



Effect of Different Protective Bases on pH Changes and Hydrogen Peroxide Microleakage During Intracoronary Bleaching

Atiyeh Feiz¹, Hessamoddin Faghihian^{2*}, Marjan Sadat Mahdaviani³

1. Dental Materials Research Center, Dental Research Institute, Department of Operative Dentistry, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

2. Dental Research Center, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

3. Dental Students Research Committee, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

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* Corresponding author:

Dental Materials Research Center,
Dental Research Institute, Department
of Operative Dentistry, School of
Dentistry, Isfahan University of Medical
Sciences, Isfahan, Iran

Email: hesamfgh95@gmail.com

ABSTRACT

Objectives: This study aimed to evaluate the pH changes and penetration of hydrogen peroxide into radicular dentin when different protective bases were used with and without a bonding agent.

Materials and Methods: In this in-vitro experimental study, 70 single-rooted bovine teeth were instrumented and obturated with gutta-percha. The gutta-percha was removed 3mm below the cemento-enamel junction (CEJ) and the teeth were divided into seven groups (n=10). In each group, 2mm base (1mm apical to the CEJ) was applied as follows: TheraCal LC, TheraCal LC plus SE Bond, Lime-Lite, Lime-Lite plus SE Bond, Ionoseal, Ionoseal plus SE Bond, and resin-modified glass ionomer (RMGI). The teeth were placed in vials containing distilled water, and pH values and molar concentration of the medium surrounding the teeth were recorded immediately after internal bleaching with 35% hydrogen peroxide. The pH values were also recorded at 1, 7, and 14 days following renewal of the medium. Data were analyzed with t-test, one-way ANOVA, and Kruskal-Wallis test.

Results: After bleaching, the medium pH became acidic in all groups. There were no significant differences among groups in the mean pH of the medium after bleaching (P=0.189). Moreover, there were no significant differences among the study groups with respect to hydrogen peroxide concentration (P=0.895).

Conclusion: Intra-orifice barriers such as light-cure resin-modified calcium hydroxide, light-cure resin-reinforced glass ionomer, and light-cure calcium silicate can be as effective as RMGI in providing coronal seal during intracoronary bleaching.

Keywords: Tooth Bleaching; Root Canal Therapy; Esthetics, Dental; Dental Materials

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INTRODUCTION

Discoloration of permanent anterior teeth subsequent to endodontic treatment causes serious esthetic problems. Root canal treatment is the main cause of intrinsic coronal discolorations due to the presence of residual pulp tissue and sealer. Compared with laminates and veneers, internal bleaching as a non-invasive esthetic technique is used to

reverse the discoloration of endodontically treated teeth to create an attractive smile. However, external root resorption at the cervical region is one of the disadvantages of internal bleaching [1,2], which occurs in 0-6.9% of the cases [1,3].

Hydrogen peroxide which is commonly used for internal bleaching leads to external cervical resorption (ECR) by degrading the

enamel and cementum structures. Preliminary studies have shown that ECR caused by internal bleaching is more common in teeth with a history of traumatic dental injury or in thermo-catalytic bleaching technique, which utilizes heat in addition to hydrogen peroxide [2,4]. However, more recent studies have asserted that ECR occurs even if thermocycling is not performed [1].

Although the exact mechanism of ECR has not yet been discovered, it has been reported that dentin permeability to hydrogen peroxide [5,6] affects the surrounding tissues, which may lead to an inflammatory process [2,7]. According to Lima et al. [2] after bleaching with hydrogen peroxide, the exposed dentin at the CEJ can be denatured and act as a foreign body. Rotstein et al. [8] also reported that bleaching agents reduce the organic content of both dentin and cementum; thus, making them more susceptible to resorption. Additionally, hydrogen peroxide releases hydroxyl radicals, which are highly toxic to the vital tissues and contribute to ECR by decreasing pH [9,10].

It has been reported that bleaching agents decrease the pH around the periodontal ligament in the cervical region, thereby, causing root resorption [10]. However, no protective barrier has been placed between the root filling materials and pulp chamber [1-3]. Since dentinal tubules extend to the incisal area, placement of a protective barrier below cemento-enamel junction (CEJ) reduces the microleakage of hydrogen peroxide into the periodontal tissue [8,11].

