Acid and Microhardness of Mineral Trioxide Aggregate and Mineral Trioxide Aggregate–like Materials

Behnam Bolhari, DDS, MSc, DoIBoE,[*†]
Mohammad Hossein Nekoofar, DDS, MSc, DoIBoE, PhD,[*†‡]
Mohammadreza Sharifian, DDS, MSc, DoIBoE,[*†] *Sholeh Ghabrai, DDS, MSc, DoIBoE,*[*†]
Naghmeh Meraji, DDS, MSc, DoIBoE,[*†]
and Paul M.H. Dummer, BDS, MScD, PhD, DDSc, FDSRCS (Ed)[‡]

Abstract

Introduction: The aim of this study was to compare the surface microhardness of BioAggregate, ProRoot MTA, and CEM Cement when exposed to an acidic environment or phosphate-buffered saline (PBS) as a synthetic tissue fluid. **Methods:** Ninety cylindrical molds made of polymethyl methacrylate with an internal diameter of 6 mm and height of 4 mm (according to ASTM E384 standard for microhardness tests) were fabricated and filled with BioAggregate (n = 30), tooth-colored ProRoot MTA (n = 30), or CEM Cement (n = 30). Each group was then divided into 3 subgroups of 10 specimens consisting of those exposed to distilled water, exposed to PBS (pH = 7.4), or exposed to butyric acid (pH = 5.4). After 1 week the Vickers surface microhardness test was performed. Statistical analysis included 2-way analysis of variance, followed by post hoc Dunnett T3 in cases with lack of homoscedasticity and Tukey honestly significant difference in cases with homoscedasticity. **Results:** The indentations obtained from the CEM Cement specimens exposed to an acidic pH were not readable because of incomplete setting. There was a significant difference between the microhardness of the materials regardless of the environmental conditions ($P < .001$). In all the environmental conditions, MTA had significantly higher and CEM Cement had significantly lower microhardness values ($P < .001$). All experimental cements had significantly higher microhardness values when exposed to PBS ($P < .001$) and had significantly lower microhardness values when exposed to butyric acid ($P < .001$). **Conclusions:** The surface microhardness of BioAggregate, ProRoot MTA, and CEM Cement was reduced significantly by exposure to butyric acid and increased significantly by exposure to PBS. In all environmental conditions, MTA had significantly higher microhardness values. *(J Endod 2014;40:432–435)*

Key Words

BioAggregate, butyric acid, CEM cement, microhardness, MTA, ProRoot MTA

The outcomes of clinical restorative procedures are influenced by the chemical and physical properties of the materials used (1). The physical properties of dental materials are influenced by several factors such as storage temperature (2), powder-to-liquid ratio (3), clinical conditions (4), condensation pressure (5), and placement technique (6).

Mineral trioxide aggregate (MTA) is used extensively in a variety of challenging endodontic therapies such as root canal treatment of immature teeth with open apices and repair of root perforations (7). Despite the various beneficial properties of MTA such as biocompatibility (8), sealing ability (7, 9), antibacterial (10), and bioactive effects (11, 12), it has a few disadvantages including difficult handling (13) and long setting time (14). Furthermore, it has been well-documented that the properties of MTA are influenced by environmental conditions (4). For example, in the presence of periradicular inflammation, MTA may be exposed to an acidic environment (15), which has a negative impact on various physical properties such as porosity, microhardness (16), sealing ability (17), and push-out bond strength (18).

BioAggregate (Innovative Bioceramix, Vancouver, BC, Canada), a white ceramic cement, is composed primarily of calcium silicate, calcium hydroxide, and hydroxyapatite and is free of aluminum and bismuth (19). BioAggregate has been shown to be biocompatible (20) and have good sealing properties when tested in a glucose leakage experimental model (21). Furthermore, it displays a similar antibacterial effect against *Enterococcus faecalis* to that of MTA (22). Not surprisingly, because of its similar biological properties, the manufacturer has recommended it for use in the same clinical situations as MTA.

CEM Cement (Yektazdandan; Bionique Dent, Tehran, Iran) is another calcium silicate–based, MTA-like hydraulic cement (23). It has been reported to be biocompatible (23), with good handling characteristics (24) that can prevent bacterial leakage and form an effective seal (24). CEM Cement has also been suggested for use in the same clinical applications as MTA (24).

