



The Effect of Bleaching Agents on the Microstructure and Surface Microhardness of Three Calcium Silicate-Based Barrier Materials

Elham Ahmadi ^a , Naghmeh Meraji ^{b*}

^a Department of Restorative Dentistry, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran ; ^b Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article Type: **Original Article**

Received: 06 Aug 2019

Revised: 23 Nov 2019

Accepted: 15 Dec 2019

Doi: 10.22037/iej.v15i1.25345

*Corresponding author:
Naghmeh Meraji, Department
of Endodontics, School of
Dentistry, Tehran University of
Medical Sciences, Tehran, Iran.

Tel: +98-21 88351173

E-mail: n-meraji@tums.ac.ir

This work is licensed under the terms
of the Creative Commons Attribution-NonCommercial-
ShareAlike 4.0 International.

ABSTRACT

Introduction: The aim of this study was to evaluate the effects of exposure to sodium perborate and H₂O₂ on the surface characteristics of MTA Angelus, Biodentine and MTA Repair HP after 1 and 6 month time intervals. **Methods and Materials:** Three calcium silicate-based cements were evaluated: MTA Angelus, Biodentine, MTA Repair HP. A total of 234 specimens were stored in Hank's balanced salt solution (HBSS) for 1 month or 6 months in which afterwards were divided into 3 groups according to bleaching agent applied: control, sodium perborate, 35% hydrogen peroxide. The microstructural changes were evaluated by scanning electron microscopy and energy-dispersive X-ray spectroscopy. The surface microhardness was also evaluated. Data were analyzed by one-way analysis of variance and Games-Howell post-hoc tests for the effect bleaching agents and hydraulic calcium silicate-based cements and t-test was for the effect of time. **Results:** Distinctive alterations with uneven depression areas, woodpecker defects and cracks were seen due to exposure to perborate and H₂O₂ on all evaluated cements. Exposure to H₂O₂ caused a decrease in Ca/Si ratio in all experimental cements. Both H₂O₂ and perborate significantly decreased the microhardness of all cement ($P < 0.05$) with H₂O₂ having a more profound effect ($P < 0.01$). A 6-month delay in exposure to bleaching agents significantly increased the microhardness of Biodentine compared to 1 month ($P < 0.001$ for both bleaching agents). **Conclusion:** Based on this *in vitro* study, H₂O₂ had more detrimental effects on MTA Angelus, Biodentine and MTA Repair HP. Sodium perborate may be a more conservative selection when considering effects on barrier materials.

Keywords: Bleaching Agent; Calcium Silicate Cement; Microhardness; Mineral Trioxide Aggregate; Scanning Electron Microscopy

Introduction

Regenerative endodontic treatment (RET) is a biologically based treatment approach for necrotic, immature permanent teeth leading to the continuation of root development and reinforcement of dentinal walls and subsequently increase of long-term tooth retention [1, 2]. A retrospective study has shown a higher survival rate of RET when compared with apexification procedures using both mineral trioxide aggregate (MTA) and Ca(OH)₂ [3]. Several unfavorable outcomes have been considered for RET including persistent clinical signs and symptoms and/or increased size of the periradicular lesion [4], collapse of barrier

material for cervical sealing into the canal [5], pulp canal calcification [6] and coronal discoloration [5, 7]. In both position statements of the American Association of Endodontists (AAE) [8] and the European Society of Endodontology (ESE) [9] tooth discoloration after RET is discussed. This tooth discoloration is due to the intracanal medicaments [10, 11] used for disinfection and calcium silicate-based cements [12-14] used as barrier material for cervical sealing. For instance, the minocycline in triple antibiotic paste (TAP) which is used as an intracanal medicaments in RET procedures causes discoloration due to its binding to calcium ions through chelation [15, 16]. Akcay *et al.* [15] demonstrated clinically visible crown discoloration with the use of TAP with doxycycline,

amoxicillin, or cefaclor. Many studies have confirmed these findings [11, 17]. When MTA is used as barrier material, the interaction of bismuth oxide with collagen present in tooth tissue [18, 19] and remaining sodium hypochlorite from canal irrigation [19, 20] results in tooth discoloration as well. Due to these interactions, yellow bismuth oxide is converted to a black precipitate [18, 20].

MTA is a tricalcium silicate cement routinely used as a barrier material for cervical sealing in RET. This cement has various suitable properties such good sealing ability [21] and marginal adaptation [22]. This cement is used in the management of root resorption due to its anti-resorptive effects and high pH [23, 24]. As it contains bismuth oxide in its composition, crown discoloration is demonstrated subsequent to its use. Biodentine (Septodont, Saint Maur des Faussés, France) is another tricalcium silicate cement which is bismuth oxide free and thus has been implicated with less tooth discoloration [14, 25, 26] owing to the replacement of bismuth oxide with zirconium oxide. Biodentine sets faster than MTA due to the addition of calcium chloride to the liquid component of this cement [27]. It has been shown that this cement is less susceptible to environmental conditions such as low pH and blood [28, 29]. Biodentine can be used as barrier material for cervical sealing in RET [30]. Other tricalcium silicate-based materials which have alternative radiopacifiers thus bismuth-oxide free are available for

clinical use such as MTA Repair HP (Angelus Soluções Odontológicas, Londrina, Brazil). This cement has been shown to have a higher calcium release rate [31] and produces a quick and effective bioactive response [32].