It has been suggested that placement of a protective cervical barrier 2mm below the CEJ prior to intracoronal bleaching is useful to inhibit ECR [13,14]. Placing an intracoronal layer of calcium hydroxide can provide an alkaline pH at the root surface, which may inhibit ECR [15] by neutralizing the acidic components [16].

Various dental materials, including hydrophilic materials, IRM, Cavit, Coltosol, zinc oxide-eugenol cement, zinc phosphate cement, temporary light-cure resin, conventional glass ionomer (GI), resin-modified glass ionomer (RMGI), and mineral trioxide aggregate (MTA) have been proposed as protective barriers for

intracoronal bleaching [12,13]. If removal of a temporary material is necessary for final restoration, 2mm glass ionomer is suggested as a standard protective layer during bleaching to remain at the site as a barrier for the final restoration.

Glass Ionomer is also used as an intra-orifice barrier to seal the root canals and inhibit the penetration of released radicals. Beckham et al. [16] found that dye penetration was significantly lower when GI was used as an intracoronal barrier in comparison with temporary endodontic restorative materials. Moreover, Wolcott et al. [17] showed that coronal microleakage was remarkably lower in the groups using GI intra-orifice barrier than the control group in which no barrier was used. Evidence shows that MTA is appropriate for successful treatment of cervical resorption [18]. The prominent feature of MTA is resistance against microleakage. It also produces a high concentration of calcium hydroxide [19,20], which may inhibit root resorption due to high alkaline potency [19]. Tooth discoloration caused by MTA is the only factor that prevents the application of MTA as a protective barrier during intracoronal bleaching [18].

Given the significance of pH around the root during non-vital tooth bleaching as well as introduction of light-curing barriers such as light-cure calcium hydroxide, light-cure resin-modified calcium silicate, and light-cure RMGI, which have easier application than the former conventional compounds such as calcium hydroxide, MTA, and glass ionomer, this study aimed to assess the effect of four light-cure intracoronal barriers containing calcium alkaline components and GI with and without applying bonding agent (BA) on the root surface pH after intracoronal bleaching with hydrogen peroxide.

MATERIALS AND METHODS

For this in vitro study, 70 recently extracted single-rooted intact bovine incisors were used (ethical approval code: IR.MUI.REC.1396.3.398). All materials applied in the study are shown in Table 1. The teeth were stored in saline. The tooth surface was cleared of any remnant tissues using a No. 11 scalpel blade (Moris, China).

Table 1. Characteristics of the materials used in the present study

Material	Chemical Composition	Manufacturer	Type	
Lime-Lite	Acrylate resin	PULPDENT, USA	Calcium and fluoride releasing, light-cured, radiopaque dental liner in a urethane dimethacrylate resin	
	Hydroxyapatite			
	Calcium hydroxide			
	Calcium phosphate tribasic			
	Photo-chemistry			
	Glass filler			
TheraCal	Portland cement 30 -50%	BISCO, Inc.	Light-cured, resin-modified calcium silicate filled liner	
	Polyethylene Glycol Dimethacrylate 10 -30%			
	Bisphenol A Diglycidylmethacryl 5 -10%			
	Barium Zirconate Powder 5-10%			
Ionoseal	Fluoroalminumsilicate,Bis-GMA, 1,6-hexanediylbismethacrylate 5-10%,TEDMA 2.5-5%	VOCO GmbH, Cuxhaven, Germany	One-Component light-cured resin reinforced glass ionomer liner	
Fuji II LC	Resin/liquid (24%wt)	PAA, HEMA, proprietary ingredient, 2,2,4-trimethylhexamethylenedicarbonate, TEGDMA	GC Corporation, Tokyo, Japan	Resin-modified glass ionomer
	Fillers (76%wt)	(flouro)alumino silicate glass		
Clearfill SE Bond	Self-etching/ primer	2-hydroxyethyl methacrylate 20-40%	Kuraray, Japan	Two-step Self-etch Light-cured Bonding agent
		10-methacryloyloxydecyl dihydrogen phosphate		
		Hydrophilic aliphatic dimethacrylate		
		Dl-camphorquinone		
		Accelerators		
		Water		
	Dyes			
	Bond	Bisphenol a diglycidylmethacrylate25-45%,		
		2-hydroxyethyl methacrylate20-40%,		
		10-methacryloyloxydecyl dihydrogen phosphate		
		Hydrophobic aliphatic methacrylate		
		Colloidal silica		
		Dl-camphorquinone		
Initiators				
Accelerators				
	Others			

