

Comparative evaluation of sealing ability and microstructure of MTA and Biodentine after exposure to different environments

Anastasia Agrafioti¹ · Nestor Tzimpoulas² · Elias Chatzitheodoridis³ · Evangelos G. Kontakiotis¹

Received: 27 May 2015 / Accepted: 23 October 2015 / Published online: 6 November 2015
© Springer-Verlag Berlin Heidelberg 2015

Abstract

Objectives The aim of this study was to evaluate the sealing ability and morphological microstructure of Biodentine in comparison to ProRoot mineral trioxide aggregate (MTA) after storage in an acidic environment.

Materials and methods Biodentine and ProRoot MTA were prepared and packed into the canal lumen of dentin disks. Twenty specimens of each material were further randomly divided into two groups according to the storage media: group A: materials with saline as storage medium; group B: materials with citric acid buffered at pH 5.4 as storage medium. The sealing ability was evaluated at 1, 3, 6, and 24 h and 1 or 3 months, using a fluid transport model for quantitative

analysis of endodontic microleakage. The morphological microstructures of the materials were also evaluated using scanning electron microscopy.

Results During the first 24 h, MTA showed greater fluid transport values than Biodentine in both environments. At the 3-month measurement, when the materials were stored in saline, MTA showed greater ability to prevent fluid movement than Biodentine ($p < 0.0001$). However, when the materials were stored in an acidic environment, no statistical significant difference was found after 3 months. After storage in saline, both materials showed an uneven crystalline surface with similar hexagonal crystals. The microstructure of Biodentine changed after exposure to citric acid, showing a relatively smooth surface with more spheroidal crystals.

Conclusions The exposure to an acidic environment, within the limits of this study, seems to result in morphological changes of Biodentine in a different manner than MTA. MTA shows good ability to prevent fluid movement over time, in both environments. The ability of Biodentine to prevent fluid movement over time was enhanced in the acidic environment.

Clinical relevance The findings of the present study could imply that both materials are indicated for use in an acidic environment.

✉ Evangelos G. Kontakiotis
ekontak@dent.uoa.gr

Anastasia Agrafioti
agrafiot@dent.uoa.gr

Nestor Tzimpoulas
n.tzimpoulas@acta.nl

Elias Chatzitheodoridis
eliasch@metal.ntua.gr

¹ Department of Endodontics, School of Dentistry, National and Kapodistrian University of Athens, 2 Thivon Str., 11527 Goudi, Athens, Greece

² Division of Endodontology, Department of Cariology, Endodontology and Pedodontology, Academic Centre for Dentistry Amsterdam, University of Amsterdam and VU University, Amsterdam, The Netherlands

³ Laboratory of Mineralogy, Petrology and Economic Geology, Department of Geological Sciences, School of Mining and Metallurgical Engineering, National Technical University of Athens, Athens, Greece

Keywords Acidic environment · Biodentine · MTA · Microstructure · Sealing ability

Introduction

Mineral trioxide aggregate (MTA) has attracted considerable attention because of its excellent biocompatibility, sealing ability, and antimicrobial properties [1, 2]. Although it was initially introduced as a material for repair of root perforations

[3], today it is used in vital pulp therapy [4, 5], as a root-end filling material [6], as an apical plug in open apex non-vital teeth [7, 8], for the treatment of dentine hypersensitivity [9], and in regenerative endodontic therapy [10, 11]. However, there are also some disadvantages associated with the use of MTA including long setting time, difficult handling, possibility of staining of tooth structure, and high cost [1, 12, 13]. Recently, a new calcium silicate-based restorative cement, Biodentine (Septodont, Saint Maur des Fosses, France), was introduced. It is a fast-setting restorative material recommended as a dentin substitute that can be used in the same applications as MTA [14].

An important factor for successful endodontic treatment is the sealing ability of the filling material [15]. An ideal material should provide a tight seal. The sealing ability of MTA was investigated in different leakage studies in vitro, using dye or fluid transport methods [6, 16–24]. However, the microleakage of Biodentine has not been adequately evaluated.

The environment in which a material is maintained could affect the marginal adaptation with dentin, the microleakage, as well as the microstructure and surface morphology of the material [25–27]. It has been reported that variations in the pH of host tissues, because of pre-existing inflammation at the time of placement, could affect the physical and chemical properties of these materials [28–31]. It has also been pointed out that surface hardness of MTA was impaired in an acidic environment [30]. Another study reported that MTA surfaces exhibited higher surface roughness in the presence of an acidic environment [32]. However, there are limited data about the effect of low pH on Biodentine [33].

The aim of this study was to evaluate the sealing ability of Biodentine in comparison to MTA, after exposure to an acidic environment, with the use of a fluid transport model for quantitative analysis of microleakage. In addition, scanning electron microscopy (SEM) was performed in order to investigate the morphological microstructure of the materials in the presence of an acidic environment.

Materials and methods

Specimens' preparation

Dentin disks from anterior bovine teeth were horizontally sectioned into 3-mm-thick slices, and the canal space of each dentin slice was enlarged to 2.6 mm in diameter, using a standardized cylindrical bur in a drilling machine with standardized vertical motion. Biodentine (Septodont, Saint Maur des Fosses, France) and white ProRoot MTA (Dentsply Tulsa Dental Specialties, Memphis, TN) were prepared according to the manufacturers' instructions and compacted into the lumen of the dentin disks, using, for Biodentine, the plastic filling instrument provided by the manufacturer and, for

MTA, a BL-S Kondenser 60/120 (B&L Biotech USA, Inc.) and paper points. Twenty specimens of each material were further divided into two groups according to the storage media: group A: materials with saline (Pharmex S.A. Pharmaceuticals, Greece) as storage medium; group B: materials with citric acid buffered at pH 5.4 as storage medium. The preparation of citric acid was carried out at the Laboratory of Mineralogy, Petrology and Economic Geology, School of Mining and Metallurgical Engineering, National Technical University of Athens. The storage of the specimens was for 24 h in contact with a saline- or citric acid-soaked piece of gauze in 37 °C, before the first fluid movement measurement.