Internal bleaching of nonvital, discolored teeth is a low-risk popular procedure. Placement of a cervical barrier before internal bleaching treatment is essential for preventing the penetration of bleaching agents into periodontal tissues through the dentinal tubules thus preventing cervical resorption [33, 34]. Kim *et al.* [10] placed a resin modified glass ionomer on the MTA barrier they used prior to the application of sodium perborate. D'Mello *et al.* [35] performed internal bleaching after removing 1mm of the MTA barrier and placing glass ionomer cement on top of it. Both studies have shown internal bleaching to be a predictable treatment option for discolored teeth after RET. However, some authors [36-38] have suggested completely removing the calcium silicate cements and replacing it with other routine cervical barrier such as glass ionomer. This act may increase the risk of disrupting the seal achieved by these cement.

Internal bleaching of nonvital, discolored teeth is a low-risk popular procedure. Placement of a cervical barrier before internal bleaching treatment is essential for preventing the penetration of bleaching agents into periodontal tissues through the dentinal tubules thus preventing cervical resorption [33, 34]. Kim *et al.* [10] placed a resin modified glass ionomer on the MTA barrier they

Table 1. Description of study variables

Variable	Definition	Qualitative		Quantitative		Variable		Scale	Measurement method
		Ordinal	Nominal	Discrete	Continuous	Independent	Dependent		
Microhrdness	Resistance of the surface of materials to deformation subsequent to loading of a pyramidal diamond indenter			*			*	Vickers Tester	N/mm2
Surface crystalline structure	Crystalline structures and morphology of the surface of materials		*				*	SEM	Crystalline morphology
Surface elements	Elements found on the surface of the material				*		*	EDAX	Percentage
Type of CSC	Type of calcium silicate cement evaluated		*			*		Observation	Angelus MTA MTA Repair HP
Type of bleaching material	Type of bleaching agent used on the surface of CSCs		*			*		Observation	H2O2 Sodium Perborate Control

SEM: Scanning electron microscope; EDX: Energy-dispersive X-ray spectroscopy evaluations

