminescence

over a period of 1month followed by marginal change over 6months. In the first 24 hours ΔL shows the change to -5.09 (L2-L1 i.e 67.45-72.54) this can probably be attributed to the contamination due to blood. The change in ΔE in this group 2A was 5.09 at the end of 24 hours which increased to 8.73 by the end of 1 month and further increased to 10.78 by the end of 6 months. This results were similar to the results obtained by **shoukouhinejad et al** in their study.[37]This suggests that contamination with blood and the material increased the discoloration to moderately perceivable change at the end of 24 hours. Contamination with blood alone produced highly distinct perceivable discoloration where as contamination with blood and white Proroot MTA produced moderately perceivable discoloration. This can be attributed to the presence of Proroot material which may be due to its structure and composition. Since the blood is contaminated with white Proroot MTA, this material could probably prevent the direct penetrability of the blood into the dentinal tissues there by reducing the discoloration due to the contamination of the blood alone at the end of 24 hours. At the end of 1month the discoloration increased to distinctly perceivable change and increased further by the end of 6 months, but at both time periods the change in ΔE was comparatively lesser than ΔE value obtained with blood, though all these specimens were in the distinctly perceivable change in discoloration level. This can be attributed to the composition of Proroot MTA which has metal constituents like bismuth, iron, aluminium and magnesium. According to **shoukouhinejad et al** this discoloration may be related in the oxidation of the iron content and remaining

composition of the set material and belongs to the calcium aluminoferrite phase of the powder.[37,19] Moreover presence of bismuth in the material as bismuth oxide, which undergoes oxidation probably destabilizes the oxygen in the compound which reacts with carbondioxide to produce bismuth carbonate that could cause discoloration [24,39]

Marciano et al[30] suggested that there is interference of bismuth oxide with dentin collagen. **Camilleri**[11] suggested that interference of bismuth oxide with sodium hypochlorite, if present on dentin, could be a cause for discoloration. This theory was supported by **lenherr et al** who compared discoloration caused by Portland cements without bismuth oxide and both types of MTA.[26]

When Biodentin was used in the present simulated vital pulp therapy study, the following changes were seen(as per the tables 9 10 11 and 12) . Addition of mixture of biodentin material within the pulp chamber in the positive control group (saline) did not produce any perceivable change during the 1st 24 hours (-.32) but showed a mild decrease in ΔL (luminescence) at the end of 1 month (-1.78)which decreased further by the end of 6th month (-2.62). This was similar to the studies done by **shoukouhinejad et al** at the end of 1 month but showed more decrease at the end of 6 months[37]. Comparing the ΔL values for the BD group with the control group it was seen that the luminescence or the value of the BD showed more decrease in the saline group than the BD group at all time periods. This may be attributed to the composition of biodentin material which does not contain bismuth oxide but

contain zirconium oxide. Zirconium oxide is a radiopacifier and would have contributed to reduce the translucency (ΔL) with resultant increase in opacity of the tooth when placed in contact with saline. The degree of luminescence change to a more decreased value in the normal saline group in the absence of any of these calcium silicate based materials and can probably be attributed to the composite restorative material used for filling the pulp chamber completely resulting in more translucency, during the entire period of study.

On comparing the ΔE values it was seen that at the end of 24 hours, the change was 0.87 which increased to 3.66 at the end of 1month and to 5.19 at the end of 6 months. This was in similar to the studies done by **Shoukouhinejad et al, Ramos et al, Mozynska et al**[31,35,37]. Change in discoloration was present after one year in the study done by **Ramos et al**,**Valles et al**[35,40]. Since the present study was conducted only for the periods of 6months, the change in discoloration was only in the moderate perceivability range, similar to saline group with no cement.

The teeth in the saline control group showed slightly more discoloration compared to the biodentin group. This can be attributed to the presence of zirconium oxide in the BD group and to the restorative composite material used in the saline control group. According to **shoukouhinejad et al**, the presence of composite resin filling the pulp chamber completely, in the absence of leakage, can cause loss of translucency changing the color parameter as recorded by the spectrophotometer[30]. But **Marciano et al** reported that the presence of composite resin had no role to play in changing

the color produced by MTA like cements. On comparing Δa that is the green/red channel the change increase from 0.2 at the end of 24 hours to 1.7 at the end of 1month and further increase to 2.09 at the end of 6 month suggesting a slow and gradual change towards the red axis. Similarly on comparing the change in Δb which is in the blue/yellow channel the change seen was 0.78 at the end of 24 hours, increased to 2.7 at the end of 1 month and gradually increased to 3.96 at the end of 6 months suggesting a slow and gradual change towards the yellow axis. This means that there is a slow discoloration occurring in BD over a period. On comparing with the saline control group the change in Δa increased from 0.22 to 2.41 and slightly increased to 2.22 at the end of 24 hours, 1 month and 6 month respectively in the control group. Δb changes were -0.49 and 3.58 and 2.98 for this same period. This shows that the change in discoloration is slowly progressive in both the color channels for BD (increasing towards red in the green/red channel and increasing towards yellowness in the blue/yellow channel). On the other hand the saline control group shows a tendency to increase in the red channel at the end of one month and slightly decreasing towards the green channel at the end of 6 month. Similarly on the blue/yellow channel, it shows increase towards yellowness at the end of 1 month and decreasing towards blue channel towards at the end of 6 months. So this slight change can be attributed probably to the property of both the composite resin material and tooth per se. The change in BD group could be attributed to the composition of BD and it does not contain bismuth oxide and has zirconium oxide. As

reported earlier zirconium oxide per se could cause change in color overtime.[35,40]

A comparative study done while using Biodentin in specimens contaminated with blood produced the falling observations. The change in luminescence ΔL decreased significantly from -2.3 at the end of 24 hours to -4.54 at the end of one month and continue to decrease at a slower rate to the end of 6th month till -5.11.When comparing this changes with the contaminated blood group alone (where no MTA like material was placed in the pulp chamber). It was seen that the ΔL changes decreased highly significantly in the first 24 hours (-14.9) and ΔL changes were -13.2 by the end of 1 month and almost remained at the same level -13.4 at the end of 6 months. On comparison between these two groups it is seen that BD has a role to play within 24 hours to reduce the luminescence. This is probably related to its composition and character of biodentin. In a recent study by **Yoldas et.al**, they reported that biodentin sets quickly in comparison to other similar materials and could start to block the penetrability of the components of blood much earlier[44]. The change in luminescence can be attributed to its composition probably zirconium oxide. The change seen at the end of one month and six months may be attributed to the slower penetration of blood components and also to more decrease in luminescence of BD. Study by **Shoukouhinejad** et al. showed similar results.[37]

On comparing ΔE values the following were the results produced due to the presence of BD. At the end of 24 hours ΔE is 2.3, after one month it

increased to 5.05 and at the end of six months ΔE gradually increases to 6.58, so that there is a perceivable change in color at the end of 24 hours which increased towards moderately perceivable change and gradually increased overtime.

On checking the changes in the blood group, the following were the changes seen, that is 16.17 at the end of 24 hours followed by 14.94 at 1 month and 14.80 at 6 months. This means that with contamination of blood the discoloration significantly is in the distinctly perceivable color change region. On comparing both these groups, it is apparent that ΔE value shows a gradual increase as time progresses with Biodentin but less than the blood contamination group. At the end of 24 hours the change is markedly high in comparison to the Biodentin group as Biodentin as increased stainability due to the leaching of its components into the tooth structure. Study by **Beatty et al** reported that biodentin, a root repair material discolors the tooth structure over 2 months to an acceptable change similar to biodentin saline control group[5].

discoloration of biodentin may take place between 6 months and 1 year[37,40]. The probable reason may be due to the inherent character of the biodentin material. On checking Δa (green/red channel) these specimens tested changed from -0.01 to 1.9 to 2.4 over the time period tested Δa in the blood group at the same periods was 2.92, 3.19 and 2.92 respectively which shows that the teeth specimens change initially from light green to a increasing red , where as staining with blood changed to a increasing red at the end of 24

hours itself which gradually increased by 1 month and showed slight change at the end of 6 months.