Standard access cavities were prepared by a #029 round carbide bur in a high-speed rotary instrument. The coronal third of the canals was flared with #5 and #6 Gates-Glidden drills (Mani, Japan). The root canals were instrumented by circumferential filing using K-files (Mani, Japan). A #60 K-file was used as the master apical file. The canals were irrigated with 5.25% hypochlorite solution during instrumentation. The root canals were dried with paper points and finally obturated with AH26 sealer (Dentsply, Germany) and gutta-percha (Meta Biomed, Korea) via cold lateral compaction technique. Access cavities were filled with Cavit (3M ESPE, USA) as temporary restoration.

After one week, the temporary fillings and 3 mm of the cervical portion of gutta-percha below the CEJ were removed by a heated plugger. The depth was confirmed by a periodontal probe. The pulp chamber was cleaned with cotton pellets impregnated with 90% alcohol. The smear layer was removed with 17% EDTA (Dentsply, USA) for 3 minutes, followed by rinsing with distilled water. In order to inhibit the penetration of any liquid, the apical foramina and apical thirds of the roots were covered with two layers of nail varnish and a thin layer of wax. The samples were divided into seven groups, with 10 samples in each group. In three groups, Clearfil SE Bond (Kuraray, Japan) and an experimental base were used. In four other groups, only the experimental base was applied. The experimental bases were Theracal LC (BISCO, IL, USA), Lime-Lite (Pulpdent, USA), Ionoseal (VOCO, Germany), and RMGI (Fuji II LC, GC, Japan). Thus, the groups included in this study were as follows: Theracal LC (group A), Theracal LC + BA (group B), Lime-Lite (group C), Lime-Lite + BA (group D), Ionoseal (group E), Ionoseal + BA (group F), and RMGI (group E).

The self-etch primer of BA was rubbed on dentin by a microbrush 1 mm apical to the CEJ for 20 seconds. Then, it was dried with mild air flow for 10 seconds in order to evaporate the solvent. The bonding agent was applied on the surface and light-cured (400mw/cm², Demetron LC, Kerr, USA) for 20 seconds after using gentle air flow. In each group, 2mm-thick experimental bases were placed 1 mm under the CEJ and light

cured for 20 seconds. The base was placed thicker on the buccal and lingual sides compared with the proximal side to follow the CEJ pattern. The composition of each experimental base is shown in Table 1.

Each tooth was separately placed in a glass container containing 10 mL of distilled water and fixed with a rubber dam such that the whole root and CEJ were immersed in distilled water and the crown and access cavity were out of the water (Fig. 1).

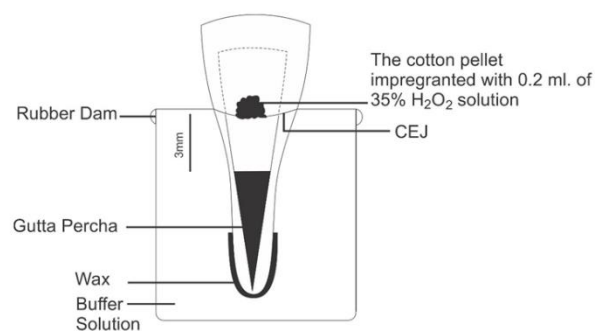
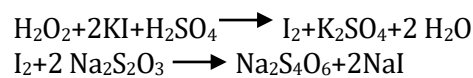


Fig 1. Schematic view of the tooth prepared to assess microleakage following internal bleaching. CEJ: Cementoenamel junction

The teeth were incubated (Behdad, Iran) at 37°C for 24 hours. Afterward, the samples were removed from the incubator, and the pH of the surrounding water was measured by a pH meter (Metrohm 744, Switzerland). Cotton pellets impregnated with 0.2 mL of 35% H₂O₂ solution were placed in each access cavity. Then, the teeth underwent heat treatment by a 1000 W light for 2 minutes. This process was repeated three times to simulate clinical bleaching. Finally, the access cavity was dried and a dry cotton pellet was placed in it. The pH of the surrounding water and concentration of H₂O₂ were immediately measured by iodometric titration method [21]. The following steps were performed to standardize iodometric pH measurement:



Later on, the samples were placed back in the incubator and the pH of the surrounding water was measured 1 day, 1 week, and 2 weeks after

bleaching (Table 2). The water was changed after each measurement.