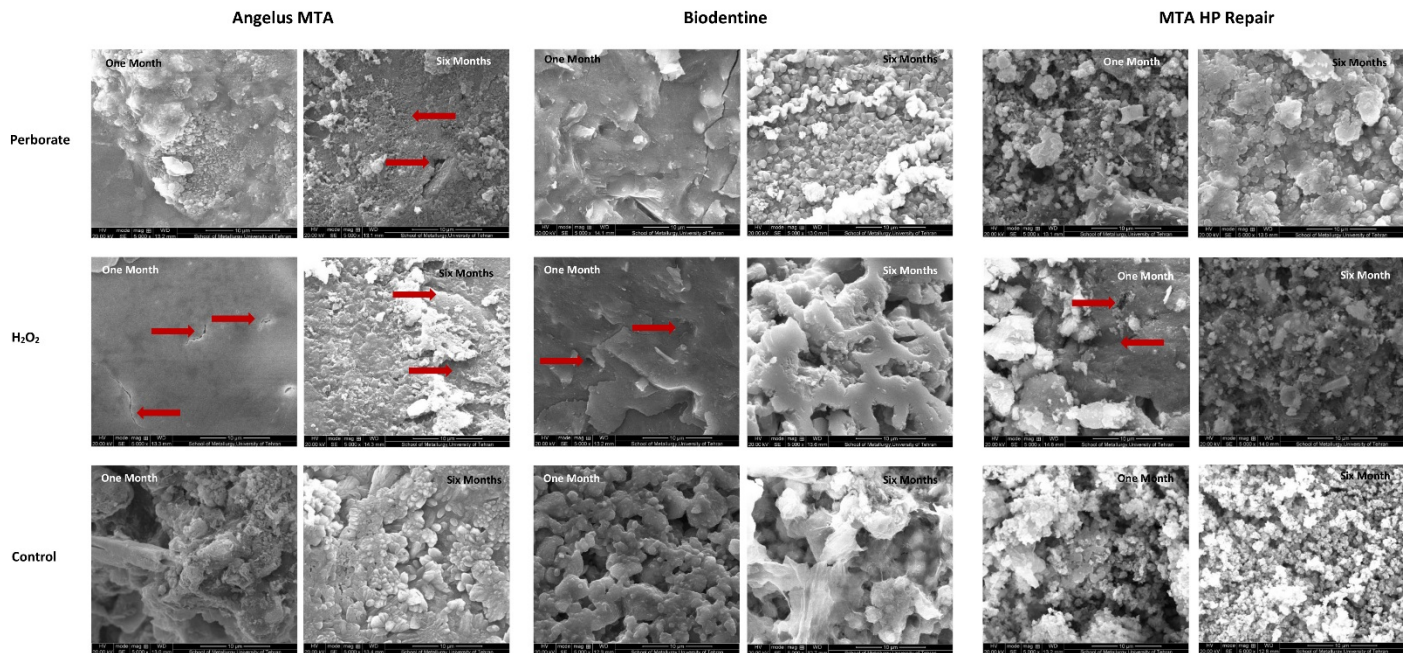


Figure 1. The scanning electron micrographs of A) Angelus MTA; B) Biodentine ; C) MTA HP Repair showing deterioration (red arrows) of the surface structure due to bleaching agents

used prior to the application of sodium perborate. D'Mello *et al.* [35] performed internal bleaching after removing 1mm of the MTA barrier and placing glass ionomer cement on top of it. Both studies have shown internal bleaching to be a predictable treatment option for discolored teeth after RET. However, some authors [36-38] have suggested completely removing the calcium silicate cements and replacing it with other routine cervical barrier such as glass ionomer. This act may increase the risk of disrupting the seal achieved by these cement.

Bleaching agents lower the pH on the root surface and subsequently cause cervical resorption [39]. The high pH of MTA and released calcium hydroxide may further protect the root and prevent cervical resorption. The sealing ability of MTA used as a cervical barrier in internal bleaching was shown to be similar to glass ionomer cement *in vitro* [34]. Therefore, it seems that considering MTA as a barrier in which a bleaching agent may be applied on in the discolored teeth due to RET is logical. However, exposure to bleaching agents may result in changes in the chemical and physical properties of these barriers (*e.g.* hardness, roughness [40], compressive strength [41], microleakage [42] and bond strength of restorative materials [43]) and subsequently affect the treatment outcomes. The aim of the current study was the evaluation of surface characteristics of MTA Angelus, Biodentine and MTA Repair HP after exposure to bleaching agents. The null hypothesis was that bleaching agents do not affect the surface characteristics of these cements.

Materials and Methods

Three hydraulic calcium silicate-based cements were evaluated in this *in vitro* study:

- MTA Angelus (Angelus Soluções Odontológicas, Londrina, Brazil)
- Biodentine (Septodont, Saint Maur des Faussés, France)
- MTA Repair HP (Angelus Soluções Odontológicas, Londrina, Brazil)

The materials were mixed according to the manufacturers' instructions and were allowed to set until final setting time which was measured using an indentation technique. Specimens measuring 10 mm in diameter and 2 mm height were prepared for each material type. Testing was performed after 1 month or 6 months of storage in Hank's balanced salt solution (HBSS). After storage time, specimens in each group were divided into three groups according to the type of bleaching agent they were exposed to:

- Not exposed to bleaching agents (control)
- Exposed to 2 g sodium perborate (Sigma-Aldrich, Chemie, Steinheim, Germany) (pH=8.5) per mL of distilled water forming a thick paste
- Exposed to 35% hydrogen peroxide (Opalescence Endo, Ultradent Products, South Jordan, UT, USA) (pH=5)
- Bleaching agents were placed over the specimens for 1 week. The following tests were performed.

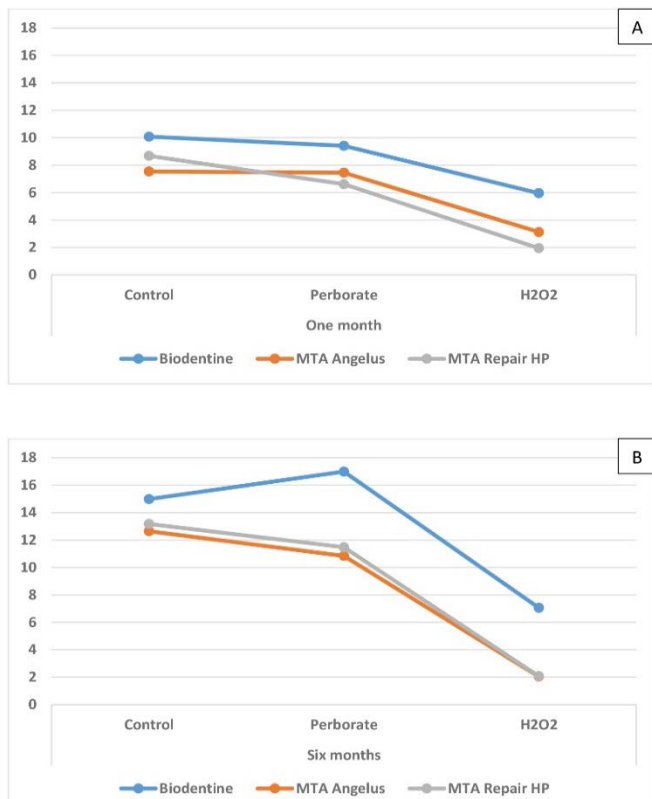


Figure 2. Ca/Si ratio of all cements decreases when exposed to H₂O₂ in the A) 1 month; B) 6 month