Δb values (change in blue/yellow channel) was -0.13, 1.16, and 3.38 at the various tested time periods for biodentin when contaminated with blood. Δb for the blood stained group for the same periods were 5.57, 6.1 and 5.57 respectively. This shows that the blood stained specimens, the teeth change to yellow which increased slightly at the end of 1 month and returned to the same level after 6 months like after 24 hours. Whereas in the biodentin group the change from initial negligible blue to minimal yellow at the end of one month gradually increasing over six months in the yellow range. Both these values Δa and Δb along with ΔE signifies that the specimens show moderately perceivable darker change in the biodentin group.

On comparison of the ERRM group the following were the values obtained for ΔL. ERRM with saline showed a decrease in ΔL to -1.27 at the end of 24 hours which increased to -1.82 after 1 month and to -2.24 after 6 months. Corresponding ΔL values for the saline group were -0.03, -2.07 and -1.1 after 24 hours, 1 month and 6 months respectively. This shows that the presence of ERRM slowly decreases the translucency (value) over the tested period of 6 months. In comparison to the saline control group, it showed gradual decrease in translucency after 1 month which changed towards lesser decrease in translucency after 6 month. This is probably attributed to the composition of the cement. ERRM does not contain bismuth oxide which is replaced by zirconium oxide and tantalum oxide (Ta_2O_5).

According to **Kohli et al**, ERRM does not induce much change in translucency and color, but in the present study ΔL changed from -1.27 to -1.82 to -2.24 for ERRM over the 6 month period suggesting that there is a slow decreased in translucency. This may be related to the composition of the cement. Addition of tantalum oxide and zirconium oxide may play a role in gradual decrease in the translucency or it may be due to the change in translucency of the composite material used.

A comparison of the ΔE values shows that for ERRM paste material ΔE increases from 1.27 to 4.49 to 5.04 respectively after 24 hours, 1 month and 6 months. In comparison with ΔE for the same periods without the ERRM material (only saline) the value are 0.63, 4.79 and 3.91 respectively. The results suggests that there is a gradual increase in discoloration from a not perceivable change at the end of 24 hours to a moderately perceivable change in discoloration after 1 month and almost remaining at the same level after 6 months. This shows that ERRM does not show much color change after 1 month, Though, it changes from not perceivable after 24 hours to moderately perceivable after 1 month. Previous studies by **Al Subait et al** did not show perceptable color change and maintained stability over a 4 month period. Study by **Beatty et al** described that ERRM paste material discolors the tooth structure over an 8 week period but this study was done on a bovine tooth model unlike the present study which was done on human teeth. Their results indicated that all the materials in their study discolored the tooth structure to a perceptable degree and ERRM showed significantly more overall change.

This may be related to the irrigation protocol followed in the present study. The results of present study was similar to the study of **Shoukouhinejad et al** at the end of 6 months showing a significantly smaller change for ERRM in the presence of salin.e[37]

On comparing Δa that is the green/red channel the change increased from -0.03 at the end of 24 hours to 2.2 at the end of 1month and further increased to 2.58 at the end of 6 months suggesting a slow and gradual change towards the red axis. Δa for the control group for the same periods increased from 0.2 to 2.41 and showed a slight decrease to 2.28. ERRM has a tendency to change towards red. Δb values at the end of 24 hours after 1 month, and after 6 months for ERRM was initially 0.001 increased to 3.4 and further increased slowly to 3.7 suggesting a slow change in discoloration towards the yellow axis in blue/yellow channel. The Δb values for the saline control group for the same time periods changed from an initial -0.49 to 3.58 and then slightly decreased to 3.0. This may be due to the irrigation protocol and the composite resin used.

In the experimental group 2C (ERRM placed over blood sample) the ΔL values were -6.05, -6.04 and -8 at the end of 24 hours, after 1 month and after 6 months respectively. ΔL values for the control group with blood contamination for the same periods were -14.9, -13.2 and -13.4respectively. These values suggest that contamination with blood decreases the translucency significantly high in the first 24 hours followed by improvement at the end of

1 month and then showed a minor variation over a period of 6 months where as placement of ERRM paste over the blood sample decreased the translucency to -6.05 at the end of 24 hours and did not show any change at the end of 1 month (-6.04) and decreased further at the end of 6 months (-8). This suggests comparing both these groups 2C with CB, maximum decrease in translucency (ΔL) was in the blood-stained control group which may be because of the components of blood flowing into the dentinal tubules and making the enamel above less translucent. The placement of the ERRM above blood prevented rapid decrease of translucency and the result is highly significant (-14.9 versus -6). Over the period of 1 month and 6 months there was a slight variation or decrease in translucency suggesting a slow and steady progressive decrease in translucency as compared to blood samples which show a marginal increase in translucency. This is probably due to the reason that the blood components are leached away very slowly from the dentinal tubules in the blood samples, but they are blocked by the ERRM cement over 6 months thereby the translucency increasing at the end of 6 months. This results were almost similar to the study done by **Shoukouhinejad et al.**

comparing the ΔE values between ERRM (2 C) and control blood group(CB) the following changes were seen at the end of 24 hours. ERRM group showed an increase in ΔE 6.05, 8.41 and after 6 months it is 10.5.For the blood-stained control group the changes were 16.17 at the end of 24 hours decreased to 14.9 after 1 month and then to 14.2 at the end of 6 months

This suggests that the sample contaminated with blood showed distinctly perceivable discoloration. color change which was almost are made constant probably due to the components of blood entering the dentinal tubules. In presence of ERRM paste material over the blood stained samples it showed moderate perceivable discoloration at the end of 24 hours which increase to distinct discoloration after one month and then increased progressively over 6 months. This may be due to absence of bismuth oxide and presence of zirconium oxide and tantalum oxide with ERRM.

The results of the present study were almost similar to the study done by **Shoukouhinejad et al**.However study done by **Beatty et al** reported that there was significant change with ERRM probably because they used bovine teeth and not related to the composition of the material per se[6]. In the study by **Kohli et al**, by ERRM did not show perceptible change in color in the tooth structure24, as the tested material in their study did not reach the pulp chamber. The discrepancy may be related to the difference in methodology. **Alsubait et al** reported that crowns on teeth restored with ERRM did not exhibit color change. **Alsubait et al** suggested that ERRM due to its short setting time and ease of handling it would be a good material of choice for coronal restoration of teeth in the esthetic zone. An earlier study reported that ERRM and also Biodentin did not cause significant discoloration in 2 months when compared with control group. Comparing the Δa and Δb values for ERRM in the presence of blood versus blood stained samples without ERRM showed the following particulars. Δa for the ERRM group increased from 0 to 3.09 to 3.6 at the end of the test period of 6 months as compared to 2.92, 3.19 and 2.92 for the blood stained control group. ERRM. This suggests that there is a slow progressive discolorations in the green/red channel with slight variations. Similarly Δb values for the ERRM group were 0.05, 4.98 and 5.77

over the time period in the presence of blood. Δb values for the control blood stained group(without ERRM) showed values of 5.57, 6.1 and 5.57. On comparison when there is blood contamination there is a tendency for the teeth to change progressively in the blue/yellow channel when ERRM is not present. The presence of ERRM over blood in the first 24 hours did not change the color of the tooth,probably this may be related to the irrigation protocol employed in the study and also to various oxides present in the ERRM paste diffusing into the dentinal tubules, thereby over powering the action of blood components.

A comparison after one month and after 6months shows that in ERRM group with blood stained, there is a drastic increase in discoloration in the yellow axis in the blue/yellow channel. On the other hand, the discolorations in the control group is towards the yellow axis and increases marginally over 1 month and then shows a slow decrease in the blue/yellow channel by the end of 6 months Both the groups almost show similar yellowish discoloration. This may related to interaction of the oxides present in ERRM with the components of blood.

The aim of the present invitro study was to evaluate and compare the change in color following placement of Calcium silicate based materials namely white Proroot MTA, Biodentin and endosequence root repair material in the pulp chamber following vital pulp therapy.

Extracted human maxillary anterior teeth with single canal were selected and access cavities were prepared and restored with composite resin after placement of one of these materials in the pulp chamber of the root

canal. The placement of these calcium silicate based materials in the pulp chamber of the tooth have a potential to discolor the teeth. Vital pulp therapy procedures involves placement of these materials in contact with blood therefore the direct effect of these materials and effect of blood on the crown discoloration caused by these materials was assessed in the present study.