Statistical analysis of the data was performed by SPSS version 23.0 (SPSS Inc. IL, USA). T-test was used to compare preoperative pH value with the mean postoperative pH values in each group. ANOVA was applied to compare the mean postoperative pH values among the experimental groups. The Kruskal-Wallis test was applied to evaluate H₂O₂ microleakage in all groups.

RESULTS

Table 2 shows the mean pH values in each of the seven groups. In all groups, the mean pH of the surrounding medium became acidic immediately and 24 hours following the bleaching procedure. However, the mean pH value increased after 1 week and continued to increase 2 weeks after bleaching. The comparison of mean pH values before and after bleaching in all stages indicated a significant difference ($P < 0.05$). However, ANOVA revealed no statistically significant difference among the experimental groups ($P = 0.189$). Moreover, there were no statistically significant differences among the groups in the amount of H₂O₂ microleakage ($P = 0.895$).

DISCUSSION

Reduction of pH on the external tooth surface during intracoronal bleaching is a key factor involved in cervical root resorption [22].

A filling material is suggested to cover the canal to seal the dentinal tubules, which are a communication path between the pulp and root surface. A filled root canal is not resistant to microleakage, and sealer and filling technique cannot effectively prevent the leakage of materials into the canal [23]. Hence, it is essential to achieve the best seal and consider the quality of the orifice base. Use of base is highly important to prevent the adverse effect of bleaching materials.

Many attempts have been made to introduce a long lasting seal. GI cement is one of the standard materials introduced for this purpose [24]. It has been stated that GI, owing to its significant sealing ability, can effectively prevent ECR when used as an intra-orifice barrier [24]. However, several new biomaterials such as calcium hydroxide, composite GI, and calcium silicates have not yet been assessed.

The results of this in vitro study showed that the pH value of the surrounding medium in all groups decreased immediately and 24 hours after bleaching and then increased for the next 2 weeks after bleaching regardless of the type of intracoronal barrier or application of BA. In this study, 35% hydrogen peroxide solution was utilized by thermo-catalytic technique to evaluate the sealing effect of the bases in spite of the fact that in most previous studies the samples were analyzed via walking bleaching technique [10].

Table 2. Mean (\pm standard deviation) of pH in each group in five different periods

Base	Before bleaching	After bleaching	1 day after bleaching	1 week after bleaching	2 weeks after bleaching	Mean pH after bleaching
Ionoseal	7.31 \pm 0.11	6.98 \pm 0.26	6.73 \pm 0.3	7.14 \pm 0.17	7.49 \pm 0.3	7.08 \pm 16
Ionoseal+BA	7.30 \pm 0.17	6.79 \pm 0.11	6.67 \pm 0.14	7.04 \pm 0.12	7.36 \pm 0.27	6.97 \pm 0.12
Calcium hydroxide	7.34 \pm 0.15	7.04 \pm 0.14	6.73 \pm 0.086	7.09 \pm 0.21	7.30 \pm 0.15	7.04 \pm 0.08
Calcium hydroxide+BA	7.24 \pm 0.06	6.91 \pm 0.06	6.73 \pm 0.14	7.14 \pm 0.1	7.40 \pm 0.16	7.06 \pm 0.08
Theracal	7.22 \pm 0.05	6.90 \pm 0.09	6.62 \pm 0.14	7.07 \pm 0.15	7.30 \pm 0.17	6.97 \pm 0.09
Theracal+BA	7.34 \pm 0.11	7.19 \pm 0.14	6.69 \pm 0.2	7.03 \pm 0.16	7.31 \pm 0.28	7.05 \pm 0.16
Resin-modified glass ionomer	7.27 \pm 0.09	6.93 \pm 0.07	6.58 \pm 0.17	7.06 \pm 0.11	7.32 \pm 0.34	6.97 \pm 0.14

BA: bonding agent

Furthermore, the thermo-catalytic technique was preferred to walking technique since in this technique the pulp chamber is filled with a liquid and heated for 3 times, thus, it is more challenging for intracoronal barrier to work efficiently [11]. Application of heat during thermo-catalytic technique causes the expansion of dentinal tubules, which increases dentinal permeability [25,26]. Increased dentinal permeability results in penetration of microorganisms into the root canal, which results in ECR [27]. In addition, hydrogen peroxide is the most important bleaching substance involved in ECR compared with other bleaching agents [1,28] because of its low pH and induction of acidic pH around the root [29].