Scanning electron microscope and Energy-dispersive X-ray spectroscopy evaluations:

For scanning electron microscope (SEM) evaluation of specimens ($n=3$ in each subgroup), the surface in contact with bleaching agents was coated with carbon and analyzed with a SEM equipped with energy-dispersive x-ray spectroscopy (EDX)(SEM-EDX 515, Phillips, Eindhoven, The Netherlands). Magnifications that were considered adequate for the characterization of the microstructure were selected, 500 \times and 5000 \times , and images were acquired.

EDX by area mode (analytic area: 0.01 mm²) was done on three parts of the material surface selected randomly. The Ca/Si ratios were worked out for all the materials and different surface treatments [44].

Microhardness testing

A total of 180 specimens ($n=10$ in each subgroup) measuring diameter of 6 mm and height of 4 mm (according to ASTM E384 Standard for microhardness tests tests [45]) were prepared. The material are allowed to set and the surfaces were polished using silicon carbide sandpaper with decreasing particle sizes of 400, 500, 800, 1000, 1200, 1500, and 2000 grit, respectively. For the purpose of facilitating indentation and minimizing the influence of sample preparation on surface microhardness, wet polishing

with minimal hand pressure was used [46]. All the specimens were placed in HBSS for either 1 month or 6 months.

Microhardness testing was performed after the use of bleaching agents for 1 week. The surface microhardness test was performed by using a Vickers Tester (Bareiss Prüfgeratebau GmbH, Oberdischingen, Germany) with a pyramidal diamond indenter by using a load of 300 g for 10 sec. According to the pilot study, this load created a clear and reliable indent in all 3 materials. Five indents were made on the surface exposed to bleaching agents of each sample at separate locations with a 2.5d (2.5 times the mean diameter of each indent) distance between indentations and from the edge of the sample (in accordance with ASTM E384 standard for Vickers microhardness test). The Vickers microhardness value was calculated by the testing machine on the basis of the following equation in which F is the load in kilogram-force, d is the mean of the 2 diagonals in mm, and HV stands for Vickers microhardness value [46].

$$HV = \frac{2f \sin \frac{136^\circ}{2}}{d^2}$$

$$HV = 1.854 \frac{f}{d^2}$$

Statistical analysis

The data were evaluated using Statistical Package for the Social Sciences software (PASW Statistics 18; SPSS Inc, Chicago, IL, USA). One-way analysis of variance and Games-Howell post-hoc tests were used for the effect bleaching agents and hydraulic calcium silicate-based cements. T-test was used for the effect of time (Table 1). A significance level of $P=0.05$ was used to perform multiple comparison tests.

Results

Scanning electron microscope and Energy-dispersive X-ray spectroscopy

The scanning electron micrographs of the test materials are shown in Figures 1A to 1C. The control groups which were not exposed to the chemicals exhibited the deposition of crystals on the material surface. These crystals indicate the presence of hydration. The use of sodium perborate and hydrogen peroxide resulted in a devoid surface for all materials cured for 1 month. Crystallization was evident in the 6 months old specimens regardless of the surface treatment. The MTA HP was the least effected by the surface treatment at all ages. The Ca/Si ratio of all cements decreases when exposed to H₂O₂ (Figure 2).