The present results revealed the following information. For the sake of comparison these specimens were divided into 2 sub groups, saline and fresh human whole blood. The calcium silicate based materials were placed in contact with either of these solutions and change in translucency and the discoloration was assessed using a spectrophotometer and the changes were evaluated with CIELAB system. On comparing the saline groups that is group 1A, 1B,1C with CS group the following were the results seen. In control group where no repair material was placed in contact with saline and restored directly with composite resin, the ΔL values (change in luminescence) decreased significantly overtime ($P<0.5$) varying from -0.33 to -2.07 and then coming back to -1.1 at the end of 6 months. When white Proroot MTA, Biodentin or endosequence root repair material was placed as a cement barrier followed by the placement of composite material, there was a significant difference in ΔL overtime. In general ΔL decreased significantly overtime with all the three materials. At the end of 24 hrs, ΔL in the saline group (no cements) and Biodentin showed no change where as Proroot MTA and ERRM presented a slight decrease in translucency (ΔL). This may be due to the

chemical reaction taking place between calcium silicate based cement and the composite resin. At the end of one month ΔL decreased significantly in all the groups including the control group which showed the change in ΔL (-2.06). Proroot showed the maximum (-2.67). ΔL in Biodentin decreased to -1.78 from initial value of -0.32; and in ERRM decreased to -1.82 from -1.27, the change in translucency was more in the control group probably due to inherent property of the composite resin and also the loss of action of NaOCl over the period of one month, which was used as an irrigant during the procedure initially.

The high change in translucency in the white Proroot MTA group can be due to the presence of metal constituents such as bismuth present as bismuth oxide. The presence of metallic oxide of zirconium in Biodentin cement could have decreased ΔL over a period of 1 month. In case of Biodentin ΔL decreased significantly from -0.32 to -1.78.

The ERRM paste causes change in translucency over the first 24 hours. This may be related to the paste form of the mix that was used where as Proroot and Biodentin were powders. The paste form probably penetrated the dentinal tubules enhancing the effect of composite resin placed over these materials and decreases the translucency in the first 24 hours. The ΔL changes from-1.27 to -1.82. Comparing ΔL at the end of six months, it was observed that in the control saline group ΔL decreased significantly from -2.06 to -1.1. This may be due to the direct contact of the composite resin material on to the

tooth surface without any cement barrier material in between. Further effect of irrigation with NaOCl could have decreased with in 24 hours and subsequently the composite material and the tooth lost its translucency over a month. Further over 6 month period the change in optical property could have increased ΔL (-1.1) at the end of 6 months.

Comparing the change over 6 months for the 3 tested groups it was seen, that maximum change was seen in the Proroot group followed by ERRM and Biodentin. In the Proroot MTA group, ΔL decreased to -2.99 from -2.67. This can be attributed to the presence of bismuth oxide present in the cement. In the Biodentin group ΔL decreased significantly from -1.78 to -2.62 but this change was less than the Proroot MTA group. This is probably because of zirconium oxide replaces bismuth oxide in this material.

In the ERRM group ΔL decreased from -1.82 to -2.24 at the end of 6 months. This slight decrease may be due to presence of additional oxides like tantalum oxide present in the cement along with zirconium oxide.

From the above discussion it can be deduced that the composition and structure of the calcium silicate based cement material plays an important factor in reducing the translucency of the teeth following vital pulp therapy. The chemical reaction between the calcium silicate based cement and composite resin also plays a role. The presence of composite resin as such changes the parameters of color as recorded by the spectrophotometer.[37] It has been stated by **Frecciae et al**[19] in their study that filling the pulp chamber

completely with light cure composite resin may cause a loss of translucency. The greater thickness of composite material could also have resulted in decreasing the translucency. The change in discoloration in all the 3 tested groups may be related to the discoloration produced by its metallic constituents, such as iron, bismuth, magnesium aluminium etc. The calcium aluminoferrite phase of the powder causes oxidation of the iron remaining in the set material possibly producing tooth discoloration.[18]

In the case of Proroot MTA cement the discoloration is associated with the presence of bismuth, which undergoes oxidation and destabilizing the oxygen which reacts with carbondioxide producing bismuth carbonate resulting in a black precipitate causing dicoloration of the tooth[23,39]. According to **Marciano et al** in the Proroot MTA there is a interference of bismuth oxide with dentin collagen[30]. According to their study, the aminoacids present in dentin collagen seems to destabilize the bismuth oxide molecule. This leads to a reaction which eventually changes its color to black there by causing reduction in translucency over a period of 6months. This discoloration is not apparent over the first 24 hours probably because of application of dentin bonding agent before placing Proroot MTA.[2]

In the Biodentin and ERRM groups bismuth oxide is replaced by zirconium oxide. Tantalum oxide is incorporated in ERRM group. A study by **Kang et al** revealed that in the absence of blood[23], the discoloration caused by bismuth oxide containing materials like Proroot MTA decreased the

translucency significantly more than the materials containing zirconium oxide like Biodentin and tantalum oxide containing materials (ERRM cement) over six months period. [4]

In the CIELAB system ΔE denotes the amount of discoloration combining the range in 2 axis namely green/red channel represented by Δa and blue yellow channel represented by Δb. The mean and standard deviation of change in color are represented in the tables 1 to 16 and graph 1 at different time period tested. From the tables it can be seen that in the control group teeth specimen in the absence of blood, the mean value was 0.63 at the end of 24 hours increasing to 4.79 after 1 month and decreasing to 3.91 after 6 months. This shows that there is not much change in color at the end of 24 hours where as after 1 month there is increase in discoloration. This discoloration is probably due to composition of the coloring pigments present in the composite resin and also due to the use of NaOCl as irrigant. As the time period increases, the discoloration decreases probably due to the structure and composition of the tooth and the penetrability of the restorative material into the dentinal tubules. Placement of barrier cement material such as white Proroot MTA, Biodentin or ERRM paste does not change the discoloration significantly at the end of 24 hours. In comparison to the saline group there is a slightly significant increase in all the 3 materials tested (white Proroot MTA 1.13, Biodentin 0.87 and ERRM 1.2). This slight increase may be due to the usual chemical reaction occurring between the different calcium silicate based

cements and composite resin. According to **Kohli et al** white Proroot MTA shows greater discoloration because this material could interact with the irrigant NaOCl due to the presence of bismuth oxide present in this material.[24] **Camilleri et al** inferred that NaOCl, reduced to sodium chloride and formed a precipitate on bismuth oxide. The absence of bismuth oxide in BD and ERRM probably produced more color stability to these materials. Study by **Valles et al** showed that materials containing bismuth oxide like wPMTA showed change to dark discoloration after light activation in an oxygen free environment where as materials such as BD and ERRM contain zirconium oxide which acts as a radiopacifier and is considered to be more color stable.[39]

ERRM seems to be causing very slightly more discoloration than BD probably because of its paste like nature penetrating more into the dentinal tubules following NaOCl irrigation. The transmission of this material through the enamel could have influence on the color change with the addition of composite resin.

