Spectrophotometric Analysis of Coronal Discolouration Induced by ProRoot MTA, Biodentine and MTA Repair HP Used for Pulpotomy Procedures

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ABSTRACT

Objective: To assess and quantify coronal tooth discolouration by ProRoot MTA, Biodentine and MTA repair HP as pulpotomy agents and to identify colour stability of these materials in presence of blood contamination.

Methods: 120 human premolar teeth were used in the study. The teeth were sectioned horizontally 1 mm apical to the cementoenamel junction. A retrograde cavity extending within 2 mm of the incisal edge was prepared. The specimens were randomly distributed as; Control: Group 1, ProRoot MTA: Group 2, Biodentine: Group 3 and MTA repair HP: Group 4. The groups werefurther subdivided on basis of exposure to saline (subgroup A) or blood (subgroup B). The access was sealed with light cured Glass ionomer cemet and the specimens were stored in artificial saliva at 37°C. The Colour change was evaluated with a spectrophotometer at: day 0 (T0), day 1 (T1), day 7 (T7), 1 month (T30), 2 months (T60), and 6 months (T180). The colour measurements were recorded using the Commission Internationale de l'Eclairage L*a*b* value.

Results: For all groups, there was a sharp increase in L* parameter at T1. At 6 months, Group 1B (Control + blood) showed maximum decrease in luminosity followed by Group 2A (ProRoot + saline) > Group 4B (MTA repair HP + blood) > Group 2B (ProRoot + blood). Group 3A (Biodentine + saline) showed the least amount of decrease in luminosity followed by Group 4A (MTA repair HP + saline) and Group 3B (Biodentine + blood). No significant difference was found in ΔE change between any of the groups from baseline to 180 days (P>0.05).

Conclusion: Relative to L* parameter, it was possible to observe a statistically significant decrease in luminosity in the Group1B (Control + blood) followed by ProRoot MTA (Group 2A and 2B) and MTA repair HP (Group 4A and 4B). Biodentine (Group 3A and 3B) showed least tooth discolouration in terms of L* parameter.

Keywords: Biodentine, blood contamination, discolouration, MTA repair HP, pulpotomy, spectrophotometer

HIGHLIGHTS

- Biodentine showed lesser tooth discolouration in terms of value as compared to ProRoot MTA and MTA repair HP.
- Intra-group comparison showed no significant difference in ΔE from baseline to 180 days.
- Blood contamination of materials caused no significant tooth discolouration.

INTRODUCTION

Over the last decade, there have been various innovations in the field of endodontic materials and techniques. However, poor aesthetic appearance of pulp therapy procedures which involve the placement of materials in the coronal third of the tooth, remains a challenging issue in clinical dentistry (1). Mineral Trioxide Aggregate (MTA) because of its favourable biocompatibility in comparison with traditional calcium hydroxide paste, is the material of choice for pulpotomy. However, it has several drawbacks such as poor handling properties, long setting time, and discolouration potential of the remaining tooth structure (1, 2). MTA was first introduced in Grey form (GMTA), which reported discolouration after its application; thus limiting its use in aesthetic areas (1, 3-5). To overcome this, formulation was modified by lowering the concentration of various metal oxides such as iron (Fe_2O_3), bismuth (Bi_2O_3), aluminum (Al_2O_3), and magnesium (MgO) implicated in colour change, and an off-white MTA formula was developed (WMTA).However, studies have shown that WMTA induces the same chromogenic effect as GMTA (6-8).

Enamel gets discoloured in presence of blood and the discolouration possibly becomes more pronounced with longer exposure time (9, 10). Histochemical analysis have shown erythrocytes to enclose the blood pigment hemoglobin, which is responsible for tooth staining (11). Various studies have reported that the blood in the pulp can deepen the colour change of MTA (7, 12).

Biodentine (Septodont, Saint-Maur-des-Fosses, France), is a bioactive dentine substitute with endodontic indications similar to those of MTA. Biodentine has advantages including favourable biocompatibility, good sealing ability, adequate compressive strength, and short setting time, which provides a significant advantage over other materials (13, 14). Studies show conflicting results regarding its discolouration, with some studies reporting colour stability while others report discolouration (14-17). Some studies also reported increased discolouration of Biodentine in presence of blood contamination (18, 19).

Recently, an MTA-based material named as MTA repair HP (Angelus Industria de Produtos Odontologicos) was introduced. One of the differences of this new material over white MTA is the replacement of the distilled water by liquid containing an organic plasticizer. This liquid provides a higher plasticity, improving handling properties of the material. Furthermore, the manufacturer claims that calcium tungstate as radiopacifier will avoid discolouration. Yet there have been no studies to confirm this claim (14).

As per discussion above, the present study aimed to investigate the discolouration potential of ProRoot MTA, Biodentine and MTA repair HP, when used as a pulpotomy capping material. The study also aimed to identify colour stability of these materials in presence of blood contamination.