The findings of this study indicated an acidic pH around all teeth treated by thermo-catalytic technique. Kehoe [10] concluded that dentinal pH and cement became more acidic after walking bleaching. Also, McCormick et al. [30] reported that acidic pH on the root surfaces following bleaching provided a proper environment for osteoclastic activity because leucocytes and osteoclasts have their optimal performance in acidic environments. Hence, acidic pH in the oral cavity can cause cervical root resorption [30].

In the present study, application and no application of BA had no significant effect on hydrogen peroxide microleakage or pH value around the root. This inefficacy could be related to the degradation of BA adjacent to hydrogen peroxide [1,28] or expansion of dentinal tubules following thermo-catalytic procedure [25,26]. However, in contrast to the results of this study, Shindo et al. [31] stated that using self-adhesive BA improved the sealing ability. This contradiction can be because bleaching agent and heat were not applied in their study and the sealing ability was assessed merely in the orifices. Liena et al. [32] also found that using BA decreased microleakage in non-vital dental bleaching, which was in contrast to the findings of the current study. This contrast might be related to no application of heat during bleaching and application of a two-step etch and rinse system.

In the present study, due to possible

destruction of intracoronal barrier while using the bleaching substance and subsequent data distortion, the pH value of the medium was measured in addition to hydrogen peroxide microleakage. In general, the amount of hydrogen peroxide microleakage in the present study was low in all experimental groups. According to Halliwell et al. [33] hydrogen peroxide $<20\text{Nmol/L}$ is safe and $>50\text{Nmol/L}$ is cytotoxic to a wide range of human cells. Since H_2O_2 levels in this study were below the hazardous range in all samples, application of intracoronal barrier might be beneficial to obtain a safe internal bleaching treatment. Thus, the efficacy of base in decreasing hydrogen peroxide diffusion cannot be explained. Yet, it is noteworthy that hydrogen peroxide level in the present study was much lower than those obtained in the two previous studied in which base was not used. It seems that base is an effective material in reducing hydrogen peroxide diffusion. This is also in line with the results of MacIsaac and MacIsaac et al. [34] reporting that an intermediate base was not used in many cases of external cervical root resorption.

RMGI does not induce tooth discoloration due to its positive features such as adhesion to tooth structure and resistance to dissolution induced by the bleaching agent. In addition, it is accepted as a cervical barrier that prevents the recurrence of caries [35]. Consistent with this study, Yui et al. [35] observed that using RMGI as an intracoronal barrier reduced the linear dye leakage in the apical direction. Liebenberg [36] also found similar results about RMGI in his study. However, slight microleakage through RMGI would be possible because of shrinkage during polymerization or wrong manipulation [37]. In our study, RMGI exhibited significantly greater pH in experimental groups than the control group. Thus, it might be able to effectively reduce hydrogen peroxide microleakage.

Lime-Lite as a light-cure calcium hydroxide in a urethane dimethacrylate resin could not prevent acidic pH after the bleaching treatment. It might be due to resin polymerization, which blocks calcium hydroxide infiltration into dentinal tubules. Although

Lambriandis et al. [38] used a calcium hydroxide layer beneath a GI base, the results of their study were similar to those of the present study. Ionoseal, a one-component light-cure resin-reinforced glass ionomer, which is a compomer-like material, was another base examined in this study. Rafeek et al. [39] evaluated the effect of Dyract AP (Dentsply, USA) application on dye microleakage and reported the same results as the present study. TheraCal, which is made of calcium silicate plus Portland cement and polyethylene glycol dimethacrylate, is very similar to MTA in composition because of existence of calcium silicate. The results of studies [13,24,40] that have used MTA as an intra-orifice barrier showed no significant difference between MTA and GI in terms of microleakage, which is in agreement with the findings of the current study. It should be mentioned that more studies with different base materials and various methods of bleaching are recommended.

CONCLUSION

Considering the results of this study regarding the pH of the surrounding medium and hydrogen peroxide microleakage, all examined materials, including RMGI, Lime-Lite, Ionoseal, and Theracal can act effectively as a cervical barrier in non-vital bleaching procedure in order to inhibit external cervical resorption.

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CONFLICT OF INTEREST STATEMENT

None declared.

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