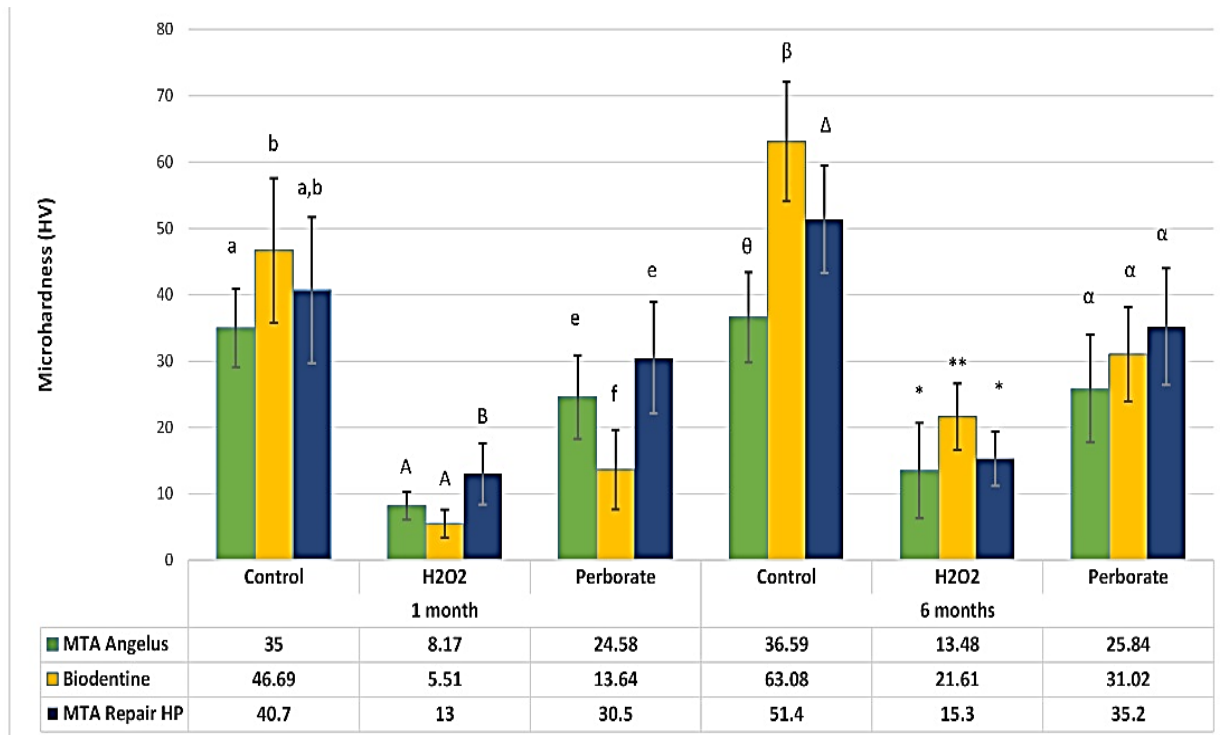


Figure 3. The surface microhardness values of different experimental groups can be seen

Microhardness results

The microhardness values of different groups are shown in Figure 3. In all three hydraulic calcium silicate-based cements regardless of the time of exposure, exposure to H₂O₂ and perborate significantly decreased the microhardness of materials in comparison with the control group ($P < 0.05$). Furthermore, the microhardness of specimens exposed to H₂O₂ were significantly lower than that of those exposed to perborate ($P < 0.01$). In the control groups, the microhardness of Angelus MTA showed no significant difference between 1 month and 6 months specimens ($P = 0.58$), whereas; in the case of Biodentine and MTA Repair HP these values significantly increased after 6 months ($P = 0.002$ and $P = 0.005$ respectively).

When comparing 1 month and 6 month exposure to perborate in Angelus MTA, no significant difference was seen ($P = 0.6$); however, in the case of exposure to H₂O₂ in this cement these values were significantly higher in 6 month exposure specimens ($P = 0.03$). In the case of Biodentine, microhardness values were significantly higher in specimens exposed to either perborate or H₂O₂ after 6 month ($P < 0.001$ in both). However, no difference was seen in the microhardness of MTA Repair HP exposed to bleaching agent when comparing their time of exposure ($P = 0.2$ in both bleaching agents).

Discussion

Application of a cervical barrier in cases in which are undergoing internal bleaching is essential for preventing cervical root resorption [47]. In many cases of tooth discoloration such as teeth undergone RET, a cervical barrier of MTA or other hydraulic calcium silicate-based cements already have been applied; therefore, removing and replacing it with other routine cervical barrier such as glass ionomer or Cavit for internal bleaching as suggested by some authors [36-38] would increase the risk of disrupting the seal.

Sodium perborate mixed with water has been reported to be the safest bleaching agent due to its low hydroxyl radical diffusion [48]. On the other hand, its effect on discoloration is limited and less than bleaching agent enduring higher hydroxyl radical diffusion. As calcium silicate-based cements release calcium hydroxide and increase the pH of the surrounding environment [31, 49] contrary to glass ionomer which has acidic pH [50], they may neutralize the adverse effects of hydroxyl radical diffused from the bleaching agents and may be more effective in prevention of cervical root resorption. Therefore, the use of more effective bleaching agents such as H₂O₂ may be considered.

According to the findings of the current study, both sodium perborate and H₂O₂ had negative effects on the surface of all