MATERIALS AND METHODS
The experiment was performed under guidelines approved by the institutional ethics committee. (GDCH/IIEC/2018-09 dated 16/04/2018)

Specimen collection
The study was conducted on 120 freshly extracted single rooted human premolar teeth. The teeth were inspected to ensure the absence of cracks, fractures, caries, and prior discolouration because of intrinsic causes. The teeth were cleansed with an ultrasonic scaler and polished with pumice and water to remove soft tissue remnants, calculus, and extrinsic stains. Radiographic images were taken to confirm that all teeth have a single canal and are devoid of resorptive defects or extensive pulp canal obliteration. The teeth were stored in 0.1% of thymol solution for 1 month before use.

Blood collection
Whole blood (12 ml) was collected from a single volunteer by venipuncture. The sterile anticoagulant coated blood collection tubes (3-4 ml) were utilized to prevent clotting to facilitate the experiment.

Sample preparation
The teeth were sectioned horizontally 1 mm apical to the cementoenamel junction. The coronal pulp was chemo-me-

chanically removed using Hedström files and 2.5% sodium hypochlorite (NaOCl) (10 ml) via retrograde access for one minute. A cavity of dimension 6x0.8 mm was prepared using a cylindrical flat end diamond bur (SF-12, Mani, Tochigi, Japan) through the retrograde access ensuring that it extends to within 2 mm of the central pit of the premolar. The teeth were then washed several times with normal saline and after drying with air, the access opening was irrigated with 20% ethylene di-amine tetra acetic acid (EDTA) for two minutes to remove the smear layer. After final 3 minutes irrigation with sodium hypochlorite, the specimens were stored in distilled water.

The specimens were randomly distributed into 4 groups, containing 30 teeth each: Group 1: Control; Group 2: ProRoot MTA; Group 3: Biodentine and Group 4: MTA repair HP.

Under each experimental group the teeth were randomly divided into 2 subgroups, containing 15 teeth each:

Group 1A: control without blood contamination

Group 1B: control with blood contamination

Group 2A: ProRoot MTA without blood contamination

Group 2B: ProRoot MTA with blood contamination

Group 3A: Biodentine without blood contamination

Group 3B: Biodentine with blood contamination

Group 4A: MTA repair HP without blood contamination

Group 4B: MTA repair HP with blood contamination

The materials were then formulated as instructed by the manufacturer, and were then used to retro-fill the sectioned teeth. A slight vertical pressure was applied with finger pluggers to fill the pulp chamber with 2 mm of the material. Dry sterile cotton pellets were loosely placed within the prepared canals from the apical access.

A micro-pipette was used to transfer 0.01 ml blood in group 1B, group 2B, group 3B and group 4B to saturate the cotton pellets. In the other groups, the cotton pellets were saturated with 0.01 ml saline transferred with help of micropipette. After the early setting phase, the access area was sealed with light cured GIC. In the control group 1A, the pulp tissue was removed from the teeth; a cotton pellet saturated with saline was introduced and then sealed with self-adhesive resin material. Whereas, in the negative group 1B, a cotton pellet saturated with 0.01ml blood was introduced and then sealed with self-adhesive resin material.

A polystyrene mould was fabricated for the teeth to stabilize the specimen and that the same site can be measured every time. An area at the mid-buccal third of the specimen was chosen to facilitate observation of any internal discolouration.

All specimens were placed into separate polyethylene tubes containing artificial saliva at pH of 5.5 and stored next to a window. The composition of artificial saliva used was sodium chloride (6.7 gs/litre), sodium bicarbonate (1.5 gs/litre), potas-

sium chloride (1.2 gs/litre), disodium phosphate (0.26 gs/litre), sodium dihydrogen phosphate (0.2 gs/litre), and bovine albumin (0.1 gs/litre) with 0.1% sodium azide as preservative. The artificial saliva was changed every 2 weeks.

Tooth Shade Assessment:

Colour change was evaluated with a reflectance spectrophotometer at 6 time points: day 0 (T0), day 1 (T1), day 7 (T7), 1 month (T30), 2 months (T60), and 6 months (T180) after material placement. T0 was the baseline value after tooth preparation but before the placement of the materials. The colour measurement was made using the L*a*b* value. Using these L*a*b* values, the differences between the colour measured at the initial time point (time 0) and those measured at the various time points (ΔE) was calculated as follows:

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

where ΔL, Δa, and Δb are the changes in L*, a*, and b*, respectively, between the initial time point and the next time point being compared.

The study was designed to compare the degree of tooth discolouration that occurs over time in specimens filled with ProRoot MTA, Biodentine and MTA repair HP in presence and absence of blood contamination. All the prepared specimens were subjected to spectrophotometric analysis using spectrophotometer (X Rite Gretag Macbeth, Berlin, Germany). The colour differences were evaluated at six-time intervals; T0, T1, T7, T30, T60 and T180 using the CIE L*a*b*[Commission Internationale de l'Eclairage (CIE)] colorimetric system.

Statistical analysis

One-way ANOVA with post hoc Bonferroni's correction were used to verify coordinate L* evolution, ΔE evaluation, considering time (T0, T1, T7, T30, T60 and T180) as the within subjects and considering groups (1, 2, 3 and 4) as between subjects effects (P<0.05). Student's T-test was applied to analyze which subgroup pairs had statistically significant differences in co-ordinate L*, ΔE (P<0.05).