At the end of 1 month wPMTA showed 4.95, BD 3.65 and ERRM 4.49. The increase in slight discoloration in the Proroot group may be related to the bismuth oxide undergoing oxidation reacting with carbondioxide to form bismuth carbonate. In BD, zirconium oxide acting as a color stabilizer is probably the cause for reduced discoloration. In the ERRM group ΔE value was more than BD and less than Proroot materials. According to **Marciano et al** presence of radiopacifier such as zirconium oxide did not produce a black

precipitation when these material contacted the collagen in dentin.[30] At the end of 6 months the saline specimens showed a slight reduction in ΔE 3.9 as compared to after 1 month (4.7). This may be due to the chromatic properties of the restorative materials and effect of dehydration of the tooth surface. ΔE in the Proroot group showed a slight increase to 5.95 at the end of 6 months as against 4.95 after 1 month. This slight increase in discoloration may be due to the continuation of the chemical reaction due to presence of bismuth oxide. **Valles et al** reported that when bismuth oxide containing cement is light activated in oxygen free environment, [39] inside the tooth it dissociates to produce metallic bismuth and oxygen. This bismuth atoms form black crystals that results in darkening of the specimens. They concluded that only bismuth containing materials showed discoloration. **Camelleri et al** hypothesized that the aminoacids present in the collagen of dentin destabilize bismuth molecules leading to a reaction causing discoloration.[10] In the case of BD the ΔE changed from 3.65 after 1 month to5.18 after 6 months. BD is a material similar to MTA is composed of zirconium oxide as radiopacifier in the powder and has calcium chloride as accelerator in liquid. Due to the presence of these materials the BD demonstrated more color stability over the 6 months period as reported by **Shoukouhinejad et al, Valles et al and Ramos et al.**[37,39,35] **Ramos et al** reported the acceptable color change at the end of 6 weeks with BD.[35] On the other hand **Beatty et al** reported greater discoloration for BD over an 8 week period[6]. This was probably because of the use of bovine teeth in their study unlike human teeth used in other studies. **Ramos et al** detected

that there was greater discoloration in BD specimens at 1 year evaluation.[35] **Shoukouhinejad et al** and **valles et al** also detected greater ΔE values after 6 months, suggesting that the discoloration of BD could take place between 6 months and 1 year. The greater change in ΔE after 6 months could be due to the inherent property of the opacifiers present in BD.

In case of ERRM ΔE did not show significant change after 6 months. (4.49 to 5.04). This slight change over a period of 6 months could be attributed to the presence of stable oxides of zirconia and tantalum present in ERRM. This findings is similar to the findings of **Kohli et al**.[24] but dissimilar to the findings of **Beatty et al** [6]who reported greater discoloration with ERRM than wPMTA. This may be related to the difference in methodology. **Alsubait et al** suggested that ERRM is a material that can be used as a cemental barrier in the esthetic zone because of its color stability.[4]

From the tables the following were the changes in Δa value. In the saline group it was 0.22 increased to 2.42 and decreased to 2.28 overtime. In wPMTA saline group it was -0.01 increasing to 2.32 and further increasing to 2.73. In the BD group Δa was 0.22 increasing to 1.7 and further increased to 2.09. In the ERRM group it was -0.03 increasing to 2.29 and further increased to 2.58. This value suggest that Δa increases for all the cemental barrier materials overtime. It shows slight decrease only for the saline control group. The results of the present study suggests that the specimens have a tendency to discolor towards the red axis in the green/red channel as time progresses. This

change is more with Proroot MTA, probably due to the presence of bismuth oxides. ERRM also shows greater change probably due to its better penetration into the dentinal tubules as it is used as paste form.

From the tables Δb were as follows .Δb for saline control group were -0.49 increasing to 3.58 and decreasing to 2.98. Δb for wPMTA is -0.16, 3.47, and 4.36. Δb for BD 0.78, 2.69 to 3.95. For ERRM 0.001, 3.41 and 3.71. This shows that these specimens had a tendency to discolor more in the yellow axis (Δb) of the blue/yellow channel over a period of six months with all the cement groups. There is a slight decrease in discoloration observed after 6 months only in the saline group, probably related to the composition of the resin and dehydration of the tooth. Highest Δb change was seen in the Proroot group, followed by ERRM and BD. There was a tendency for greater change in Δb values for BD after 6 months. This suggests that all teeth undergo progressive yellowish discoloration overtime, when the cemental barrier materials were used and composite resin is placed over it.

Overall the results of the present study on ΔE, that is the discoloration of the tooth structures increased for various cemental barrier materials over different time periods. In the specimens not contaminated by blood reveals that wPMTA causes discoloration of the tooth structure over time. This results are similar to studies done by **Valles et al, Felman et al, Alsubait et al** and **shoukouhinejad et al**.[39.18,4,37] wPMTA has 75% Portland cement, 20% bismuth oxide and 5% calcium sulphate. This bismuth oxide when exposed to

light in an oxygen free environment dissociates to form metallic bismuth. This metallic bismuth that is formed are black in color and is responsible for the change in color and is responsible for the discoloration of the tooth. The use of NaOCl as irrigant causes color change in Proroot MTA. NaOCl gets reduced to sodium chloride displacing its oxygen. This oxygen reacts with bismuth oxygen reducing it to metallic bismuth.[11] Hence this color change is apparent at the end of 24 hours. **Marciano et al** showed that the collagen in dentin reacts with bismuth oxide destabilizing it and forming a black precipitate.[30] This in turn causes dark staining of the material throughout the dentinal tubules. Enamel being relatively translucent material, the transmission of these material discoloration through the dentinal tubules induces overall tooth discoloration. [4]

The group containing BD in the present study showed color stability overtime. Previous study by **Valles et al**[40] showed that BD maintains its color stability over a time period of 6 months in different laboratory condition. BD contains zirconium oxide which replaces bismuth oxide and is a radiopacifier. Marciano et al found that the presence of zirconium oxide and calcium tungstate present in BD exhibited the stability in color[30] , even when placed in contact with the collagen in dentin. However BD undergoes discoloration after 6 months to 1 year for reasons unknown and this time period was not part of this study. Regarding ERRM it is a premixed calcium silicate based material that sets when it comes in contact with moisture. ERRM material when used

as a cemental barrier over specimens not contaminated with blood did not induce any color change exceeding the perceivability threshold (ΔE>2) at the end of 24 hours. ERRM also contains zirconium oxide as a radiopacifier and has tantalum oxide additionally. Earlier studies by **Kohli et al**[24] did not show perceptable color change in tooth structure when ERRM was used. However significantly greater color change was observed in a previous study by **Beatty et al**[6]. The results of the present study are similar to **Kohli et al** studies where human teeth are used to evaluate the discoloration as against bovine teeth used by **Beatty et al**. The teeth restored over ERRM also has additional advantage of short setting time and ease of handling.

In the present study, some amount of tooth color change was observed in the normal saline group (without any calcium silicate based cement barrier material). Due to absence of any barrier material, the access cavities were restored with a greater thickness of composite resin material as compared to the experimental control groups. This may cause a loss of translucency and the presence of composite resin per se changes the parameters recorded by the spectrophotometer used in the present study.[19]The presence of barrier reduced the thickness of composite resin material and comparatively produced a significant difference in discoloration (ΔE) with the experimental groups . So in the absence of blood the cemental barrier materials BD and ERRM exhibited less tooth discoloration compared to wPMTA.

On comparison of group 2A,2B and 2C with CB group the following were the results seen with respect to change in translucency (ΔL) and change in discoloration (ΔE) based on ΔL Δa and Δb. The parameter ΔL, change in translucency observed through the mean and standard deviation at each time period as per the tables 8,12 and 16 and graphs 2 were as follows . Contamination of the pulp chamber with blood and direct placement of the composite resin into the pulp chamber caused the ΔL to decrease significantly at the end of 24 hours (-14.9). after 1 month it shows slight increase (-13.27) and did not show significant difference after 6 months (-13.4). The most significant difference observed in this group after one day. The whole blood sample placed resulted in a rapid development of diffuse pink-red discoloration. The freshly prepared erythrocyte concentrated blood solution was the likely cause for the initial color change due to the transmission of the red-crimson hue on the blood through the tooth structure. Overtime this may change to a dark brown hue, due to the physiologic degradation of the erythrocytes and its interaction with light. A study by **Marin et al**[28] suggests that the main cause of tooth discoloration was pulpal haemorrhage. So to simulate bleeding due to pulpal haemorrhage , freshly prepared erythrocyte concentration blood solution was placed on the foam by light dropping method of the specimen. The pigment responsible for staining the tooth arises from the haemoglobin within the erythrocytes[28] ,the staining was greatest in the dentin layer and closest to the pulp chamber and spreading to the dentin with some discoloration of enamel. Placement of composite resin probably caused

penetration of the constituents or components of blood through the composite resin causing significant decrease in the translucency of the tooth structure. Since the components of the blood diffuses slowly into the enamel the degree of translucency improved after 30 days and did not show any significant change further. So when comparing ΔL for the experimental groups in the wPMTA the decrease in translucency were -5.09 at the end of 24 hours, increasing to -8.71 and then marginally decreasing to -8.11 over time. This may be probably due to the presence of bismuth present in this material which forms black crystals causing a greater decrease in translucency but less significantly than the control group. According to **Felman et al**[18] possible change for the significant change in translucency may be due to the interaction between the components of blood and the unset wPMTA. The slow hydration of the wPMTA may cause the absorption and subsequent haemolysis of erythrocytes from the adjacent tissue thus producing a loss of translucency in both the wPMTA and tooth.