In various clinical applications such as repair of root perforation, Bioaggregate, MTA, and CEM Cement are often applied in contact with tissue fluids such as serum and blood. The adjacent tissue fluid may have a normal pH or might have lower pH levels because of infection and inflammation (15). On the hand, if the inflammatory process in

From the *Dental Research Center, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran; †Department of Endodontics, School of Dentistry, Tehran University of Medical Sciences, Tehran, Iran; and ‡Endodontology Research Group, School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom.

Address requests for reprints to Dr Mohammad Hossein Nekoofar, Endodontology Research Group, School of Dentistry, College of Biomedical and Life Sciences, Cardiff University, Cardiff CF14 4XY, United Kingdom. E-mail address: nekoofarmh@cf.ac.uk
0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2013.10.014

the adjacent tissue is controlled by endodontic treatment, the pH will return to slightly alkaline (pH = 7.4) within 7 days (4) or less (16). Therefore, during the setting process the surface of materials may be exposed to acidic or slightly alkaline pH levels.

In this study to simulate infectious and normal *in vivo* conditions, butyric acid and phosphate-buffered saline (PBS) have been used. Butyric acid is one of the by-products of the metabolism of anaerobic bacteria, the dominant bacteria in endodontic infections (25). Therefore, to simulate infectious situations in laboratory studies the use of butyric acid has been suggested (16, 18). PBS is a simulated tissue fluid containing phosphate (26) that can be used for the purpose of mimicking normal *in vivo* conditions in laboratory studies (4, 27).

The aim of this study was to compare the surface microhardness of Bioaggregate (batch# VRO 1004-102), tooth-colored ProRoot MTA (batch #11004158; DENTSPLY Tulsa Dental, Tulsa, OK), and CEM Cement (batch #C100501) in an acidic environment and/or PBS. The null hypothesis was that exposure to an acidic environment will not affect the surface microhardness of these materials, an indicator of the progress of the hydration and setting process (4), and that exposure to PBS will have no effect on surface microhardness.

Materials and Methods

Ninety cylindrical molds made of polymethyl methacrylate with an internal diameter of 6 mm and height of 4 mm (according to ASTM E384 Standard for microhardness tests) were fabricated by CNC laser cutting (LaserProI; GCC, New Taipei City, Taiwan).

Bioaggregate, tooth-colored ProRoot MTA, and CEM Cement were prepared by mixing 1 g powder with 0.33 mL associated liquid of each material supplied by the manufacturer.

Each group of 30 specimens was divided into 3 subgroups according to the environmental condition: (1) exposure to distilled water, (2) exposure to PBS (pH = 7.4) (Merck, Darmstadt, Germany), or (3) exposure to 1 mmol/L butyric acid (pH = 5.4) (Merck).

Before cement placement, each mold was filled with distilled water, PBS, or butyric acid according to the specific subgroup, and then the liquid was aspirated after 20 seconds. The molds were then filled with the prepared cements by using minimal pressure (28) and wrapped in gauze soaked with distilled water, PBS, or butyric acid on the top and bottom of the samples according to the subgroup and incubated at 37°C in 100% relative humidity for 1 week.

In a pilot study, it was observed that the pH level of the gauze covering the samples changed after 14 hours; therefore, the pieces of gauze were replaced every 12 hours after checking the pH levels (pH strip Panpeha; Sigma-Aldrich, Munich, Germany).

The samples were then polished by 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.

The surface microhardness test was performed by using a Vickers Tester (Bareiss Prufgeratebau GmbH, Oberdischingen, Germany) with a pyramidal diamond indenter by using a load of 300 g for 10 seconds. According to the pilot study, this load created a clear and reliable indent in all 3 materials. Five indents were made on the polished surface of each sample at separate locations with a 2.5× d (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.

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

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

Statistical analysis included 2-way analysis of variance, followed by post hoc Dunnett T3 in cases with lack of homoscedasticity and Tukey honestly significant difference in cases with homoscedasticity.

Results

The results are summarized in Table 1 and Figure 1. Because of lack of hardening of the CEM Cement specimens exposed to butyric acid, the indentations obtained were not readable.