RESULTS

Inter-group comparisons
Coordinate L*

No statistically significant difference was found was found in L* coordinate at T0, T1 and T7. At T30, Group3A (Biodentine + saline) showed statistically less change as compared to Group 4B (MTA repair HP +blood). At T60, Group 2A (ProRoot MTA + saline)statistically showed more discolouration as compared to Group 3A (Biodentine + saline) and 4A (MTA repair HP + saline). Also, at T60 Group 4B (MTA repair HP + blood) showed statistically more significant change as compared to Group 3A (Biodentine + saline). At T180, Group 1B (control + blood) showed the maximum decrease in luminosity followed by Group 2A (ProRoot MTA + saline), Group 4B (MTA repair HP + blood) and Group 2B (ProRoot MTA + blood). Group 3A (Biodentine + saline) showed least decrease in luminosity followed by Group 4A (MTA repair HP + saline) and Group 3B (Biodentine + blood), respectively (Fig. 1, Table 1).
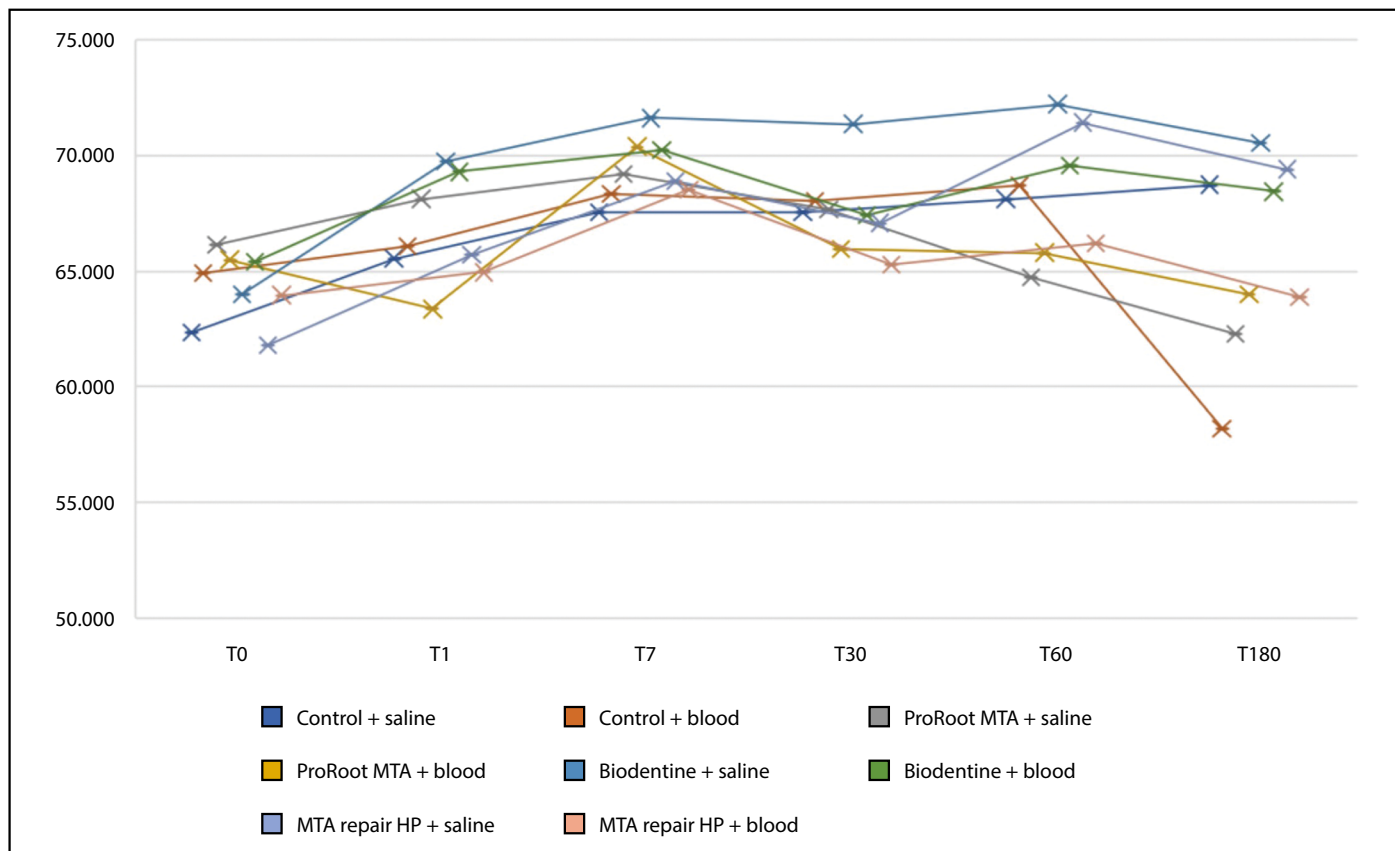