The change in ΔL for the BD group were -2.3 at the end of 24 hours decreasing to -4.54 after 1 month and further slightly decreasing to -5.11 at the end of 6 months. BD found to have the least discoloration potential among the materials tested in the present which was similar to the study of **Yoldas et al**[44]. They reported that the BD has faster setting time and higher solubility than wPMTA. Since setting time is significantly faster, the cement particles of BD would start to block the components of blood more quickly. The results of the

present study confirmed this as after 24 hours ΔL decreases only to -2.3 as compared to the control group showing -14.9. However this study was done on bovine teeth unlike the present study which was done on human teeth and requires further research. Decrease in ΔL observed after 1 month and after 6 month is probably related to its quicker setting time and better sealing ability. On comparing ΔL in the ERRM group it was -6.05, -6.04 and -8 over various time periods. In comparison with blood stained group there was significant difference in ΔL over time. Unlike BD the ΔL decreased significantly in the first 24 hours. This may be due to the use of paste form in this group which probably would have carried the staining components of blood while penetrating the dentinal tubules in the first 24 hours, thereby causing the significant decrease in translucency (-6.05). This is found to be more when comparing wPMTA. After 1 month there is slow change and translucency decreases slowly over the period of 6 months to -8, almost similar to Proroot which decreases more by the end of 1 month and stabilizes after 6 months(-8.11). The presence of zirconium oxide in both BD and ERRM cements could have played a role. Further studies are needed to authenticate these changes through ΔL. In the present study ΔL showed significant decrease in all specimens contaminated with blood. [37]

On comparison of ΔE that is change in discoloration, in the blood stained group without any cemental barrier ΔE increased to 16.1 at the end of 24 hours and then decreased marginally to 14.9 and 14.8 after 1 month and

6 months respectively. This may be due to the interaction of the components of the blood probably hemoglobin causing permeability through the dentinal tubules and reaching enamel. The slight decrease in discoloration after 1 month and 6 months is not statistically significant and may be related to the dissociation of the components of blood. In case of Proroot MTA ΔE showed increase to 5.09 which further increased to 8.73 to 10.77 over the tested time periods. This meant that there was moderately perceivable color change at the end of 24 hours which increased to distinct change in discoloration after 1 month. The change in the color can be attributed to reduction of Bismuth Oxide to Bismuth metal forming a black crystal which further reacts, with components of blood which had already penetrated the dentinal tubules. According to **Marciano et al**,[30] the amino acids present in the dentinal collagen may destabilize Bismuth Oxide molecule causing a reaction and changing it into black. One of the possible mechanism of the increased tooth discoloration by White Pro-root MTA may be due to the oxidation of iron content present in the set material.[37] The iron oxide and the components of hemoglobin may be a cause of increased discoloration over 1 month and continuing to increase after 6 months. In the case of Biodentin group, the change in discoloration was just perceivable at the end of 24 hours which increased to moderately perceivable discoloration (2.3) at the end of 6 months (5.04 to 6.57). The probable reason for mild change in discoloration initially to moderate change later may be related to the faster setting time of the cement, preventing the components of the blood to increase discoloration[44]. After 6

months only a significantl smaller color change was noted. For the experimental group ERRM the change ΔE increased from 6.05 to 8.41 to 10.5 over the various time period. All the specimens tested in this group showed moderately perceivable discoloration which increased to distinctly perceivable discoloration after a month. After 6 months ΔE further increased to 10.5. This may be due to the use of ERRM in the paste form. This may enter the dentinal tubules along with the blood components resulting in moderately perceivable discoloration at the end of 24 hours. However the color change seen with ERRM was almost similar to pro-root MTA after 1 month and beyond . Studies by **Beatty et al**[6] done on bovine teeth also reported similar results. Further studies needs to be done on ERRM especially when contaminated by blood. However study by **Alsubait et al** [4]suggest that ERRM does not induce discoloration and is recommended for use on anterior teeth prior to placing the coronal restoration.

On comparing Δa in the control group (CB) which increased from 2.9 to 3.19 and then decreased to 2.92. On the whole the change in Δa was towards the red axis in the green/red channel which is probably due to the penetrability of the components of the blood through the dentin into the enamel. In the case of wPMTA it varies from 0.12 to 0.34 to 2.82. Over the tested time period, Δa changed from its base value to red axis only after 6 months probably the change into red axis is not due to the presence of bismuth oxide. In the case of BD over the time period the changes in Δa is -0.02, 1.88

and 2.4 respectively. The change in color was from the base of green to mild red in the green/red channel. This may be correlated with faster setting time preventing change of color in the first 24 hours and to mildly perceivable color change after 6 months probably due to the interaction of zirconium oxide in the blood components. On comparing Δa values of ERRM it can be noted that Δa increases from 0.005 to 3.09 and further to 3.6 after 6 months. This is again probably related to the paste form of the cement. As discussed earlier, there is change in color towards the red axis after 1 month. On overall comparison, all these specimens tend to discolor towards the red axis.

On comparison of Δb that is change in color in the blue/yellow channel in the group contaminated with blood only. Δb increased from 5.6 to 6.1 and returned to 5.6 after 6 months. This shows that the teeth changed color into yellow axis significantly by the end of 24 hours and continues to remain almost at the same level. In the wPMTA group it changed from -0.08 (initial blue) to 0.58 (initial yellow) to 6.51 (distinctly yellow) by the end of 6 months. BD showed the minimum change from the base of -0.12 to 1.5 to 3.38 over the tested time period and becoming yellowish after 6 months. Δb in ERRM changed from initial very mild yellow from 0.05 to 4.98 to 5.77 after 6 months showing a greater tendency to yellow discoloration after 1 month and shows the maximum change among the materials tested after 1 month. The change in color in the control group may be associated due to the interaction between the pigments of the erythrocytes with the bonding agent used with the

composite resin, probably being the cause for no further change later. After 6 months the wPMTA showed the maximum change towards the yellow axis(6.51). This may be related only to the interaction of bismuth oxide with the dentinal collagen. According to **Alsubait et al [4]** this tooth discoloration induced by wPMTA cannot be easily avoided. **Akbari et al [2]** suggests applying 2 layers of dentin bonding agent to occlude the dentinal tubules before placing wPMTA to prevent this discoloration.

On overall comparison the ΔE of materials increased significantly when contaminated with blood at different time periods. There was significant change in the discoloration irrespective of the type of material, the presence of blood and the time factor. Hence it can be concluded that contamination with blood significantly increased discoloration with all the materials. **Lenherr et al[26]** showed that Proroot MTA was associated with greater color change after contamination with blood and its degree of discoloration increased overtime. Similar results were reflected in the present study to wPMTA and also to ERRM. The exact mechanism for the discoloration is not yet known. The probable reason is due to the penetration of erythrocytes into the tooth structure and leaching its components causing discoloration [18]. The porosities occurring during the setting of these material can lead to discoloration of the material [2,32]. So it is suggested to use these materials after achieving complete haemostasis. The composition and structure of the material may also play an important role in determining its discoloration potential and depends on its

metal constituents[37]. Further the thickness of composite resin filling the pulp chamber directly or above the cemental barrier may also have an effect due to the seepage of components.[37,19]

Overall comparison of the present study showed that in the presence of blood contamination there is no significant difference between the materials tested with the least discoloration seen with Biodentin.

The main aim of this present invitro study was to see the amount of coronal tooth discoloration following the placement of 3 currently available materials in the pulp chamber during vital pulp therapy (Pro root MTA , BD, ERRM paste) due to contamination with and without blood.