There was a significant difference between the microhardness of the materials regardless of the environmental conditions ($P < .001$). In all the environmental conditions, MTA had significantly higher microhardness values, followed by BioAggregate and CEM Cement ($P < .001$).

All specimens exposed to PBS had significantly higher microhardness values than those exposed to distilled water and butyric acid ($P < .001$), and all specimens exposed to butyric acid had significantly lower microhardness values than those exposed to PBS and distilled water ($P < .001$).

Discussion

Microhardness testing is based on evaluating the resistance of materials to deformation (29). This property is influenced by several fundamental properties of materials such as tensile strength, modulus of elasticity (29), and the stability of their crystal structure (29); it has an inverse relationship with porosity (2). Microhardness tests are used for evaluating the quality and progression of the hydration process and as an indicator of the setting process (4, 16).

In this study the effects of acidic pH and PBS on the surface microhardness of ProRoot MTA and 2 MTA-like materials were

TABLE 1. Mean, Minimum, and Maximum Microhardness Values of Each Group

	Vickers microhardness								
	Distilled water			PBS (pH = 7.4)			Butyric acid (pH = 5.4)		
Material	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
MTA	83.5 ± 10.2	60.7	108.8	94.1 ± 11.6	76.0	122.8	56.7 ± 8.9	42.9	73.8
Bioaggregate	28.1 ± 4.4	20.8	37.8	35.4 ± 4.3	23.6	46.2	8.9 ± 1.4	6.4	11.9
CEM Cement	6.0 ± 1.1	3.6	9.0	9.2 ± 3.6	1.0	3.1	0	0	0

Differences between all groups were significant ($P < .001$).

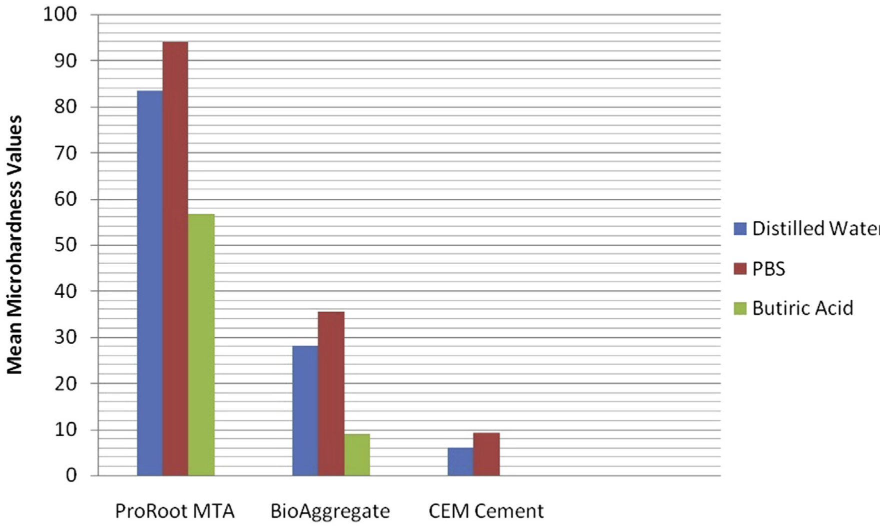

Figure 1. Mean surface microhardness values of BioAggregate, ProRoot MTA, and CEM Cement when exposed to distilled water, PBS, and butyric acid. Differences between all groups were significant ($P < .001$).

evaluated along with a control group at neutral pH (water). PBS (pH = 7.4) and butyric acid (pH = 5.4) were applied to simulate the environmental conditions that these cements may be exposed to. The surface microhardness of Bioaggregate, ProRootMTA, and CEM Cement was greater when exposed to PBS. Studies using x-ray diffraction and scanning electron microscopy have revealed that when Bioaggregate, ProRootMTA, and CEM Cement are exposed to phosphate-containing solutions, carbonated apatite crystals, also known as biologic apatite, are formed on their surfaces (27, 30, 31). These crystals represent the mineral constitute of hard tissues such as bone, dentin, and cementum (31); therefore, the increase in microhardness values after exposure to PBS may be due to the formation of these carbonated apatite crystals. Therefore, for future laboratory studies, storage of calcium silicate–based cements in PBS to ensure optimum hydration could be advantageous.