Figure 1. Mean value of 'L' at each time of observation

TABLE 1. Mean values of 'L' at each time of observation

Time	Group	Mean±SD	P value
T0	Group 1A	62.347±6.544	0.203
	Group 1B	64.933±5.418	
	Group 2A	66.160±4.566	
	Group 2B	65.493±5.659	
	Group 3A	64.020±3.895	
	Group 3B	65.420±4.712	
	Group 4A	61.800±3.536	
	Group 4B	63.973±5.216	
T1	Group 1A	65.553±6.780	0.053
	Group 1B	66.093±8.589	
	Group 2A	68.127±4.362	
	Group 2B	63.367±5.612	
	Group 3A	69.753±4.429	
	Group 3B	69.300±6.427	
	Group 4A	65.733±4.655	
	Group 4B	64.967±5.995	
T7	Group 1A	67.560±6.929	0.690
	Group 1B	68.360±8.717	
	Group 2A	69.220±3.607	
	Group 2B	70.393±5.928	
	Group 3A	71.633±4.670	
	Group 3B	70.240±6.705	
	Group 4A	68.900±5.603	
	Group 4B	68.547±6.037	
T30	Group 1A	67.567±5.628	0.048*
	Group 1B	68.047±6.384	
	Group 2A	67.673±2.954	
	Group 2B	65.973±4.857	
	Group 3A	71.367±5.430	
	Group 3B	67.433±3.891	
	Group 4A	67.087±3.663	
	Group 4B	65.293±4.588	
T60	Group 1A	68.127±7.059	<0.001**
	Group 1B	68.707±4.134	
	Group 2A	64.747±3.855	
	Group 2B	65.800±2.849	
	Group 3A	72.220±4.267	
	Group 3B	69.573±2.831	
	Group 4A	71.427±4.063	
	Group 4B	66.200±6.587	
T180	Group 1A	68.747±6.598	<0.001**
	Group 1B	58.193±4.194	
	Group 2A	62.293±4.086	
	Group 2B	64.020±3.033	
	Group 3A	70.553±3.379	
	Group 3B	68.453±4.706	
	Group 4A	69.413±3.325	
	Group 4B	63.893±5.264	

One-Way ANOVA: *P<0.05; Significant; **P<0.001; Highly significant

TABLE 2. Mean values of 'Delta E' at each time of observation

Time	Group	Mean±SD	P value
T1	Group 1A	3.860±1.148	<0.001**
	Group 1B	4.707±1.274	
	Group 2A	4.180±1.058	
	Group 2B	4.540±1.074	
	Group 3A	5.240±0.703	
	Group 3B	7.947±1.308	
	Group 4A	4.053±0.802	
	Group 4B	3.913±0.770	
T7	Group 1A	1.920±0.531	<0.001**
	Group 1B	5.000±0.896	
	Group 2A	2.200±0.740	
	Group 2B	5.527±0.678	
	Group 3A	2.700±0.711	
	Group 3B	2.660±0.780	
	Group 4A	2.987±0.334	
	Group 4B	4.140±0.738	
T30	Group 1A	1.080±0.803	<0.001**
	Group 1B	2.107±0.894	
	Group 2A	2.107±1.108	
	Group 2B	4.807±0.646	
	Group 3A	1.753±0.722	
	Group 3B	4.847±0.976	
	Group 4A	4.147±1.011	
	Group 4B	4.513±0.936	
T60	Group 1A	2.233±0.863	<0.001**
	Group 1B	4.133±0.954	
	Group 2A	3.760±0.990	
	Group 2B	4.460±0.860	
	Group 3A	4.120±1.100	
	Group 3B	4.500±1.059	
	Group 4A	4.420±0.994	
	Group 4B	4.560±1.250	
T180	Group 1A	1.747±0.913	<0.001**
	Group 1B	8.340±0.745	
	Group 2A	4.547±1.425	
	Group 2B	2.307±0.554	
	Group 3A	2.520±0.963	
	Group 3B	4.733±0.990	
	Group 4A	4.167±0.804	
	Group 4B	4.187±0.865	

One-Way ANOVA: *P<0.05; Significant; **P<0.001; Highly significant

Delta E

Group 3A (Biodentine + saline) and 3B (Biodentine + blood) showed most change in ΔE value at T1 as compared to all the other groups. Group 3B (Biodentine + blood) showed the maximum difference in ΔE and was statistically different to all groups. At T7, Group 1A (control + saline) showed least change. Group 2B (ProRoot MTA + blood), and Group 1B (control + blood)show maximum difference significant to all groups except to each other. ΔE of Group 2A (ProRoot MTA + saline) was less than that of Group 2B (ProRoot MTA + blood) and Group 3A (Biodentine + saline). Group 4B (MTA repair HP + blood) had the third highest change which was significant to all except Group 2A (ProRoot MTA + saline) and Group 4A (MTA repair HP + saline). At T30, Group 1A (control + saline) showed the least change whereas Group 3B (Biodentine + blood) showed the maximum change. At T60, all groups showed perceptible colour change except Group 1A (control + saline). At T180, Group 1A (control + saline) showed the least change whereas Group 1B (control + blood) showed the most significantchange. Group 2A (ProRoot MTA + saline) was showed significantly more change as compared to Group 2B (ProRoot MTA + blood) and Group 3A (Biodentine + saline) (Fig. 2, Table 2).