For the simulation of vital pulp therapy extracted human upper anterior teeth were used. Anterior teeth were selected because they had wide root canal diameter which would facilitate placement of the test materials easily. The apical part of each roots was removed perpendicular to its long axis till only 5mm of the root remained as this would facilitate easy placement of plastic white foam into the root canal. The apical end was sealed with resin to prevent leakage of the testing solution. 2-3 drops of testing solution either saline or freshly collected whole blood was syringed into the root canal from the coronal end to simulate a clinical situation for vital pulp therapy. Following this the specimens were divided into 2 sub groups (1-saline and 2- blood contaminated group), 3 teeth from each sub group served as control. The pulp chamber and the access cavity were restored using visible light cured

composite resin material after applying self etch bonding agent. Self etch bonding agent was used to prevent the contact of the etchant with the testing solution in the root canal which could probably interfere with the discoloration.

The methodology followed by this study was similar to the method followed by **Shoukouhinejad** et al.[37] In the 2 subgroups 1 and 2 one of the three selected calcium silicate based cement material was placed in the pulp chamber as per manufacturers instruction to about 1.5mm depth simulating the clinical situation of placing a barrier material. The rest of the access cavity was sealed with temporary filling material (MD temp) for 24 hours to facilitate the setting of the barrier material placed. Following this visible light cured composite resin was used to replace the temporary filling material similar to the placement followed in the control groups but over the barrier material. Preparing the access cavity and irrigation with 5.25% sodium hypochlorite and 17% EDTA, ensures removal of the pulpal remnants from the pulp chamber and root canal. This prevented the interference of the left out tissue during the course of the study while using spectrophotometer. Fresh human whole blood was used in the present study to see the effect of the blood on the discoloration of tooth , following the placement of barrier materials. This model was designed to simulate the clinical situation of vital pulp therapy. However no obturation was done here which was done in previous studies. [2,23]

In the present study a spectrophotometer was used to assess the color change. Spectrophotometer measures the reflection coefficient of light with in the wavelength of visible spectrum used for color and translucency measurements. The spectrophotometer calculates the changes in color and translucency using the CIELAB system. The specimens for measurements were mounted on the acrylic resin block and entire labial surface was exposed to measure the various parameters used in this study. The color of the crowns was measured in a dark room and the surface of the specimens are illuminated by the light source kept at an angle of 90 degree from vertical axis at a distance of 10 cm from the specimen surface . This provided a shadow free surface and helped in correct measurement of the surface color change. Measurement was performed on the entire labial surface to prevent any variation that could arise at different levels of tooth (cervical level is darker yellowish than the incisal level). The measurement was done at 4 time intervals.

1. Before the placement of any material into the pulp chamber
2. 24 hours after cemental barrier material (except in control groups) where only composite resin was placed)
3. 1 month after the placement of cemental barrier material
4. 6 months after the placement of cemental barrier material

The change in color expressed as ΔE^* for each specimen at each time interval was calculated using the following equation

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

In this equation L* parameter calculates the value or the change in translucency ranging from 0(black) to 100(white), a* indicates greenness/redness, (-ve value indicates green and positive value indicates red) b* indicates blueness/yellowness ((-ve value indicates blue and positive value indicates yellow).

On overall comparison of the 2 subgroups (saline and blood) the following were the changes observed. The ΔL in all the experimental groups (both subgroups) decreased significantly over various time periods($P<0.5$). On comparing the saline group with the blood contaminated specimens, the maximum decrease in translucency was seen in the blood contaminated specimens without any cemental barrier material and the least decrease in translucency (ΔL) was observed in the control saline group where the composite resin was kept in contact with the floor of the pulp chamber and to an increased depth from the access cavity to the pulp chamber. A study by **Frecciae et al** [19]suggested that filling the pulp chamber completely with composite resin may cause loss of translucency (ΔL). The results of the present study were also supportive of this statement as it can be seen that there is a decrease in translucency (ΔL) after 1 month. Most probably the irrigation done with 5.25% sodium hypochlorite would have induced a mild bleaching effect on this group of teeth in the first 24 hours. The change in translucency (ΔL) over 1 month may be due to the slow neutralisation of the bleaching

effect . In case of trauma to the teeth, it can cause haemorrhage into the pulp chamber which can result in severe changes in tooth color and translucency. [28]

According to **Yoldas et al**[44], blood components following pulpal haemorrhage flow into the dentinal tubules and become visible, thereby reducing the translucency cause darkening of the tooth.

According to **Shoukouhinjad et al** [37]the access cavity in the control groups were restored with a greater thickness of composite resin material in comparison to the testing groups. The presence of thick layer of composite resin per se could have changed the translucency.[19]

On comparing the 3 experimental cements used in this study it was seen that there was significant decrease in translucency (ΔL) with all the materials contaminated with blood in comparison with saline control groups. But , from the tables it could be seen that the decrease in translucency was much less in all the three experimental groups in comparison to the control blood groups without any cemental barrier.

According to **Yoldas et al**[44] who stated that red blood cells or erythrocytes are responsible for the discoloration and change in translucency . They suggested that the reason for the only blood group to show highest change in translucency (maximum decrease in ΔL) could be explained due to the different cemental barrier materials blocking the blood components

influx.The results of the present study for the 3 cemental barrier material tested seems to be supportive of this statement.

The results of the present study revealed that contamination with blood significantly increased the discoloration associated with MTA like materials.

On comparing the saline sample and blood sample without any intervening cemental materials, ΔE showed less than perceivable discoloration at the end of 24 hours and increased to moderately perceivable discoloration after 1 month with a slight reduction in discoloration after 6 months. On the other hand in the blood contaminated group where there was maximum change in ΔE showing ΔE to be at 16.1 at the end of 24 hours, decreasing to 15 after 1 month.

This shows that contamination with blood increases to distinctly perceivable discoloration and almost remains at that level. This can be attributed to the results that following dental trauma there is pupal haemorrhage, thereby causing the blood components to permeate into the dentinal tubules to become visible in the first 24 hours. The present study is an invitro study and is not identical to the clinical condition. The use of whole blood served as the efficient model for the assessment of tooth discoloration. The discoloration reported in the present study was a quantitative change evaluated by the spectrophotometer used and need not match the clinical scenario.

In the absence of blood all the cemental barrier materials tested did not show any perceivable change at the end of 24 hours but showed moderately perceivable change after 1 month and further increased marginally in 6 months. In the case of Biodentin group the change in discoloration ΔE increased significantly after 6 months (5.18) as compared to 1 month (3.65). According to a study be **Valles et al [40]**, it was seen that white MTA produced significant change in teeth and Biodentin maintained stability over 6 months.Their findings suggested a rapid and severe pattern of discoloration due to the cemental material. **Lenherr et al[26]** and **Felman et al[18]** have reported that White MTA induces gray tooth discoloration within 1 week and similar results were produced in the present study at the end of 1 month.

in pro-root MTA was responsible for the discoloration. Bismuth Oxide when exposed to higher temperature or some form of light radiation in the absence of oxygen, it dissociates into metallic bismuth and oxygen. This metallic bismuth exists as black crystals causing darkening of the sample.The samples were filled with pro-root MTA and covered with composite resin in the present study, in an oxygen free atmosphere and evaluated under artificial light.

Valles et al[44] studied the stability of five calcium silicate based barrier materials and concluded that only Bismuth Oxide containing material showed discoloration.

Camilleri [11] in her study observed the change in White MTA and Bismuth Oxide which was caused by sodium hypochlorite . She postulated that the reduction of Bismuth Oxide to bismuth metal could have resulted in the color change. She also suggested that oxidation of Bismuth Oxide produces Bismuth Carbonate which is light sensitive and could cause discoloration. In the present study, the early change seen after 24 hours in the blood contaminate samples could have been due to the irrigation of the specimens with sodium hypochlorite for the White MTA group.

Marciano et al [30] investigated on color stability in bovine teeth and used wPMTA sealed with composite resin and found that wPMTA maintained its original color . It could probably be due to the reaction of the aminoacids present in dentin collagen which destabilizes bismuth molecules causing discoloration. In the present study in comparison, Biodentin group showed more color stability over the tested time period. Study by **Valles et al**[40] deduced that stability in Biodentin is maintained in different laboratory conditions overtime. This stability is attributed to the presence of Zirconia replacing Bismuth Oxide.