evaluated cements by creating defects and cracks on the surface and altering the chemical composition. These deteriorating effects were exacerbated when H₂O₂ was applied. An increase in O and Si levels and decrease in Ca was when the materials were exposed to H₂O₂. This was in accordance to other literature on this topic [42, 51]. These findings can be attributed to the low pH of H₂O₂ and oxygen bubbling produced during its application. Exposure of calcium silicate-based cements to acidic pH after setting can lead to chemical degradation of hydrates and the release of the Ca ions, thus, can decrease the Ca/Si ratio of these cements [42]. Higher levels of O detected on the surface of these cements may be attributed to the oxygen bubbling effect of H₂O₂ or incomplete removal of the bleaching agent from the surface of the cements [52]. Evaluation of chemical changes in different depths from the surface is suggested and may enlighten new facts. Exposure to both H₂O₂ and perborate also significantly decreased the microhardness values of all three evaluated cements with H₂O₂ causing significantly more decrease. Mohebbi *et al.* [53] evaluated the effect of acidic and alkaline pH on the microhardness of MTA and CEM Cement and reported a decrease in microhardness compare to the control group after exposure to both acidic and alkaline pH, however, the decrease was higher in acidic pH. The results of our study are similar to that of the study of Mohebbi *et al.* [53] as H₂O₂ has an acidic pH and perborate has an alkaline pH. Serin Kalay [54] also reported a significant decrease in the surface microhardness of MTA after application of H₂O₂, however, reported no significant effect for sodium perborate. This difference between the results of the two studies in the case of sodium perborate can be due to differences in the protocol of use of bleaching agents. In our study, bleaching agents were only used once for a one-week duration, however, in the study of Serin Kalay, bleaching agents were repeatedly used for three times on every fourth day. Keskin *et al.* [41] showed a reduction in the compressive strength of various calcium silicate cements after exposure to the mixture of sodium perborate with 30% hydrogen peroxide. Decrease in surface microhardness may be due to detrimental changes seen on surface morphology and composition of these materials [55].

Delayed exposure to bleaching agents (up to 6 months) only compensated the adverse effects of bleaching agents on Biodentine. Therefore, in the case of MTA Angelus and MTA Repair HP, increasing the time interval between cement placement and application of bleaching agents did not have beneficial effects.

Conclusion

H₂O₂ and sodium perborate exhibited adverse effects on the surface characteristics of MTA Angelus, Biodentine and MTA Repair HP. These effects were more severe when exposed to H₂O₂. Since the delayed exposure to bleaching agents (up to 6 months) compensated the adverse effects on Biodentine, it seems that postponing internal bleaching in the case of Biodentine may be beneficial.

Acknowledgement

This study was supported by a grant from Tehran University of Medical Sciences (grant no. 96026935310)

Conflict of Interest: 'None declared'.

References

1. Wigler R, Kaufman AY, Lin S, Steinbock N, Hazan-Molina H, Torneck CD. Revascularization: a treatment for permanent teeth with necrotic pulp and incomplete root development. *J Endod.* 2013;39(3):319-26.
2. Hargreaves KM, Diogenes A, Teixeira FB. Treatment options: biological basis of regenerative endodontic procedures. *J Endod.* 2013;39(3 Suppl):S30-43.
3. Jeeruphan T, Jantarat J, Yanpiset K, Suwannapan L, Khewsawai P, Hargreaves KM. Mahidol study 1: comparison of radiographic and survival outcomes of immature teeth treated with either regenerative endodontic or apexification methods: a retrospective study. *J Endod.* 2012;38(10):1330-6.
4. Bukhari S, Kohli MR, Setzer F, Karabucak B. Outcome of Revascularization Procedure: A Retrospective Case Series. *J Endod.* 2016;42(12):1752-9.
5. Dabbagh B, Alvaro E, Vu DD, Rizkallah J, Schwartz S. Clinical complications in the revascularization of immature necrotic permanent teeth. *Pediatr Dent.* 2012;34(5):414-7.
6. Chen MY, Chen KL, Chen CA, Tayebaty F, Rosenberg PA, Lin LM. Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. *Int Endod J.* 2012;45(3):294-305.
7. Nosrat A, Homayounfar N, Oloomi K. Drawbacks and unfavorable outcomes of regenerative endodontic treatments of necrotic immature teeth: a literature review and report of a case. *J Endod.* 2012;38(10):1428-34.
8. American Association of Endodontists. Clinical considerations for a regenerative procedure. 2018 [22 July 2018]; Available from: http://www.aae.org/uploadedfiles/publications_and_research/research/currentregenerativeendodonticconsiderations.pdf.