Intra-group comparisons

In the Control group, significant difference in L* and ΔE coordinate between Group 1A (control + saline) and Group 1B (control
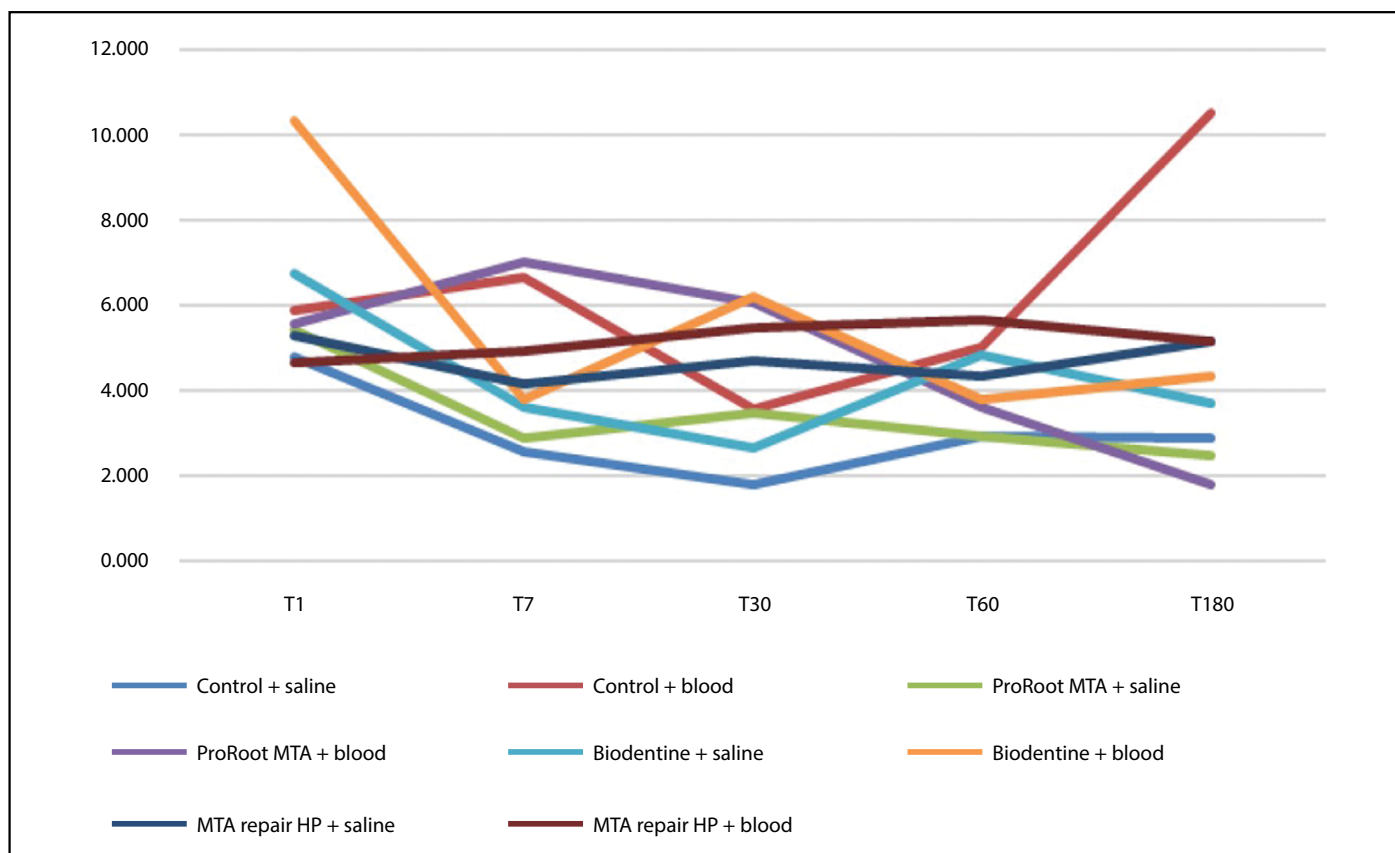
Figure 2. Change in ΔE at each time of observation

+ blood) was observed at T180. Significant values in difference in ΔE was seen at T7, T30, T60 and T180. Thus, it can be inferred that Group 1A (control + saline) remained constant but Group 1B (control + blood) was changing throughout but maximum change was found at T180. In ProRoot MTA group, significant difference in L* and ΔE coordinate between Group 2A (ProRoot MTA + saline) and Group 2B (ProRoot MTA + blood) was observed at T1. Significant values in difference in ΔE was seen between T7, T30, T60 and T180. Thus, it can be inferred that change was observed in both groups, however no significant change was identified from T0-T180. L* coordinate change was more in Group 2A (ProRoot MTA + saline) as compared to Group 2B (ProRoot MTA + blood) but was not statistically significant. In Biodentine group, significant difference in L* and ΔE coordinate between Group 3A (Biodentine + saline) and Group 3B (Biodentine + blood) was found at T30. Significant values in difference in ΔE was observed between T7, T30, T180 and T0-T180. L* coordinate change was more in Group 3A (Biodentine + saline) as compared to Group 3B (Biodentine + blood) but was not statistically significant. In MTA repair HP group, significant difference in L* and ΔE coordinate between Group 4A (MTA repair HP + saline) and Group 4B (MTA repair HP + blood) was found at T60 and T180. Significant values in difference in ΔE was observed between T7. Thus, it can be inferred that L* and ΔE coordinate decreased more for Group 4B (MTA repair HP + blood) as compared to Group 4A (MTA repair HP + saline) but was statistically significant only at T60 and T180. However, the perceptible colour change was similar for both the groups and showed no statistical difference (Fig. 3 and Fig. 4).

