Coronal Discoloration Related to Bioceramic and Mineral Trioxide Aggregate Coronal Barrier in Non-vital Mature Teeth Undergoing Regenerative Endodontic Procedures

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ABSTRACT

Objectives: This study aimed to compare the coronal discoloration related to the application of two coronal sealing materials: Bioceramic (BC) root repair material (RRM) putty and mineral trioxide aggregate (MTA).

Materials and methods: A total of 16 patients (divided into two subgroups, each one included 8 patients) had undergone regenerative endodontic procedures (REPs). Disinfecting the canals was achieved by the application of the double-antibiotic paste (DAP) "metronidazole and ciprofloxacin" to exclude minocycline discoloration effect; after 3 weeks, the medicament was removed, apical bleeding was induced till the formation of blood clot in the root canal, and then a coronal sealing was created. Bioceramic RRM putty was used in eight patients, while gold standard "MTA" was used in another eight patients. Finally, resin modified glass ionomer (RMGI) was applied followed with resin composite matching the shade of the treated teeth. Shades were measured immediately after procedures and through organized follow-up (3, 6, 9, and 12 months). Data were collected according to Commission International de l'Eclairage (CIE-LAB) and then ΔE values were calculated. A two-way statistical analysis of variance was performed depending on Student's *t* and Chi-square tests at 95% ($p = 0.05$).

Results: Coronal discoloration accompanied with MTA was clinically perceptible ($\Delta E > 3.3$) through all periods. Coronal discoloration was slightly recognized in the BC group. However, there was no significant difference between the used two materials through studied periods ($p > 0.05$). There was incrementally increasing of discoloration through sequential organized follow-up periods.

Conclusion: As a result of this study, BC RRM putty could be a good alternative material to MTA through REPs.

Clinical significance: Pulp REPs are predictable therapies, but discoloration is still considered as a one of the most unfavorable complications; so, this study highlights on this outcome to obviate it.

Keywords: Bioceramic, Discoloration, Mineral trioxide aggregate, Regenerative endodontic procedures.

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INTRODUCTION

Root canal therapy is a traditional method used to remove pulp tissue and replace it by inorganic materials. Therefore, there is a need to regain vitality of the dental pulp to save teeth as long as possible, where tissue engineering could lead to reproduce as same as original pulp tissue with similar morphology and function.¹ Pulp regenerative endodontic procedures (REPs) are based on three basic elements, namely stem cells, scaffolds, and growth factors, and require two conditions, such as effective disinfection and apical foramen with proper size.² Regenerative endodontic procedures prolong on two visits, where the canal would be filled with induced blood clot after efficient disinfection with a medicament of calcium hydroxide or triple antibiotic paste (TAP) after mechanical preparation accompanied by sodium hypochlorite.³ Non-vital mature teeth with/without apical periodontitis are traditionally treated with nonsurgical root canal therapy which has predictable outcomes. Recently, successful treatments of mature necrotic teeth with/without apical periodontitis were obtained with REPs, and the results were considered positive if therapies led to elimination of clinical signs/symptoms and healing of apical periodontitis.^{4,5} Despite a high success rate of REPs with regard to resolving signs and symptoms of disease and periodontal healing, discoloration is still considered one of the most important unfavorable complications.^{6,7} There is certainly a relationship between discoloration and minocycline used in TAP.^{8,9} Therefore, the use of double-antibiotic paste (DAP) "without minocycline" was

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suggested as an alternative medicament because DAP discoloration is not perceptible by the human eye.¹⁰ Mineral trioxide aggregate is a calcium silicate-based reparative material used in REPs as a coronal barrier, it is characterized by biocompatibility, marginal sealing, and biological properties, but one of the most unfavorable

