Original Article

Evaluation of peroxide release during nonvital bleaching using three different coronal barriers: An *in vitro* study

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Abstract

Background: Peroxide from bleaching agents can cause external cervical resorption. An intracoronal barrier is used to prevent leakage of bleaching agents into the periradicular space.

Aim: This study aims to determine and compare the amount of peroxide released, during non vital bleaching at the end of 1st and 3rd day using Glass ionomer cement (GIC), Mineral Trioxide aggregate (ProRoot MTA) and Biodentine as intracoronal barriers.

Materials and Methods: Forty-five single-rooted teeth were selected for the study and root canal therapy was performed. Three millimeters of the coronal gutta-percha were removed and according to the coronal barrier placed, samples were divided into Group A: GIC, Group B: ProRoot MTA, and Group C: Biodentine. Nonvital bleaching was done using sodium perborate and 30% H_2O_2. Peroxide released at the end of the 1st and 3rd day was analyzed using potassium iodide and ultraviolet spectrophotometer.

Statistical Analysis: This was done using the Wilcoxon matched pair test and the Kruskal–Wallis test.

Results: No significant difference in intergroup comparison at the end of 1st and 3rd day, respectively ($P > 0.05$), a significant difference was found in the MTA group at follow-up dates ($P < 0.05$).

Conclusion: All the three tested materials (GIC, MTA, and Biodentine) may be preferred as intracoronal barrier for nonvital bleaching.

Keywords: Biodentine; bleaching agents; nonvital bleaching; ProRoot mineral trioxide aggregate; tricalcium silicate

INTRODUCTION

Discoloration is one of the consequences occurring as a result of necrosis of the pulp. Intracoronal or nonvital bleaching is a conservative procedure done for discolored orthodontically treated teeth.[1,2] Various bleaching agents such as 30% hydrogen peroxide (H_2O_2), sodium perborate with 30% H_2O_2, and sodium perborate with distilled water are used.[3] One of the most common disadvantages of nonvital bleaching is the risk of external cervical root resorption. This is probably due to the diffusion of the highly concentrated oxidizing agents to the pericemental area, thereby causing cementum degradation, inflammation, and osteoclast accumulation which is because of the acidic pH of the bleaching agents.[4-6] As a result, it is recommended to use an intracoronal cervical barrier, to

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prevent the leakage of highly concentrated peroxide to the periodontal ligament area. Several materials have been used as intracoronal barriers such as glass ionomer cement (GIC), intermediate restorative material, and composite resin restorations.[7] One of the disadvantages of using temporary restorative material is the need to remove it before placing a final restoration. GICs have been used traditionally, at a thickness of 2 mm which acts as a base for the final restoration.[8] Mineral trioxide aggregate (MTA) is a calcium silicate cement that was originally used as a root-end filling material.[9] MTA has numerous other clinical applications such as pulp capping, pulpotomy, treatment of internal root resorption, apexogenesis, apexification, and perforation repair.[10-12]

Numerous studies have demonstrated better sealing ability of MTA with regard to GIC.[13,14] "Biodentine" (Septodont) is a calcium silicate-based cement that came into the market in 2009 and had been specifically designed to be used as a "dentin replacement" material. However, studies have reported that it can also be used in root perforations, apexification, resorptive lesions, pulp capping, and as a retrograde filling material in endodontic surgery.[15,16]

This study has been done to evaluate the amount of leakage of peroxide after nonvital bleaching using GIC, MTA, and Biodentine as intracoronal barriers. Thus, the null hypothesis states that there is no significant difference between the amount of peroxide released from the experimental samples when using GIC, MTA, and Biodentine as intracoronal barriers during nonvital bleaching using sodium perborate and 30% H_2O_2.

MATERIALS AND METHODS

The study followed the modified CONSORT guidelines for *in vitro* studies. Forty-five single-rooted teeth without caries, restorations, fractures, or anomalies were included in the study. All the soft and hard tissue deposits on the teeth were thoroughly cleaned, rinsed, and stored in saline. Teeth with root caries and anatomical deformities, cracks, root fractures, cervical abrasion, immature teeth, multiple or curved canals, or teeth with previous endodontic treatment were excluded from the study. The sample size was calculated on the estimated population mean and standard deviation based on the previous studies[3] and anticipated mean-based pilot study. The estimated population mean was taken as 0.263 and standard deviation as 0.1312 and anticipated mean as 0.16. The sample size at 0.05 alpha level and power of 0.80 was found to be a minimum of 13. Hence, the sample size was taken as 15 per group.