DISCUSSION

The current study showed maximum decrease in luminosity in Group 1B (Control + blood) followed by Group 2A (ProRoot + saline) > Group 4B (MTA repair HP + blood) > Group 2B (ProRoot + blood). Whereas Biodentine showed the least amount of decrease in luminosity over the experimental period. No significant difference was seen in ΔE change between any of the groups from baseline to 180 days.

In the clinical practice, the clinicians frequently encounter teeth with exposed dental pulps which under specific circumstances can be treated with direct pulp capping. Recently calcium silicate–based cements have gained popularity for dealing with pulp exposure (20). However, many products are aesthetically unstable and may even induce changes in the dental tissue (5, 21, 22). Most theories about the mechanism of tooth discolouration include oxidation because of the heavy metal oxides (i.e., iron or bismuth) contained in cements. One possible mechanism of tooth discolouration by white MTA is related to the oxidation of the iron content of the set material, which is attributed to the calcium alumino-ferrite phase of the powder (5). Another reason could be attributed to bismuth oxide which when exposed to light in an oxygen-free environment, dissociates into dark-coloured crystals of metallic bismuth and oxygen. Discolouration can also occur when materials containing bismuth oxide encounter sodium hypochlorite and oxidize into bismuth carbonate, which is sensitive to light and causes discolouration (2). Thus newer calcium silicate cements were introduced in the market with altered radio-opacifiers such as zirconium oxide (Biodentine) and calcium tungstate (MTA repair HP).
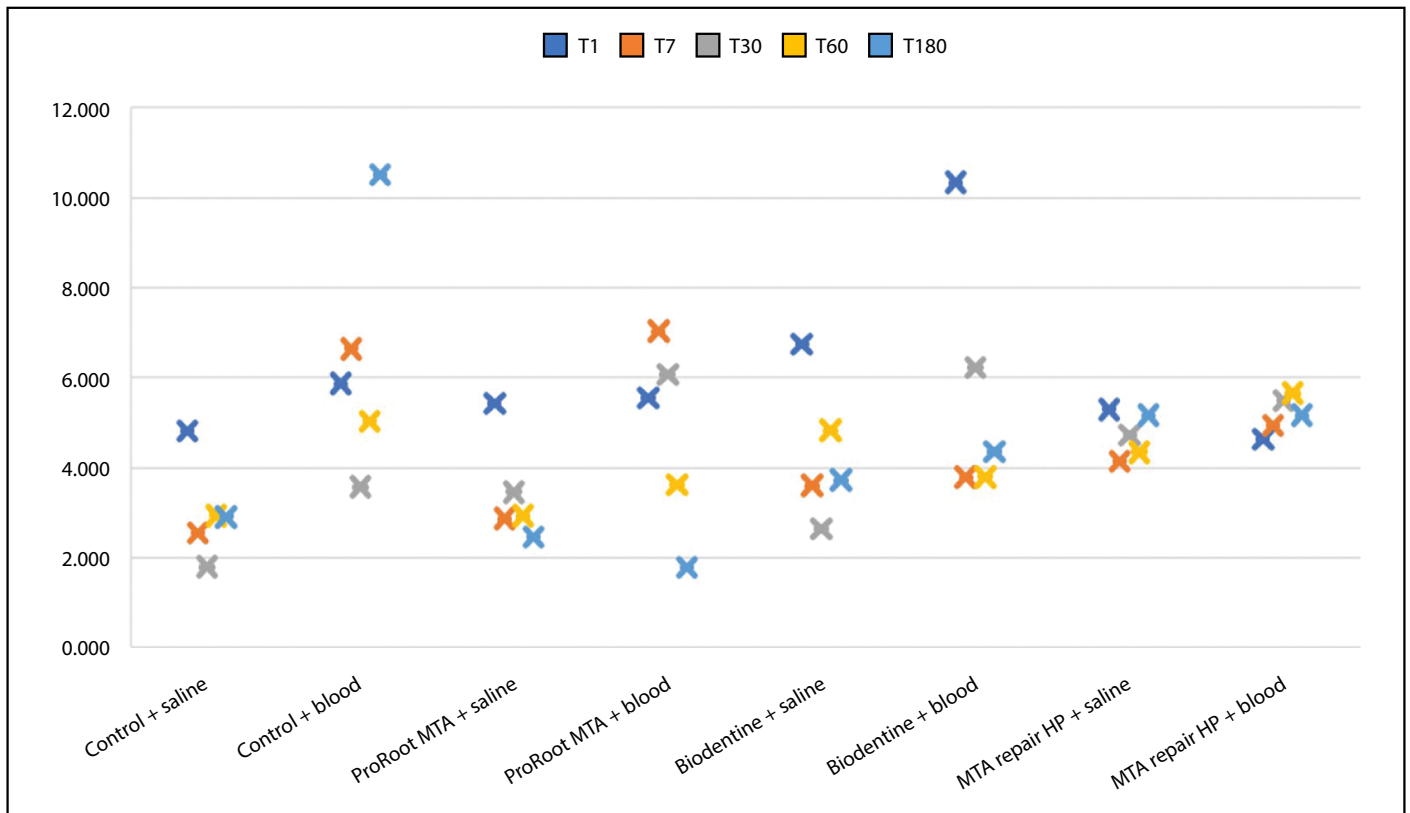
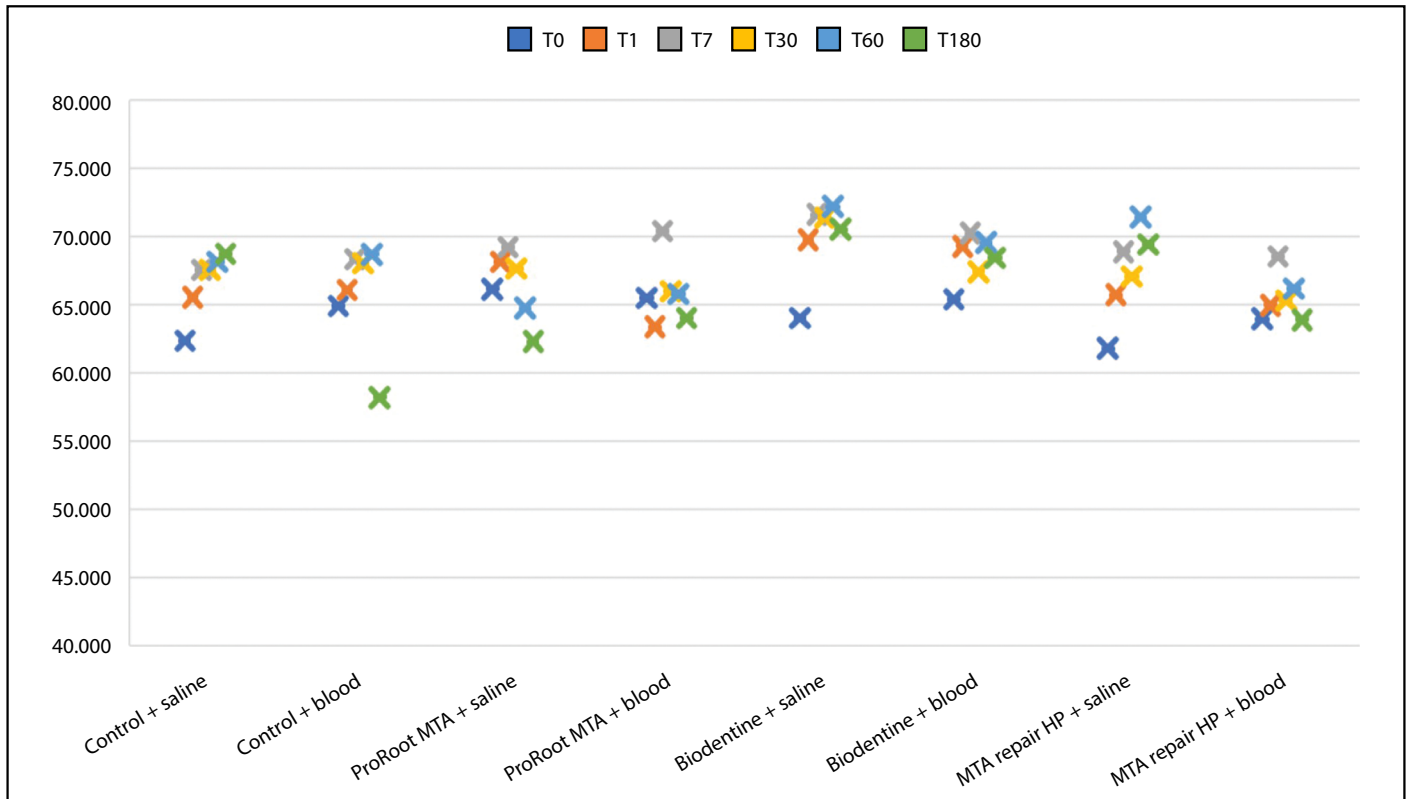**Figure 3.** ∆E values of experimental groups at different measurement points