properties is discoloration involvement.¹¹ Discoloration is probably attributed to the coronal sealing material; calcium silicate is the standard material used in this purpose, and mineral trioxide aggregate (MTA) is the most type of calcium silicate which is used in REPs as a gold standard material.¹² Other types of calcium silicate like Biodentine, bioceramic (BC) root repair material (RRM) putty, and calcium-enriched mixture cement are also used in REPs.¹³ Although some literature reviews showed limited staining potential of some calcium silicate materials on dental tissue, other reviews showed high perceptible discoloration related to calcium silicate materials. However, the incidence of discoloration was due to oxidation of the metal elements like iron, bismuth, or manganese which are ingredients included in the calcium silicate materials.¹⁴ Ethylenediaminetetraacetic acid (EDTA) has limited evidence on discoloration on dental tissues and is recommended as a final irrigation solution in REPs according to American Association of Endodontics (AAE).¹⁵ The blood clot is the most popular matrix used in REPs;¹² when accumulated hemoglobin of blood undergo metabolism, hematin molecules precipitate in dentin tubules and lead to staining,¹⁶ as well as possibility of calcium silicate material discoloration when they were in contact with blood.¹⁷ There are many tricks to prevent or at least diminish discoloration in REPs, like place the coronal barrier “coronal sealing material” more apically, and this region of tooth structure will be covered by bone or gingiva. A double layer of dentin bonding agent (DBA) is recommended in the access cavity, to diminish of the penetration of tinctorial components into dentinal tubules to avoid discoloration.^{8,18} Nevertheless, the application of DBA does not prevent the clinically perceptible coronal color change completely, but it reduces the tooth discoloration significantly.¹⁹ Therefore, the aim of this clinical study was to compare incidence of discoloration after REPs with two different materials for coronal sealing: the first material is MTA, and the second one is BC RRM putty to perceive potential role of coronal sealing material in posttreatment discoloration.

MATERIALS AND METHODS

This study was conducted in the dental clinics of faculty of dentistry in Damascus University for 14 months in total (from August 2018 to October 2019). It involved 16 patients who have a non-vital tooth (by traumatic/carious reasons) with single root canal with/without periodontitis disease. Regardless of the location (superior/inferior), the regenerative treatment decision was taken, and the patients were informed by full details, and consents were taken regard special clear ethical approval.

All patients had undergone similar procedures under rubber dam isolation starting from straight access cavity. Then, instrumentation with ProTaper® Universal till F3 (Dentsply Maillefer, Switzerland), apical manual enlargement to size of 0.60,⁵ synchronizing with disinfection of canals by sodium hypochlorite 1.5% (NaOCl; 20 mL/canal, 5 minutes), followed by saline and then 17% EDTA (20 mL/canal, 5 minutes) according to AAE,¹⁵ and final irrigation by saline and EDTA were used to remove a smear layer from dentinal walls. Then, the canals were dried with sterilized paper points. Double-antibiotic paste (metronidazole and ciprofloxacin 0.25 mg of each drug) mixed with distilled water (0.50 mL) to a concentration of 1 mg/mL as a temporary medicament was inserted into the canal and ensured to be below cementum–enamel junction (CEJ)²⁰ and then access sealing was done by glass ionomer (Fuji IX GP; GC, Japan). In the second visit (after 3 weeks), every patient was checked up with exclusion of any signs/symptoms and then the medicament was

flushed out by copious irrigation by saline and then 20 mL of 17% EDTA accompanied by ultrasonic activation to ensure the removal of medicament as much as possible. Then, bleeding was evoked by introducing precurved #15 K file 3 mm beyond the apex with motion of clockwise/contraclockwise; after the bleeding fulfillment till the orifice of canals and waiting about 10–15 minutes, the blood clot was formed, and its stability was checked at the CEJ level or lower than that. All patients had undergone the same previous steps till the coronal sealing step where they were divided into an experimental group and a control group ($n = 8$ teeth per group). The control group had white MTA (ProRoot; Dentsply Tulsa, USA) as the coronal barrier material followed by resin-modified glass ionomer (Ionoseal; VOCO, Germany) and resin composite filling (Filtek Z250 XT; 3M ESPE, USA) (Figs 1 and 2), whereas the experimental group had BC RRM putty (TotalFill; FKG, Switzerland) as the coronal barrier followed by the same aforementioned materials (Figs 3 and 4). VITA Easyshade® Advance 4.0 (VITA, Germany) was calibrated before using each time and then it is used to measure the shade in the central region of the buccal surface of each treated tooth twice at each time immediately after the procedures and through organized follow-up visits (3, 6, 9, and 12 months), and the mean of the two measurements was calculated. Data were collected according to CIE-LAB (1976), which is a uniform color scale designed to perceive accurate colors, where L , a , and b are color values; (L^*) indicates to value of lightness or darkness on a scale from black (0) to white (100); (a^*) indicates to progression from red (+80 a^*) to green (80 a^*); and (b^*) indicates to progression from yellow (+80 b^*) to blue (80 b^*). The color difference is calculated by the following equation:

Figs 1A to D: Mineral trioxide aggregate as a coronal barrier on the upper right central incisor: (A) After application; (B) After 3 months; (C) After 6 months with good healing; (D) After 9 months with superior healing

Figs 2A to B: Discoloration after application of mineral trioxide aggregate as a coronal barrier on the lower right lateral incisor: (A) After application; (B) After 6 months with noticeable discoloration

Figs 3A to D: Bioceramic as a coronal barrier on the upper left lateral incisor: (A) After application directly; (B) After 3 months with no difference; (C) After 6 months with preliminary healing indication; (D) After 9 months with superior healing

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta A)^2 + (\Delta B)^2}$$

Or

$$\Delta E = \sqrt{(L_i - L_0)^2 + (a_i - a_0)^2 + (b_i - b_0)^2}$$

Then, ΔE values were calculated and compared between intervals in the same groups and between two groups from each other.

ΔE discoloration less than 3.3 is considered not perceptible (no discoloration effect), and ΔE discoloration equal/over 3.3 is considered perceptible (discoloration effect).^{21,22}

Statistical Analysis

SPSS software was used to evaluate data (PASW Statistics 13; SPSS, Inc., Chicago, IL, USA), to evaluate the role of the coronal barrier type on discoloration among four intervals with the same scaffold type regardless of the gender, age, and tooth location. The data were analyzed using Student's *t* test and Chi-square test. The level of statistically significant difference was set at the confidence level of 95% and *p* value = 0.05. *L**, *a**, and *b** color factor's values were measured in five different periods (after treatment directly, after 3 months, after 6 months, after 9 months, and after 1 year) for each treated tooth in the sample. The human perceptibility threshold was set to 3.3 units to determine which differences were clinically visible. ΔE values were calculated, and clinical validity of ΔE was determined (unacceptable discoloration and acceptable discoloration). Then, the effects of the coronal barrier material and studied period on ΔE values and clinical validity of ΔE frequencies were studied, and the analysis results are shown in the Results section.

ΔE Values Study

Effect of coronal barrier material on ΔE values according to the studied period:

An independent sample *t* test was applied to know if there were significant differences in ΔE values between MTA group and BC group according to the studied period as shown in Table 1. All *p* values were greater than 0.05. So, at the confidence level of 95%, there were no significant differences in ΔE mean values between MTA group and BC group whatever the studied period was (after 3 months, after 6 months, after 9 months, and after 1 year) in the studied sample.

Effect of studied period on ΔE values according to the restorative material:

A paired sample *t* test was applied to know if there were pairwise significant differences in ΔE values between the four studied periods (after 3 months, after 6 months, after 9 months, and after 1 year) according to the coronal barrier material as shown in Figure 5.

Paired sample *t* test results (Table 2):

Figs 4A and B: Discoloration after application of bioceramic as a coronal barrier on the upper right central incisor: (A) After application directly; (B) After 6 months with preliminary discoloration

Table 1: Independent sample *t* test results

Studied period	Studied variable = ΔE			Significant difference
	Mean difference	<i>t</i>	<i>p</i> value	
After 3 months	1.27	1.172	0.261	No
After 6 months	1.54	1.392	0.185	No
After 9 months	1.62	1.526	0.149	No
After 1 year	1.87	1.727	0.106	No

The values of *p* were greater than 0.05 when comparing ΔE values between after 6 months and after 9 months periods in the MTA group. So, at the confidence level of 95%, there were no significant differences in ΔE values between after 6 months and after 9 months periods in the MTA group in the studied sample. All other *p* values were lower than 0.05. So, at the confidence level of 95%, there were pair-wise significant differences in the corresponding mean values between the corresponding studied periods. Positive algebraic sign of mean differences indicates that statistically:

ΔE values after 6 months, after 9 months, and after 1 year were greater than those of after 3 months period whatever the studied coronal barrier material was.