9. Galler KM, Krastl G, Simon S, Van Gorp G, Meschi N, Vahedi B, Lambrechts P. European Society of Endodontology position statement: Revitalization procedures. *Int Endod J*. 2016;49(8):717-23.
10. Kim JH, Kim Y, Shin SJ, Park JW, Jung IY. Tooth discoloration of immature permanent incisor associated with triple antibiotic therapy: a case report. *J Endod*. 2010;36(6):1086-91.
11. AlSaeed T, Nosrat A, Melo MA, Wang P, Romberg E, Xu H, Fouad AF. Antibacterial Efficacy and Discoloration Potential of Endodontic Topical Antibiotics. *J Endod*. 2018;44(7):1110-4.
12. Felman D, Parashos P. Coronal tooth discoloration and white mineral trioxide aggregate. *J Endod*. 2013;39(4):484-7.
13. Ioannidis K, Mistakidis I, Beltes P, Karagiannis V. Spectrophotometric analysis of coronal discoloration induced by grey and white MTA. *Int Endod J*. 2013;46(2):137-44.
14. Bhavya B, Sadique M, Simon EP, Ravi SV, Lal S. Spectrophotometric analysis of coronal discoloration induced by white mineral trioxide aggregate and Biodentine: An in vitro study. *J Conserv Dent*. 2017;20(4):237-40.
15. Akcay M, Arslan H, Yasa B, Kavrik F, Yasa E. Spectrophotometric analysis of crown discoloration induced by various antibiotic pastes used in revascularization. *J Endod*. 2014;40(6):845-8.
16. Tanase S, Tsuchiya H, Yao J, Ohmoto S, Takagi N, Yoshida S. Reversed-phase ion-pair chromatographic analysis of tetracycline antibiotics. Application to discolored teeth. *J Chromatogr B Biomed Sci Appl*. 1998;706(2):279-85.
17. Jagdale S, Bhargava K, Bhosale S, Kumar T, Chawla M, Jagtap P. Comparative evaluation of coronal discoloration induced by two triple antibiotic revascularization protocols when used at varying depths of temporary sealing material at the end of varying time periods. *J Conserv Dent*. 2018;21(4):388-93.
18. Marciano MA, Costa RM, Camilleri J, Mondelli RF, Guimaraes BM, Duarte MA. Assessment of color stability of white mineral trioxide aggregate angelus and bismuth oxide in contact with tooth structure. *J Endod*. 2014;40(8):1235-40.
19. Meraji N, Bolhari B, Sefideh MR, Niavarzi SJDH. Prevention of Tooth Discoloration Due to Calcium-Silicate Cements: A Review. 2019;10(1):4-8.
20. Camilleri J. Color stability of white mineral trioxide aggregate in contact with hypochlorite solution. *J Endod*. 2014;40(3):436-40.
21. Shahriari S, Faramarzi F, Alikhani MY, Farhadian M, Hendi SS. Apical Sealing Ability of Mineral Trioxide Aggregate, Intermediate Restorative Material and Calcium Enriched Mixture Cement: A Bacterial Leakage Study. *Iran Endod J*. 2016;11(4):336-40.
22. Torabinejad M, Smith PW, Kettering JD, Pitt Ford TR. Comparative investigation of marginal adaptation of mineral trioxide aggregate and other commonly used root-end filling materials. *J Endod*. 1995;21(6):295-9.
23. Kusgoz A, Yildirim T, Alp CK, Tanriver M. Management of root resorption with mineral trioxide aggregate complicated by a luxation injury: report of a case with six-year follow-up. *J Pak Med Assoc*. 2017;67(1):134-6.
24. Shahi S, Ghasemi N, Rahimi S, Yavari HR, Samiei M, Janani M, Bahari M. The Effect of Different Mixing Methods on the pH and Solubility of Mineral Trioxide Aggregate and Calcium-Enriched Mixture. *Iran Endod J*. 2015;10(2):140-3.
25. Mozynska J, Metlerski M, Lipski M, Nowicka A. Tooth Discoloration Induced by Different Calcium Silicate-based Cements: A Systematic Review of In Vitro Studies. *J Endod*. 2017;43(10):1593-601.
26. Beatty H, Svec T. Quantifying Coronal Tooth Discoloration Caused by Biodentine and EndoSequence Root Repair Material. *J Endod*. 2015;41(12):2036-9.
27. Grech L, Mallia B, Camilleri J. Investigation of the physical properties of tricalcium silicate cement-based root-end filling materials. *Dent Mater*. 2013;29(2):e20-8.
28. Ashofteh Yazdi K, Ghabraei S, Bolhari B, Kafili M, Meraji N, Nekoofar MH, Dummer PMH. Microstructure and chemical analysis of four calcium silicate-based cements in different environmental conditions. *Clin Oral Investig*. 2019;23(1):43-52.
29. Sheykhrezae MS, Meraji N, Ghanbari F, Nekoofar MH, Bolhari B, Dummer PMH. Effect of blood contamination on the compressive strength of three calcium silicate-based cements. *Aust Endod J*. 2018;44(3):255-9.
30. Topcuoglu G, Topcuoglu HS. Regenerative Endodontic Therapy in a Single Visit Using Platelet-rich Plasma and Biodentine in Necrotic and Asymptomatic Immature Molar Teeth: A Report of 3 Cases. *J Endod*. 2016;42(9):1344-6.
31. Guimaraes BM, Prati C, Duarte MAH, Bramante CM, Gandolfi MG. Physicochemical properties of calcium silicate-based formulations MTA Repair HP and MTA Vitalcem. *J Appl Oral Sci*. 2018;26:e2017115.
32. Jimenez-Sanchez MDC, Segura-Egea JJ, Diaz-Cuenca A. Higher hydration performance and bioactive response of the new endodontic bioactive cement MTA HP repair compared with ProRoot MTA white and NeoMTA plus. *J Biomed Mater Res B Appl Biomater*. 2019;107(6):2109-20.
33. Harrington GW, Natkin E. External resorption associated with bleaching of pulpless teeth. *J Endod*. 1979;5(11):344-8.
34. Vosoughhosseini S, Lotfi M, Shahmoradi K, Saghiri MA, Zand V, Mehdipour M, Ranjkesh B, Mokhtari H, Salemmilani A, Doosti S. Microleakage comparison of glass-ionomer and white mineral trioxide aggregate used as a coronal barrier in nonvital bleaching. *Med Oral Patol Oral Cir Bucal*. 2011;16(7):e1017-21.
35. D'Mello G, Moloney L. Management of coronal discoloration following a regenerative endodontic procedure in a maxillary incisor. *Aust Dent J*. 2017;62(1):111-6.
36. Belobrov I, Parashos P. Treatment of tooth discoloration after the use of white mineral trioxide aggregate. *J Endod*. 2011;37(7):1017-20.
37. Jang JH, Kang M, Ahn S, Kim S, Kim W, Kim Y, Kim E. Tooth discoloration after the use of new pozzolan cement (Endocem) and mineral trioxide aggregate and the effects of internal bleaching. *J Endod*. 2013;39(12):1598-602.