Figure 4. L* values of experimental groups at different measurement points

In the present study, the teeth were irrigated with 2.5% NaOCl and EDTA to remove the smear layer which was in accordance to the study conducted by Valles et al.14 It has been documented that NaOCl has a very significant impact on the pigmentation caused by calcium silicate–based cements as its residues could penetrate into dentin to a depth of 77–300 µm

(5, 23). NaOCl not only damages collagen but also contributes to opening of the lumen of the tubules. Consequently, the residual hypochlorite and the extended surface of collagen can get into contact with the calcium-silicate cements and, in the presence of bismuth oxide, increase the amount of greater discolouration (18). The present study also used blood contamination of the calcium silicate cements as a factor for discolouration. Various studies have shown that the blood in the pulp can deepen the colour change of the MTA and therefore MTA should be applied only after perfect hemostasis (2). Blood significantly increases the value of ΔE; the amount of change depends on the type of material used and the passage of time (5). Various studies have used whole blood to stain teeth, with centrifugation commonly used to encourage erythrocytic extravasation into the dentinal tubules to promote and accelerate tooth discolouration (14, 19) Centrifugation was not used in this study, hence the pattern of tooth discolouration in the control group with blood contamination may reflect in vivo intra-pulpal hemorrhage better and thus its aesthetic consequences (1). The mechanisms by which WMTA impacts on coronal tooth discolouration and those by which blood exacerbates this discolouration are currently unknown. One possible mechanism may relate to the oxidation and incorporation of the remaining iron content within the WMTA powder into the calcium alumino-ferrite phase of the set WMTA cement (18, 24). Another mechanism may be the interaction between erythrocytes and the unset WMTA. The slow hydrating process of WMTA may permit the absorption and subsequent hemolysis of erythrocytes from the adjacent pulpal tissue, thus resulting in both material and subsequent tooth discolouration. Further research is required to elaborate more on the different mechanisms responsible for this finding (7).

In this study, the colour change was evaluated using CIE L*a*b values. A spectrophotometer was chosen over a colorimeter as the latter measures colour of flat surfaces wherein tooth surfaces are usually not flat (7). In terms of visual perception, the descriptive analysis of L* value indicates a darkening effect (6). Some degree of tooth colour change wherein L* values shifted towards 100 (white) was found in all groups. The factors responsible for the lightening of the teeth are uncertain. Possibly, the sodium hypochlorite canal irrigation may have induced a mild bleaching effect, which has affected all teeth in the study and thus been a constant for all the experimental groups. This rebound effect was also found in studies by Felman et al. (7) and Shokounijad et al. (18). In this study a significant decrease was measured in all experimental groups with increasing time except the control group 1A. The maximum decrease of the L* values was observed in the control group 1B at T180. This delayed decrease of luminosity may be due to the time taken for the breakdown of the hemoglobin in the red blood cells from uncentrifuged blood contrasting prior studies which used centrifuged RBC concentrate to encourage erythrocytic extravasation (1, 15, 18). Similarly, Marin et al. (9) reported that dentine stained by blood initially stained red, whereas later it showed a dark-brown hue. The authors hypothesized that this change may have resulted from the formaldehyde fixation process used in their experimental protocol. However, the present experiment used no fixation, and it is possible

that this shade change reflects the interaction between light and the physiologic degradation of the internalized erythrocytes via hemolysis (4, 25). The alterations observed for L* parameter showed decreased value in groups with blood contamination (Group 1B, 3B and 4B) as compared to groups without blood contamination (Group 1A, 3A and 4A) at T180. However, there was no statistical significance between the L* values of Biodentine group (3A and 3B) at T180. At T180 ProRoot MTA showed more decrease in the non-contaminated group 2A as compared to the blood contaminated group 2B, although the difference was not statistically significant. Namazikhah et al. (26) demonstrated that the microstructure of the materials shows pH-dependent porosities. These porosities may uptake blood components and may be responsible for the observed discolouration. The MTA repair HP showed a statistical difference between its blood contaminated group 4B and 4A at T60 and at T180. Guimarães et al. (27) reported that presence of the plasticizer in MTA HP might increase its solubility and porosity. This could be related to our results that MTA repair HP could absorb liquids such as blood thus causing more discolouration.

All groups showed perceptible colour change >3 at T1 which could be due to the bleaching action of sodium hypochlorite. In the ProRoot MTA group, perceptible colour change was recorded at T60, which shows that WMTA does not cause short term discolouration, but the discolouration gradually increases over time. This finding was in accordance with studies conducted by Akbari et al. (28) and Ionnidis et al. (6), who found that WMTA discolouration exceeded the perceptible threshold at 6 months and 3 months, respectively. However, the ProRoot MTA subgroup B with blood contamination showed perceptible colour change immediately within T1-T7 as established by Felman et al. (7) in his study. This may be explained by the slow hydrating process of WMTA which may permit the absorption and subsequent hemolysis of erythrocytes from the adjacent pulpal tissue, thus resulting in both material and subsequent tooth discolouration. In the Group 3A, perceptible colour change was recorded only at T60 which was in accordance with the study by Beatty et al16 which showed discolouration of Biodentine over 8 weeks. In Group 3B, the difference of ΔE exceeded the perceptible change only at T30 which agreed with the study conducted by Shokouhinejad et al. (18), which is the only other study that compares discolouration of Biodentine in presence of blood. Lastly, in the MTA repair HP group, both subgroups showed discolouration above the perceptible level of >2.6 at T7 which gradually increased over a period which could be attributable to its organic plasticizer. Longer evaluation periods, new material combinations, different experimental conditions and in vivo studies are warranted to investigate different aetiological factors for tooth discolouration and ways to prevent it when applied for direct pulp capping and pulpotomy procedures.

CONCLUSION
Within the limitations of the present study, it can be concluded that:

- Relative to L* parameter, it was possible to observe statistically significant decrease in luminosity in the Blood group

followed by ProRoot MTA and MTA repair HP. Biodentine showed lesser tooth discolouration in terms of value.

- No significant difference was identified in ΔE change between any of the groups from baseline to 180 days.

- There was no significant difference between tooth discolouration with materials in the presence of blood.

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