ΔE values after 9 months and after 1 year values were greater than those of after 6 months whatever the studied restorative material was.

ΔE values after 1 year were greater than those of after 9 months whatever the studied restorative material was.

ΔE values for both materials increased statistically while studied period increased in the studied samples.

Clinical Validity of ΔE Study

Clinical validity of ΔE determination results according to coronal barrier material and studied period are shown in (Table 3 and Fig. 6).

Effect of coronal barrier material on clinical validity of ΔE according to the studied period:

An independent Chi-square test was applied to know if there were significant differences in clinical validity of ΔE frequencies between MTA group and BC group according to the studied period as shown in Table 4. All *p* values were greater than 0.05. So, at the confidence level of 95%, there were no significant differences in clinical validity of ΔE frequencies between MTA group and BC group

whatever the studied period was (after 3 months, after 6 months, after 9 months, and after 1 year) in the studied sample.

Effect of studied period on clinical validity of ΔE according to the coronal barrier material:

A Cochran's *Q* test was applied to know if there were significant differences in clinical validity of ΔE frequencies between the four studied periods (after 3 months, after 6 months, after 9 months, and after 1 year) according to the coronal barrier material as shown in Figure 7.

Cochran's *Q* Test Results

Both *p* values were greater than 0.05 (Table 5). So, at the confidence level of 95%, there were no significant differences in clinical validity of ΔE frequencies between the four studied periods (after 3 months, after 6 months, after 9 months, and after 1 year) whatever the studied restorative material was (MTA group and BC group) in the studied sample.

RESULTS

The average ΔE values according to the coronal barrier materials and studied period in each subgroup are shown in Table 6 and Figure 8.

The outcomes showed that discoloration after MTA application was above clinically perceptible threshold ($\Delta E > 3.3$) in all follow-up visits, whereas the coronal discoloration after 3.6 months of BC use was below clinically perceptible threshold ($\Delta E < 3.3$) and then the discoloration increased slightly above clinically perceptible threshold ($\Delta E > 3.3$) after 9 and 12 months. The results revealed that considering the type of the coronal barrier material on crown discoloration was significantly higher in the MTA group compared with the BC group. However, no significant difference existed between MTA and BC groups ($p > 0.05$) at the confidence level of 95%. Regarding the effect of intracanal scaffold "blood clot," the results showed that the type of coronal barrier (MTA or BC) had no significant effect on coronal discoloration ($p > 0.05$) whatever the studied period was. The reason beyond this result was although there was superiority of ΔE after MTA application rather than ΔE after BC application in all studied periods (Fig. 8), there was fairly a large standard deviation in both groups, especially in the MTA group, which relates to dispersion of values and huge disparity between minimum and maximum values in each group; for example, there is a large range between 1.71 and 9.56 in the MTA group and 1.28 and 5.69 in the BC group which led to the dispersion

Fig. 5: Mean values of ΔE according to the studied period and coronal barrier material

Table 2: Paired sample *t* test

Studied variable = ΔE					
Coronal barrier material	Compared periods	Mean difference	<i>t</i>	<i>p</i> value	Significant difference
MTA group	After 6 months and after 3 months	0.48	3.202	0.015	Yes
	After 9 months and after 3 months	0.85	3.063	0.018	Yes
	After 1 year and after 3 months	1.38	4.982	0.002	Yes
	After 9 months and after 6 months	0.37	2.153	0.068	No
	After 1 year and after 6 months	0.91	5.383	0.001	Yes
	After 1 year and after 9 months	0.54	4.832	0.002	Yes
BC group	After 6 months and after 3 months	0.21	3.456	0.011	Yes
	After 9 months and after 3 months	0.49	8.451	0.000	Yes
	After 1 year and after 3 months	0.79	9.120	0.000	Yes
	After 9 months and after 6 months	0.28	4.696	0.002	Yes
	After 1 year and after 6 months	0.58	8.354	0.000	Yes
	After 1 year and after 9 months	0.30	6.586	0.000	Yes