Marciano et al [30]reported that cemental barrier materials containing Zirconium Oxide exhibited color stability even in the presence of blood. A study by **Shokouhinejad et al**[37] revealed that in the cabsence of blood, Biodentin had greater color stability over a period of 6 months compared to White MTA. However in the present study pro-root MTA showed only a

slightly more discoloration as compared to Biodentin. In the blood contaminated group, the discoloration for the Proroot specimens increased appreciably overtime as compared to Biodentin. A study by **Butt et al**[8] reported that Biodentin sets faster and has higher solubility as compared to other White MTA.They suggested that because of the significantly faster setting time, Biodentin could start to block blood components more quickly. A study by **Valles et al**[39] reported that Bismuth Oxide when exposed to light in an oxygen free atmosphere it darkens, but remains white or transparent in the presence of oxygen. Further it was reported that the presence of Bismuth Oxide to 20% in pro-root MTA showed darkest discoloration.

This is in agreement with the present study the pro-root MTA showed maximum discoloration when contaminated with blood, as compared to the other two materials.

ERRM is a premixed Calcium silicate based cement available as putty and paste form. In the present study paste form was used. Previous studies by **Kohli et al**[24] and **Beatty et al**[6] have reported contrasting results about ERRM on the change in color.

Study by **Alsubait et al**[4] showed that the color change produced by ERRM did not exceed the perceptibility threshold. Probably, this results was obtained because the putty form of ERRM was used in that study. ERRM is a material containing Zirconium Oxide as a radiopacifier and also contains

tantalum oxide. The absence of Bismuth Oxide and the presence of Zirconium Oxide contributes to more color stability for ERRM.

Zirconium Oxide does not produce a black precipitate when it contacts the dentinal collagen. The results of the present study revealed that ERRM when not contaminated, with saline showed similar color stability with Biodentin. Hence the presence of zirconium oxide in both the cements could have contributed to the color stability. When the ERRM material came in contact with blood it seems to behave similar to Proroot MTA showing greater discoloration at all periods of time.

In spite of containing zirconium oxide instead of bismuth oxide the discoloration seen with both these materials (Proroot MTA and ERRM) was high and probably associated to its penetrability as ERRM was used in a paste form. This means that the role of penetration coefficient of different barrier materials needs to be studied. Another reason for increase in discoloration associated with ERRM may be due to the extent of blood components entering the dentinal tubules following pulpal haemorrhage.

Beatty et al [6]did a study on the potential ability of 3 barrier materials to induce tooth discoloration in bovine teeth. According to this study all the tested materials discolored the tooth structure.

A study by **Lenherr et al**[26] revealed that most discoloration or staining occurs in the cervical third of the tooth. It was observed in that study that

Proroot MTA produced marked great discoloration at the cervical level where as Biodentin and ERRM did not show perceptible color change in that region. However this study was done on bovine tooth dentin which has significantly higher tubule density than human root dentin. Further that study showed irrigation with sodium hypochlorite causes a dark brown discoloration in bismuth containing material such as Proroot MTA. The sodium hypochlorite is reduced to sodium chloride and the oxygen available seems to be the cause for discoloration. Since Proroot was the only material containing bismuth, it showed more discoloration in the first 24 hours, in the present study.

According to **Valles et al**[39] and supported by **Kohli et al**[24] the penetration property of teeth, of various materials into the dentinal tubules has been proposed for the cause of discoloration. The transmission of these materials through the remaining enamel and dentin is also a reason. So based on these properties, the penetrability of ERRM paste into the dentinal tubules could have been a cause for the significant increase in discoloration associated with this material. ERRM in saline control group behaves similar to Biodentin group where as it behaves like Proroot MTA in blood contaminated group. According to a study done by **Alsubait et al**[4]the crowns of the tooth/ teeth in the WPMTA group revealed grayish discoloration where as when ERRM was used the tooth did not exhibit any change. The additional advantages of short setting time and ease of handling present in ERRM may help in reducing the discoloration.

It has been shown from earlier studies [18,6,26] and the present study, that it is the presence of blood that compounds the discoloration ability of barrier materials and blood alone also has potent staining ability.[6,19,28] The objective of the present study was to evaluate the discoloration potential of various cemental barrier materials by themselves and also when they are placed in close proximity to blood and its components.

From the results of the present study it can be concluded that all cemental barrier materials tested has an ability to significantly decrease the translucency and increase the discoloration of the tooth structure , over a six months period. A human tooth model simulating vital pulp therapy procedure was used in this study.

Fresh human whole blood was used to assess the stainability effect of blood on tooth discoloration following the placement of cemental barrier materials. A spectrophotometer was used as the measurement tool and the changes in translucency and discoloration were quantitatively measured using the CIELAB system.

The results of the present study revealed that the contamination with blood produced significant decrease in translucency and increase in discoloration following the placement of calcium silicate based barrier materials. Aside from blood contamination, other important factors determining the discoloration potential are the structure and composition of these barrier materials. Filling the pulp chamber completely with composite

resin may also cause a reduction in translucency of the tooth. The presence of blood components penetrating the dentinal structures have an effect on discoloration. The change in color parameters (ΔL, Δa, Δb and ΔE) reported in the present study does not necessarily match the color change clinically. Therefore further investigations needs to be done to compare the discoloration produced by these cemental barrier materials in a clinical setting and extrapolate the results.

Summary

SUMMARY

The aim of the present invitro study was to analyse and compare the change in discoloration after the application of various calcium silicate based cemental barrier materials when used in vital pulp therapy procedures. The 3 calcium silicate based materials used in the present study were white Proroot MTA, Biodentin and Endosequence root repair material in vital pulp therapy and were compared at the end of 3 different time periods namely after the end of 24 hours, after 1 month and after 6 months. 66 extracted human anterior teeth with single canals were used. Access cavities was prepared and were filled with one of the 3 barrier materials placed in the pulp chamber in contact with plastic white foam soaked either with saline or blood. The control groups were filled with composite resin without the presence of any barrier material directly contacting the pulp chamber. To simulate the bleeding associated with vital pulp therapy, the plastic white foam soaked with fresh human whole blood was used and this enable to assess the amount of tooth discoloration following placement of resin/cemental barrier material.

A spectrophotometer in the visible spectrum wavelength (190nm to 900nm) was used for color analysis. The entire labial surface of the specimen was focussed on the spectrophotometer to analyse color by measuring the 3 parameters of color using CIELAB system of measurement. The value of L* indicates translucency (ΔL) or lightness of the material. The a* indicated the location of the color in the green (-a)/red (+a) channel. B* indicated the

location on the blue (-b)/yellow (+b) channel denoted by a numerical value. The total magnitude in color difference between 2 objects is expressed numerically as ΔE values. The measurement of ΔE denoted the perceivable color change. The various numerical values obtained for the 3 different materials and 2 control groups was recorded at the 3 different time periods. From the values obtained ΔE was calculated and the amount of discoloration evaluated. All the values obtained were tabulated and compared using repeated measures ANOVA, Bonferroni post hoc test and paired t test using SPSS software.

Conclusion

CONCLUSION

The aim of the present invitro study was to evaluate the discoloration of tooth following the use of various calcium silicate based materials during simulated vital pulp therapy.

From the results of the present study the following were the conclusions:

1. Three calcium silicate based cements, used in the present study wPMTA, BD and ERRM paste caused tooth discoloration
2. The change in tooth discoloration is related to the composition of the 3 calcium silicate based materials.
3. Contamination with blood significantly produced change in discoloration of the tooth when these three materials were used as cemental barrier material during vital pulp therapy.
4. Extracted fresh human anterior teeth were used in this study to compare the discoloration of teeth during simulated vital pulp therapy
5. Composite resin material was used to assess discoloration following placement of cemental barrier material in the pulp chamber.
6. Plastic white foam served as a good receptacle to place saline/blood simulating the root canal in vital pulp therapy
7. Saline sample served as a positive control to check the color change in the absence of the barrier material, when composite resin was directly placed in the pulp chamber.