In the present study the exposure of the cements to an acidic environment decreased their microhardness values (Fig. 1). These results are consistent with previous studies (4, 16) and reflect the inverse effect of an acidic environment on the hydration process of these hydraulic cements.

As a result of exposure to acidic pH and in accordance with the findings of Namazikhah et al (16), surface microhardness values of the cements in the present study were significantly lower. The lack of needle-like ettringite (hydrated calcium-aluminum-sulfate) crystals on the surface of ProRoot MTA as a result of exposure to acidic pH (4, 32) and increase in porosity (16) have been suggested as reasons of decreased surface microhardness.

According to the results of the current study, BioAggregate had lower microhardness values compared with MTA. This cement, unlike MTA, does not contain aluminate (19). The addition of aluminate to silicate-based cements such as MTA improves their hardening (33). Furthermore, ettringite crystals form as a result of the reaction of aluminate and sulfate phases during the hydration process (34); therefore, BioAggregate is likely to lack ettringite crystals, and this may explain the reason why it had lower microhardness values in comparison with MTA.

CEM Cement had significantly lower microhardness values among the materials. This may be due to the specific chemical composition of this cement (24, 35), which requires more research.

Conclusion

Exposure to an acidic environment had an adverse effect on the surface microhardness of BioAggregate, ProRoot MTA, and CEM Cement, whereas exposure to PBS significantly increased their surface microhardness values. Among the 3 materials evaluated, ProRoot MTA had the highest microhardness values. Therefore, use of ProRoot MTA when environmental conditions suggest an acidic pH may be preferred.

Acknowledgments

Supported by a grant from Tehran University of Medical Sciences and Health Services (grant no. 17714).

The authors deny any conflicts of interest related to this study.

References

1. Main C, Mirzayan N, Shabahang S, Torabinejad M. Repair of root perforations using mineral trioxide aggregate: a long-term study. J Endod 2004;30:80–3.
2. Saghiri MA, Lotfi M, Joupari MD, et al. Effects of storage temperature on surface hardness, microstructure, and phase formation of white mineral trioxide aggregate. J Endod 2010;36:1414–8.
3. Bentz DP. Influence of water-to-cement ratio on hydration kinetics: simple models based on spatial considerations. Cement Concrete Res 2006;36:238–44.
4. Lee YL, Lee BS, Lin FH, et al. Effects of physiological environments on the hydration behavior of mineral trioxide aggregate. Biomaterials 2004;25:787–93.
5. Nekoofar M, Adusei G, Sheykhrezae M, et al. The effect of condensation pressure on selected physical properties of mineral trioxide aggregate. Int Endod J 2007;40:453–61.
6. Basturk FB, Nekoofar MH, Günday M, Dummer PM. The effect of various mixing and placement techniques on the compressive strength of mineral trioxide aggregate. J Endod 2013;39:111–4.
7. Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review—part II: leakage and biocompatibility investigations. J Endod 2010;36:190–202.
8. Koh ET, McDonald F, Pitt Ford TR, Torabinejad M. Cellular response to mineral trioxide aggregate. J Endod 1998;24:543–7.
9. Pelliccioni GA, Vellani CP, Gatto MR, et al. Proroot mineral trioxide aggregate cement used as a retrograde filling without addition of water: an *in vitro* evaluation of its microleakage. J Endod 2007;33:1082–5.
10. Al-Hezaimi K, Al-Shalan TA, Naghshbandi J, et al. Antibacterial effect of two mineral trioxide aggregate (MTA) preparations against *Enterococcus faecalis* and *Streptococcus sanguis in vitro*. J Endod 2006;32:1053–6.