Table 3: Clinical validity of ΔE frequencies

Studied period	Coronal barrier material	<i>n</i>			Percentage		
		Unacceptable discoloration	Acceptable discoloration	Total	Unacceptable discoloration	Acceptable discoloration	Total
After 3 months	MTA group	5	3	8	62.5	37.5	100
	BC group	3	5	8	37.5	62.5	100
After 6 months	MTA group	5	3	8	62.5	37.5	100
	BC group	3	5	8	37.5	62.5	100
After 9 months	MTA group	6	2	8	75.0	25.0	100
	BC group	3	5	8	37.5	62.5	100
After 1 year	MTA group	6	2	8	75.0	25.0	100
	BC group	3	5	8	37.5	62.5	100

of values. Although there was no significant difference between the studied materials, the type of the coronal barrier should be taken into consideration and its composition especially when in contact with blood which itself is considered as a discoloration causative factor, because the mean values of crown discoloration were higher in the MTA group compared with the BC group in all

follow-up periods. In other words, the use of MTA as an internal coronal barrier after blood clot formation led to clear coronal discoloration which was more than the clinically noticeable value, whereas BC application led to slight coronal discoloration with lower level compared with MTA. There is no significant difference existed between 6 months and 9 months in the MTA group

Fig. 6: Percentage of acceptable discoloration

Table 4: Chi-square test

Studied variable = ΔE				
Studied period	n	Chi-square	p value	Significant difference
After 3 months	16	1.000	0.317	No
After 6 months	16	1.000	0.317	No
After 9 months	16	2.286	0.131	No
After 1 year	16	2.286	0.131	No

Fig. 7: Percentage of acceptable discoloration frequencies

Table 5: Cochran's Q test results

Coronal barrier material	n	Cochran's Q	p value	Significant difference
MTA group	8	3.000	0.392	No
BC group	8	0	1.000	No

($p > 0.05$), whereas a significant difference existed between all other intervals in both MTA and BC groups, and therefore, the incremental increase in discoloration through sequential organized follow-up periods is clear.

DISCUSSION

As the significant effect of discoloration is related to sodium hypochlorite with high concentrations in classic root canal therapy,¹² as well as the AAE recommendations to avoid high concentrations

Table 6: ΔE descriptive statistics according to the coronal barrier material and studied period

Studied variable = ΔE							
Studied period	Coronal barrier material	n	Mean	Standard deviation	Standard error	Minimum	Maximum
After 3 months	MTA group	8	4.29	2.72	0.96	1.71	9.56
	BC group	8	3.02	1.43	0.50	1.28	5.69
After 6 months	MTA group	8	4.77	2.84	1.00	2.14	10.56
	BC group	8	3.23	1.31	0.46	1.58	5.74
After 9 months	MTA group	8	5.13	2.70	0.96	2.15	10.43
	BC group	8	3.51	1.33	0.47	1.69	5.94
After 1 year	MTA group	8	5.67	2.79	0.99	2.48	11.18
	BC group	8	3.81	1.23	0.44	2.21	6.06

Fig. 8: Mean values of ΔE according to the coronal barrier material and studied period

of sodium hypochlorite in REPs to eliminate its toxic effect on stem cells in apical papilla,¹⁵ the NaOCl could be excluded from discoloration factors.

Within the limits of this study, which implicate study circumstances, patients behavior, and human error factor, discoloration is elicited by both coronal barrier materials accompanied by blood clot scaffold; so, the blood scaffold is accused to be the first responsible factor, and this could be explained by a previous explanatory mechanism.^{23,24} After hemolysis of erythrocytes from blood, the hemoglobin and hematin molecules may penetrate and accumulate in dentinal tubules,^{23,24} and while the closeness of the coronal third of the root to the cervical third of the crown, that might reflex the root discoloration onto the crown.²⁵ Because of transition of Fe^{2+} (within red color) to Fe^{3+} (within dark brown color) in the center of heme group of blood through a natural redox reaction, and that could be the reason of discoloration of nearby root dentin,²⁶ in addition to blood absorption into the fresh unsetting MTA which was located in the cervical region, by mechanism of iron ions releasing and interactions of calcium allumino ferrate embedded in porous calcium silicate materials which resorb pigmentary elements of blood,⁹ and that may be secondary possible reason for discoloration.²⁵