Access cavity was prepared on all teeth using No. 6 round bur and long flat end tapered fissure bur. Working length was established using a No. 10 k file (Dentsply M access, Maillefer Instruments Holding Sarl, Switzerland), 1 mm short of the actual tooth length. Root canals were cleaned and shaped using NeoEndo Flex rotary file system (Orikam Healthcare India Pvt. Ltd, Gurugram, Haryana, India) up to the size of 35 with a taper of 0.06. 2.5% sodium hypochlorite and 17% ethylenediaminetetraacetic acid were used intermittently for 2 min to irrigate the canals. The final rinse was done using normal saline. The canals were dried using absorbent paper points and coated with AH Plus sealer (Dentsply, De Trey GmBH, Konstanz, Germany). Obturation was done with gutta-percha (Dentsply, Maillefer, Dentsply, India Pvt. Ltd, India) using the single cone obturation technique. The access cavities of all the samples were restored with temporary restorative material (Cavit G, 3M Deutschland GmbH, 3M ESPE, Germany). The teeth were incubated at 37° C for 7 days. After 7 days, the access cavities were revisited and 3 mm of gutta-percha filling was removed from the canal using heated hand pluggers with labial cementoenamel junction (CEJ) as the reference point.

Samples were divided into three groups according to the intracoronal barrier material they received, as follows: Group A – GIC (GC gold label Universal Restorative, GC Corporation, Tokyo, Japan) (n = 15); Group B – ProRoot MTA, Dentsply, Tulsa Dental Specialties, Johnson City, TN, USA (n = 15); and Group C – Biodentine (Septodont, Saint–Maur-des-Fosses, France) (n = 15).

The materials were manipulated according to the manufacturer's instructions. For the ProRoot MTA group, a piece of cotton soaked in saline was placed over the material and the cavity was sealed with temporary restorative material for 24 h. All the teeth were radiographed to check the placement of barrier material [Figure 1]. The outer root surfaces including apical foramina were covered with modeling wax and painted with two layers of nail varnish. Modeling wax was used to separate the teeth into crown

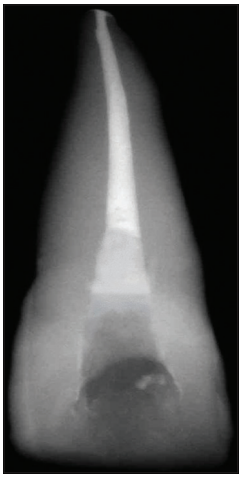

Figure 1: Radiograph of sample tooth after placement of the intracoronal barrier

and root portion. After 24 h, access cavities were reopened and nonvital bleaching procedure was done with a mixture of sodium perborate and 30% H_2O_2 (Rankem, Avantor Performance Materials India Limited, Thane, Maharashtra, India) (2 g: 1 mL). Following this, the cavities were sealed with temporary restorative material. Then, it was suspended into plastic tubes containing 2 mL of distilled water such that the entire root including CEJ was immersed in the distilled water for 3 days at 37° C. The amount of peroxide released from the samples in distilled water was assessed at the end of 1st day and 3rd day using potassium iodide (KI) solution and ultraviolet (UV) spectrophotometer.

Spectrophotometric analysis is a method to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through sample solution. Ten percent aqueous solution of KI was used in this study. It is based on KI oxidation by H_2O_2 in the acidic medium according to the following equations:

$$2\,I^- + 2\,H^+ + H_2O_2 \rightarrow I_2 + 2\,H_2O$$

$$I_2 + I^- \rightarrow I_3^-$$

This triiodide ion gives the characteristic yellowish color. H_2O_2 was determined using spectrophotometry by absorbance of iodide at 390 nm.[17] The standard calibration curve of percentage concentration of peroxide versus percentage absorbance was obtained using serial dilutions of 30% H_2O_2 stock solution and UV spectrophotometer (Spectrascan UV 2600, Double Beam UV-VIS Spectrophotometer, Chemito Instruments Pvt. Ltd., Mumbai, India). The amount of peroxide in the experimental samples was determined by comparing them to the standard calibration curve at the end of 1st day and 3rd day. Two hundred microlitere of KI solution was added to 2 mL of the solution and checked in UV spectrophotometer at a mean wavelength of 390 nm.

Statistical analysis

Statistical analysis was done using the Wilcoxon matched pair test and Kruskal–Wallis test. The *P* value was kept at a significance level of 0.05. All the statistics have been calculated and computed using IBM Statistical Package for the Social Sciences (SPSS) version 20 (IBM SPSS Statistics for Windows. Armonk, NY: IBM Corp).

RESULTS

The Kruskal–Wallis test for intergroup comparison of mean peroxide levels on day 1 revealed no statistically significant difference between the Group A, B, and C ($P > 0.05$), with the highest being in Group B and lowest in Group C. Intergroup comparison of mean peroxide level on day 3 revealed no statistically significant difference between the three groups [$P > 0.05$, Table 1]. The Wilcoxon matched pair test for intragroup comparison of mean peroxide level of day 1 and day 3 revealed no statistically significant difference ($P > 0.05$) in Group A and Group C, whereas statistically significant difference was found in Group B [$P < 0.05$, Table 2].