11. Maeda H, Nakano T, Tomokiyo A, et al. Mineral trioxide aggregate induces bone morphogenetic protein-2 expression and calcification in human periodontal ligament cells. J Endod 2010;36:647–52.
12. Gandolfi MG, Taddei P, Tinti A, Prati C. Apatite-forming ability (bioactivity) of ProRoot MTA. Int Endod J 2010;43:917–29.
13. Johnson BR. Considerations in the selection of a root-end filling material. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 1999;87:398–404.
14. Dammaschke T, Gerth HU, Zuchner H, Schafer E. Chemical and physical surface and bulk material characterization of white ProRoot MTA and two Portland cements. Dent Mater 2005;21:731–8.
15. Nekoofar MH, Namazikhah MS, Sheykhrezae MS, et al. pH of pus collected from periapical abscesses. Int Endod J 2009;42:534–8.
16. Namazikhah MS, Nekoofar MH, Sheykhrezae MS, et al. The effect of pH on surface hardness and microstructure of mineral trioxide aggregate. Int Endod J 2008;41: 108–16.
17. Saghiri MA, Lotfi M, Saghiri AM, et al. Effect of pH on sealing ability of white mineral trioxide aggregate as a root-end filling material. J Endod 2008;34:1226–9.
18. Shokouhinejad N, Nekoofar MH, Iravani A, et al. Effect of acidic environment on the push-out bond strength of mineral trioxide aggregate. J Endod 2010;36: 871–4.
19. Park JW, Hong SH, Kim JH, et al. X-Ray diffraction analysis of white ProRoot MTA and Diadent BioAggregate. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109: 155–8.
20. De-Deus G, Canabarro A, Alves G, et al. Optimal cytocompatibility of a bioceramic nanoparticulate cement in primary human mesenchymal cells. J Endod 2009;35: 1387–90.
21. Leal F, De-Deus G, Brandao C, et al. Comparison of the root-end seal provided by bioceramic repair cements and White MTA. Int Endod J 2011;44:662–8.
22. Zhang H, Pappen FG, Haapasalo M. Dentin enhances the antibacterial effect of mineral trioxide aggregate and bioaggregate. J Endod 2009;35:221–4.
23. Mozayeni MA, Milani AS, Marvasti LA, Asgary S. Cytotoxicity of calcium enriched mixture cement compared with mineral trioxide aggregate and intermediate restorative material. Aust Endod J 2012;38:70–5.
24. Asgary S, Shahabi S, Jafarzadeh T, et al. The properties of a new endodontic material. J Endod 2008;34:990–3.
25. Eftimiadi C, Buzzi E, Tonetti M, et al. Short-chain fatty acids produced by anaerobic bacteria alter the physiological responses of human neutrophils to chemotactic peptide. J Infect 1987;14:43–53.
26. Marques MRC, Loebenberg R, Almukainzi M. Simulated biological fluids with possible application in dissolution testing. Dissolution Technol 2011;18:15–28.
27. Shokouhinejad N, Nekoofar MH, Razmi H, et al. Bioactivity of EndoSequence root repair material and Bioaggregate. Int Endod J 2012;45:1127–34.
28. Nekoofar MH, Stone DF, Dummer PM. The effect of blood contamination on the compressive strength and surface microstructure of mineral trioxide aggregate. Int Endod J 2010;43:782–91.
29. Chandler H. *Hardness Testing*, 2nd ed. Materials Park, OH: ASM International; 1999:1–13.
30. Parirokh M, Asgary S, Eghbal MJ, et al. The long-term effect of saline and phosphate buffer solution on MTA: an SEM and EPMA investigation. Iran Endod J 2007;2:81–6.
31. Tay FR, Pashley DH, Rueggeberg FA, et al. Calcium phosphate phase transformation produced by the interaction of the portland cement component of white mineral trioxide aggregate with a phosphate-containing fluid. J Endod 2007;33:1347–51.
32. Kayahan MB, Nekoofar MH, Kazandag M, et al. Effect of acid-etching procedure on selected physical properties of mineral trioxide aggregate. Int Endod J 2009;42: 1004–14.
33. Andersen MD, Jakobsen HJ, Skibsted J. Characterization of white Portland cement hydration and the CSH structure in the presence of sodium aluminate by ^{27}Al and ^{29}Si MAS NMR spectroscopy. Cement Concrete Res 2004;34:857–68.
34. Gandolfi MG, Van Landuyt K, Taddei P, et al. Environmental scanning electron microscopy connected with energy dispersive x-ray analysis and Raman techniques to study ProRoot mineral trioxide aggregate and calcium silicate cements in wet conditions and in real time. J Endod 2010;36:851–7.
35. Asgary S, Eghbal MJ, Parirokh M, et al. Comparison of mineral trioxide aggregate's composition with Portland cements and a new endodontic cement. J Endod 2009; 35:243–50.