8. Fresh human whole blood served as a negative control and had an effect on the change in color when composite resin was placed in contact with it.
9. Spectrophotometer served as a stable external light source for color analysis without causing any shadow, and at a wavelength of 190nm to 900nm in the visible spectrum.
10. Placing the tooth specimen on resin blocks enabled to visualize the entire labial surface under spectrophotometer for color analysis.
11. Color change was analysed using the commission internationale de l'eclairage. (CIE) L*, a*,b* system. Quantitative analysis of the color change (ΔE) could be calculated and was found to be reliable.
12. The CIELAB coloring system was used to evaluate the change in discoloration (ΔE), the change in the parameters of color in the 3 axes denoted by ΔL for change in translucency, Δa for change in color in the green/red channel and Δb for change in color in the blue/yellow channel.
13. The normal saline/blood groups used in the present study in the absence of calcium silicate based material served as controls to compare discoloration with the 3 tested materials over 3 different time period.
14. In the saline control group direct composite resin was placed in the pulp chamber without any intervening cemental barrier material.

15. In the absence of blood and cemental barrier material, the study revealed no change in translucency and any perceivable change in the first 24 hours.
16. After 1 month the translucency decreased significantly which resulted in moderately significant increase in discoloration. Parameters of colors changing after 1 month is due to thickness and inherent properties of the composite resin material.
17. After 6 months there was an increase in translucency and associated decrease in discoloration overtime. The composite resin used, has a tendency to lighten and served as a good control in the present study.
18. The direct contact of composite resin with contaminated blood showed the maximum highly significant change in the values of ΔE, ΔL, Δa and Δb at all time periods.
19. It is the presence of blood components that produced maximum changes in the 1^{st} 24 hours. Blood alone has potent staining ability due to the components of erythrocytes which are capable of penetrating into the dentinal tubules and reaching enamel
20. After 1 month and 6 month there is a significant change in the parameters of color (increase in translucency and reduction in the discoloration in the red and yellow axes).
21. The thickness of the Composite per se does not play any role in reducing the discoloration after 1 month and 6 months. The slight

reduction in discoloration is attributed to the diffusion of the components of the resin into enamel.

22. It is logical to consider this experimental group as the positive control as it showed the maximum changes
23. The color changes associated with wPMTA was the highest among the materials tested especially after blood contamination
24. The composition of the wPMTA material is an important factor in determining its discoloration potential. White PMTA used in the study is an iron containing calcium aluminoferrite cement and also has bismuth.
25. Bismuth oxide in wPMTA is the cause for the discoloration of tooth. The discoloration is much less than the blood contaminated control group (which has the maximum).
26. At the end of 24 hours in wPMTA, ΔE showed moderate discoloration when contaminated with blood (ΔL decrease significantly) as compared to saline group.
27. After 1 month discoloration increased with wPMTA (significant decrease in translucency) in comparison to saline group.
28. After 6 months the discoloration continues to increase with wPMTA (negligible decrease in translucency)
29. The components of blood mixing with bismuth oxide causing a reaction is the cause of increased discoloration for wPMTA.

30. The application of BD increased the discoloration moderately(decrease the translucency minimally) in the saline group.
31. Placement of BD over contaminated blood increased the discoloration (with significant decrease in translucency).
32. The change in parameters of color was more with BD in the blood stained group than the control group.
33. The specimens in BD group showed significantly lesser changes in all parameters in comparison to wPMTA in both the groups.
34. The specimens in BD group showed moderately perceivable change in discoloration over 6 months (with negligible change in translucency) in the saline control group.
35. BD specimens showed moderately significant discoloration (significant change in translucency) over the 6 months period.
36. The changes in parameter of color in BD subgroup was lesser than the wPMTA specimens overtime.
37. The absence of bismuth oxide in BD results in significantly lesser changes on the parameters of color.
38. The presence of radioopacifier zirconium oxide in BD is probably the cause for lesser discoloration potential with BD.
39. The change in the chromic value due to the presence of zirconium oxide produced more color stability in BD.
40. In the 1^{st} 24 hours, changes in the color parameter are significantly lesser for BD in comparison to wPMTA.

41. The faster setting time of BD probably would start to block blood components more quickly in the 1st 24 hours.
42. The new ERRM paste is a zirconium oxide and tantalum oxide containing materials.
43. Changes in the parameters of color was slightly more significant for ERRM in comparison to the saline group.
44. ERRM showed highly significant change in ΔL and ΔE as compared to the control blood group.
45. The change in discoloration was less at all time periods with ERRM / Blood in comparison to the control blood group
46. The changes in color parameter with ERRM and wPMTA was almost similar overtime in the saline group.
47. In the blood contaminated group ERRM showed slightly increased tendency to discolour in the 1st 24 hours in comparison to wPMTA.
48. The paste form of ERRM caused more penetration and resultant color change in the 1st 24 hours in comparison to wPMTA
49. The better penetrability of ERRM probably caused the blood components to penetrate more in the 1st 24 hours.
50. The change in color parameters in ERRM was slightly less than wPMTA over time.
51. ERRM paste caused slight increase in discoloration(more decrease in translucency) in comparison to BD in the 1st 24 hours in the absence of blood.

52. After 1 month ERRM paste produced slightly more discoloration than BD in saline group.
53. After 6 months BD specimens showed slightly more progressive discoloration than ERRM in the absence of blood.
54. ERRM showed significant increase in discoloration in comparison to BD when contaminated with blood at all times (greater decrease in translucency).
55. The paste form of ERRM causing more penetration is the cause for more discoloration. The change in discoloration with ERRM/blood was significantly lesser than blood alone.
56. The ability of the components of the blood to penetrate more with ERRM paste is the reason for increased discoloration associated with ERRM in the presence of blood.
57. The a* indicated by a numerical value denoted the location of the different specimens tested to be in the red (+a) axes of the green to red channel.
58. The b* denoted by a numerical value indicating the change in color in the blue/yellow channel showed that all specimens were in the yellow axes.
59. The controlled group specimens contaminated with blood showed Δa to be in the red axes and Δb to be in the higher gradient of yellow axes.

60. The controlled group specimens contaminated with blood showed Δa to be in the red axes and Δb* to be in the higher gradient of yellow axes
61. The values of a* and b* was least with biodentin without contamination of blood for the entire tested periods
62. The b* was the least in the saline control group over the entire period
63. Between the two materials BD and ERRM paste, in the absence of blood , tooth discoloration is due to ERRM relatively more than BD.
64. Between the two materials BD and ERRM paste, the discoloration produced by ERRM is significantly more for BD overtime.
65. The two materials BD and ERRM paste both contains zirconium oxide as radioopacifier.
66. Between the two groups BD and ERRM paste increased penetrability of ERRM on blood components seems to be the cause for increased discoloration seen with ERRM over BD overtime.
67. In conclusion the present study did not show significant difference in color change between the materials tested in the absence of blood. BD exhibited the least discoloration at all times in comparison to wPMTA and ERRM paste, as it does not contain bismuth oxide which is replaced by zirconium oxide.
68. On the other hand even though ERRM contains zirconium oxide the change in color parameters produced was almost similar to wPMTA when contaminated with blood.

69. The components of blood (coloring pigments in erythrocytes) seems to play a major role in causing discoloration of teeth following trauma or pulpal therapy.
70. The composition and properties of the cemental barrier materials are influenced due to the penetrability of blood components in inducing discoloration.

Further studies on the role of blood components in causing discoloration of tooth following trauma or vital pulp therapy and their influence on various newer cemental barrier materials needs to be studied.

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Annexures

ANNEXURE -I

RAGAS DENTAL COLLEGE & HOSPITAL
(Unit of Ragas Educational Society)
Recognized by the Dental Council of India, New Delhi
Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai
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TO WHOM SO EVER IT MAY CONCERN

Date: 05-02-2019,
Place: Chennai

From
The Institutional Review Board,
Ragas Dental College & Hospital,
Uthandi,
Chennai – 600119.

The dissertation topic titled **"AN INVITRO STUDY TO EVALUATE AND COMPARE TOOTH DISCOLORATION AFTER THE APPLICATION CALCIUM SILICATE BASED CEMENTS IN THE PRESENCE OR ABSENCE OF BLOOD USING SPECTROPHOTOMETER"** submitted by **Dr. G.SASTAMI** has been approved by the Institutional Review Board of Ragas Dental College & Hospital.

Dr. N.S. AZHAGARASAN, M.D.S.,
Member Secretary,
Institutional Review Board,
Ragas Dental College & Hospital,
Uthandi,
Chennai – 600 119.

ANNEXURE –II

URKUND

Urkund Analysis Result

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Significance: 5 %

Sources included in the report:

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