DISCUSSION

The most common disadvantage of intracoronal or nonvital bleaching is the chance of external cervical root resorption. This is because peroxide from the bleaching agents leaches out of the tooth to the periradicular space and causes inflammation and cementum degradation.[5] In this study, sodium perborate is used with 30% H_2O_2 as the bleaching agent. Studies have reported that a significant amount of

Table 1: Intergroup comparison - day 1 and day 3

Follow-up	Group	*n*	Mean concentration (%)	SD (%)	Minimum (%)	Maximum (%)	Kruskal–Wallis test - *P*
Day 1	Group A	15	0.14	0.19	0.00	0.44	0.385 (NS)
	Group B	15	0.20	0.15	0.04	0.45	
	Group C	15	0.12	0.11	0.00	0.29	
Day 3	Group A	15	0.095	0.10	0.00	0.22	0.957 (NS)
	Group B	15	0.098	0.12	0.00	0.31	
	Group C	15	0.13	0.15	0.00	0.39	

*Group A: GIC, Group B: ProRoot MTA, Group C: Biodentine. NS: Statistically nonsignificant difference, $P<0.05$: Statistically significant difference (significant), SD: Standard deviation, GIC: Glass ionomer cement, MTA: Mineral trioxide aggregate

Table 2: Intragroup comparison at follow-up dates

Group	Concentration follow-up	*n*	Mean concentration (%)	SD (%)	Minimum (%)	Maximum (%)	Wilcoxon matched pair test - *P*
Group A	Day 1	15	0.14	0.19	0.00	0.44	0.273 (NS)
	Day 3	15	0.095	0.10	0.00	0.22	
Group B	Day 1	15	0.201	0.15	0.04	0.45	0.012 (significant)
	Day 3	15	0.098	0.12	0.00	0.31	
Group C	Day 1	15	0.12	0.11	0.00	0.29	0.753 (NS)
	Day 3	15	0.13	0.15	0.00	0.39	

*GroupA: GIC, Group B: ProRoot MTA, Group C: Biodentine. NS: Statistically nonsignificant difference, $P<0.05$: Statistically significant difference (significant), SD: Standard deviation, GIC: Glass ionomer cement, MTA: Mineral trioxide aggregate

peroxide is being released from the above combination.[3] Here, the peroxide analysis was done at the end of 1st day and 3rd day. Zoya *et al*.[18] reported that the highest amount of peroxide is released after 24 h of bleaching and gradually decreases with time which is consistent with our study. In this study, there is no significant difference ($P > 0.05$) found between the experimental groups concerning leakage of peroxide at the end of 1st day and 3rd day. Hence, the null hypothesis is accepted.

Barrieshi-Nusair and Hammad compared GIC and MTA as orifice plugs to check the microleakage of dye into the canal and found that GIC has more microleakage compared to MTA and stated that MTA may be preferred intracoronal barrier following root canal treatment to prevent coronal microleakage.[19] In the present study, the amount of peroxide released after the 1st day was the highest in the MTA group, which may be attributed to the incomplete setting of MTA. According to studies, the complete setting of MTA occurs after 21 days.[20] The difference in the Biodentine group when compared to ProRoot MTA when evaluated at1st day after setting could be due to the lower setting time of Biodentine and the formation of calcium or phosphate-rich crystalline deposits, which minimize the gap between the tooth and the coronal barrier material.[21,22] The relatively high leakage of ProRoot MTA observed during the initial 24 h can also be due to the longer setting time of MTA which is similar to the study done by Nabeel *et al*.[23] Although the peroxide concentration at the end of 1st day is higher in the case of ProRoot MTA group compared to GIC and Biodentine group, the results were not statistically significant, which is consistent with the study done by Tselnik *et al*.[24] They reported in their study that the amount of leakage was comparable between the GIC and white MTA groups when microbial leakage test was done. Similar results have been shown by Vosoughhosseini *et al*. which showed no difference in the protein leakage test comparing GIC and MTA. It is further stated that, because of the higher alkalinity of MTA and release of calcium hydroxide, it can protect the root surface from resorption.[25]

At the end of 3rd day, peroxide release decreased in both Group A and Group B. The increased leakage value in the Biodentine group may be because of the formation of a high pH solution containing Ca^{2+}, OH, and silicate ions, with nucleation of calcium hydroxide particles with precipitation of calcium silicate hydrated gel. Furthermore, the increase in peroxide release is not statistically significant when compared on day 1 and day 3. Kucukkaya Eren *et al*. in their study reported that H_2O_2 may produce bubbling when in contact with the surface of calcium silicate cements; this oxygen bubbling could be the reason for the more porous structure of Biodentine.[22] At the end of the 3rd day, the concentration in the MTA group decreased significantly which signifies that with time the structural integrity of MTA increases and also the pH increases causing hindrance to root resorption. Nevertheless, it was also found that the difference between all the groups was not statistically significant. Hence, the use of all the three types of material as a coronal barrier is justified. The limitations of the study are the small sample size and that clinically relevant variables could not be studied due to the *in vitro* design, and further studies are required to assess the release of peroxide from the bleaching agents in a clinically relevant scenario.

CONCLUSION

The results of the study showed that the initial release of peroxide was more in the MTA group compared to other groups but gradually it decreased significantly. Moreover, it was also found that all the three materials (GIC, ProRoot MTA, Biodentine) were comparable regarding the release of peroxide. Hence, all the three materials are suitable as intracoronal barrier for nonvital bleaching.

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Conflicts of interest
There are no conflicts of interest.

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