38. Timmerman A, Parashos P. Bleaching of a Discolored Tooth with Retrieval of Remnants after Successful Regenerative Endodontics. *J Endod.* 2018;44(1):93-7.
39. Rotstein I, Friedman S. pH variation among materials used for intracoronar bleaching. *J Endod.* 1991;17(8):376-9.
40. Mourouzis P, Koulaouzidou EA, Helvatjoglu-Antoniades MJQI. Effect of in-office bleaching agents on physical properties of dental composite resins. 2013;44(4).
41. Keskin C, Sariyilmaz E, Kele SA. The effect of bleaching agents on the compressive strength of calcium silicate-based materials. *Aust Endod J.* 2018.
42. Tsujimoto M, Ookubo A, Wada Y, Matsunaga T, Tsujimoto Y, Hayashi Y. Surface changes of mineral trioxide aggregate after the application of bleaching agents: electron microscopy and an energy-dispersive X-ray microanalysis. *J Endod.* 2011;37(2):231-4.
43. Attin T, Hannig C, Wiegand A, Attin R. Effect of bleaching on restorative materials and restorations--a systematic review. *Dent Mater.* 2004;20(9):852-61.
44. Camilleri J, Cutajar A, Mallia B. Hydration characteristics of zirconium oxide replaced Portland cement for use as a root-end filling material. *Dent Mater.* 2011;27(8):845-54.
45. Hetzner DWJM, Microanalysis. Microindentation hardness testing of materials using ASTM e384. 2003;9(S02):708-9.
46. Bolhari B, Nekoofar MH, Sharifian M, Ghabrai S, Meraji N, Dummer PM. Acid and microhardness of mineral trioxide aggregate and mineral trioxide aggregate-like materials. *J Endod.* 2014;40(3):432-5.
47. Gokay O, Ziraman F, Cali Asal A, Saka OM. Radicular peroxide penetration from carbamide peroxide gels during intracoronar bleaching. *Int Endod J.* 2008;41(7):556-60.
48. Lou EK, Cathro P, Marino V, Damiani F, Heithersay GS. Evaluation of Hydroxyl Radical Diffusion and Acidified Thiourea as a Scavenger during Intracoronar Bleaching. *J Endod.* 2016;42(7):1126-30.
49. Natale LC, Rodrigues MC, Xavier TA, Simoes A, de Souza DN, Braga RR. Ion release and mechanical properties of calcium silicate and calcium hydroxide materials used for pulp capping. *Int Endod J.* 2015;48(1):89-94.
50. Smith DC, Ruse ND. Acidity of glass ionomer cements during setting and its relation to pulp sensitivity. *J Am Dent Assoc.* 1986;112(5):654-7.
51. Kucukkaya Eren S, Aksel H, Uyanik O, Nagas E. Effect of Various Bleaching Agents on the Surface Composition and Bond Strength of a Calcium Silicate-based Cement. *Oper Dent.* 2018;43(6):613-8.
52. Samiei M, Janani M, Vahdati A, Alemzadeh Y, Bahari M. Scanning Electron Microscopy and Energy-Dispersive X-Ray Microanalysis of Set CEM Cement after Application of Different Bleaching Agents. *Iran Endod J.* 2017;12(2):191-5.
53. Mohebbi P, Asgary S. Effect of pH on physical properties of two endodontic biomaterials. *J Conserv Dent.* 2016;19(3):212-9.
54. Serin Kalay T. Effects of intracoronar bleaching agents on the surface properties of mineral trioxide aggregate. *Odontology.* 2019;107(4):465-72.
55. Meraji N, Camilleri J. Bonding over Dentin Replacement Materials. *J Endod.* 2017;43(8):1343-9.

Please cite this paper as: Ahmadi E, Meraji N. The Effect of Bleaching Agents on the Microstructure and Surface Microhardness of Three Calcium Silicate-based Barrier Materials. *Iran Endod J.* 2020;15(1): 23-30. Doi: 10.22037/iej.v15i1.25345.