It has been shown that the discoloration is caused by blood contamination exacerbated with the use of calcium silicate materials which contain bismuth oxide (MTA), and the higher discoloration

level could be attributed to color changes of coronal barriers itself which were located in contact with blood.^{9,17,26}

The high bond strength of BC compared with MTA might be the preventive factor to limit leaking of the blood pigmentary elements into the dentin, where the premixed BC putty compared with separate powder and liquid for MTA and the thickening filler agents "zirconium oxide" added to BC to improve physical properties that make it as a putty form might also resulted in higher bond strength,²⁷ and as this material has been developed with adhesion formation of tag-like structures inside the dentinal tubules as micromechanical bonding to dentin,²⁸ all that could be the other possible reason to make discoloration with BC materials lower than that with MTA.

Either gray MTA or white MTA was attributed to perceptible crown discoloration,^{29,30} due to higher content of bismuth which is a radiopacifier agent.¹⁴ Nevertheless, the staining of teeth treated with white MTA was obvious in *in vitro* studies.³¹

Bismuth is linked to calcium silicate hydrate, then leaked, and decomposed from material, for this reason the discoloration would increase gradually with time,³² add to that the bismuth carbon complex; which resulting from interaction of bismuth oxide released from calcium silicate with carbon dioxide which suspected to be existed in blood clot during its setting.³³

So, MTA itself is a doubtful factor that leads to teeth discoloration, and the results of this study affirmed that indeed,

where most of the samples that underwent MTA exhibited higher color change compared with BC samples. The bismuth oxide molecule in MTA may be destabilized by amino acids in dentin collagen and that eventually lead to color change to black.³⁴ Also, bismuth precipitates as dark sediment when it interacts with collagen especially after irrigation with EDTA which exposes collagen matrix of dentinal walls.³⁵ Moreover, in this study, the pulp chamber walls were never sealed with DBA, which is a preventive step that could save tooth structure from bismuth migration and deny any more prospective discoloration.²⁵

In the limitations of this study, BC was less involved in discoloration compared with MTA, and this result resembles the result of a previous study.³¹ Nevertheless, there was no significant differences between BC and MTA when in contact with blood, and this results agree with Shokouhinejad et al.'s study.¹⁷ Shokouhinejad et al. found that the type of coronal barrier materials (MTA or Biodentine) had no significant effect on coronal discoloration, whereas the type of the scaffold was considered as the responsible influential factor for discoloration,²⁵ and the results agree to some extent with the present result.

An incremental increase in discoloration between sequential organized follow-up periods was observed in both groups, and this result is in accordance with a previous study;¹⁰ however, the present result contradicts with Shokouhinejad et al.'s study which is based on DBA sealing of the pulp chamber and led to nonsignificant increase in discoloration till 6 months.²⁵ The proposed limit for color difference was admitted at 3.3 in this study, whereas it was up to 3.7 in many other studies,^{10,30} and this difference may contribute to make lower discoloration values as a considerably perceptible value.

Generally, discoloration is a patient-oriented outcome, and there are many reasons implicated beyond it; starting from sodium hypochlorite, provisional medicament, scaffolds, and coronal barrier materials, add to that external factors such as patient's oral habits and level of oral care with regard to the nature of received nutrients. So it is not fair to impute this consequence for a single factor.

CONCLUSION

Under the conditions of this study, there is no significant difference between the discoloration following the application of MTA and BC RRM putty through follow-up intervals. Mineral trioxide aggregate could be used with caution, whereas BC RRM putty could be used as a preferable alternative material for sealing the coronal part of canal through REPs with the lowest discoloration possibility. Further (*in vitro*–*ex vitro*) studies are required to determine the most implicated reasons beyond discoloration in REPs to inhibit this unfavorable outcome.

CLINICAL SIGNIFICANCE

Pulp REPs are predictable therapies, but discoloration is still considered one of the most unfavorable complications. So, this study highlights on this outcome to obviate it and utilize new suggested materials.

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