In the positive control group ($n=5$), no material was used. In the negative control group ($n=5$), a 3-mm-thick MTA apical plug was placed and then the entire surface was covered by two coats of nail varnish.

Fluid movement measurements

The specimens were fitted in the fluid transport device and measurements were recorded at 1, 3, 6, and 24 h and 1 or 3 months, by the method described previously by Wu et al. [18]. The root specimens were attached to a plastic tube and the connections were coated with composite resin. A second rubber tube was attached to this plastic tube, and the tube was filled with deionized water on either side of the specimen. The end of the system was connected to a 20- μ L glass capillary tube 170 mm long (Haak, Waller-Graf & Co., Werlheim, Germany). All connections were closed tightly by twisting pieces of stainless steel wire. An air bubble was introduced through the open end of the glass capillary. Finally, a headspace pressure of 60 kPa (0.6 atm) was applied. The same examiner assessed visually the movement of the air bubble. The fluid movement was measured using the fluid transport model, and the fluid transport results (F , Flow) were expressed in microliter per hour and divided into three categories: (1) $F=0$ μ L/h (no fluid movement—NF), (2) $0<F<20$ μ L/h (slight fluid movement—SF), and (3) $F>20$ μ L/h (gross fluid movement—GF).

Following the assessment at 24 h, the specimens were detached from the measuring apparatus and they were kept in 37 °C and 100 % humidity between measurements.

Scanning electron microscope analysis

For the morphological evaluation, five new specimens for each group were prepared as described above and kept under the same storage conditions. The surface structural organization of each specimen was examined through a scanning electron microscopy (SEM) approach. Samples were attached on aluminum stubs wet and uncoated and were immediately analyzed in low-vacuum mode (between 1 to 30 Pa) to minimize any changes caused to the sample materials, such as stress induced by the quick loss of humidity from the samples.

Table 1 Number of root sections by material-storage combination and time point

Material-storage	Number of root sections																	
	t_1 (1 h)			t_2 (3 h)			t_3 (6 h)			t_4 (24 h)			t_5 (1 month)			t_6 (3 months)		
	NF	SF	GF	NF	SF	GF	NF	SF	GF	NF	SF	GF	NF	SF	GF	NF	SF	GF
Biodentine-saline	10	0	0	8	0	2	8	0	2	6	2	2	2	2	6	0	4	6
MTA-saline	2	2	6	2	2	6	0	6	4	0	6	4	6	2	2	6	4	0
Biodentine-citric acid	8	0	2	6	2	2	6	0	4	6	0	4	0	4	6	6	0	4
MTA-citric acid	0	4	6	0	4	6	0	4	6	0	6	4	4	4	2	4	4	2

NF no fluid movement, SF slight fluid movement, GF gross fluid movement

Low vacuum was also used to eliminate charging after the samples have dried. The scanning electron microscope is a Jeol 6380LV (JEOL Ltd., USA), which was operated at 15 kV, a working distance of 20 mm, and a beam current of about 1 nA. The specimens were examined systematically and representative images of each area were obtained.

Statistical analysis

The Kruskal-Wallis test was used to investigate the impact of each material-storage combination fluid transport values versus time assessment. Additionally, post hoc analysis with the use of Mann-Whitney *U* test was applied for the evaluation of the differences in transport fluid values in the different groups of the study. The Friedman test was also used for comparison between the repeated fluid transport measures. Also, post hoc analysis with the use of Wilcoxon signed rank test was performed for assessing the differences between time points. All reported probability values (*p* values) were compared to a significant level of 5 %. The analyses of coded data were carried out using IBM SPSS software version 21.0.

Results

Fluid movement measurements

The fluid transport results of the materials/storage combination at the different time intervals are shown in Table 1. At each time interval, NF was recorded in the negative control

group, whereas in the positive control group, GF was recorded.

Up to the 24-h measurement, MTA stored in saline as well as in citric acid showed greater fluid transport values than Biodentine (Table 2). However, at 24 h, the difference in the fluid transport values of both materials was not statistically significant in either storage medium (Table 2).

At the 3-month measurement, the MTA stored in saline showed statistically significant greater ability to prevent fluid movement than Biodentine in the same storage ($p < 0.0001$). However, when materials were stored in an acidic environment, no statistical significant difference was found at the 3-month measurement (Table 2).

Biodentine stored in saline leaked more over time ($p < 0.008$), whereas the fluid movement values of MTA stored in saline were statistically significantly lower at the 3-month interval when compared to the 24-h measurement ($p < 0.015$) (Table 3).

When the materials were stored in an acidic environment, no statistical significant differences were found regarding their ability to prevent fluid movement between the 24-h and the 3-month measurements (Table 2).

SEM analysis

Representative surfaces of freshly mixed Biodentine and MTA, as well as after storage in different environments, are shown in Fig. 1. Freshly mixed Biodentine showed small and more developed hexagonal crystal structure compared to freshly mixed MTA (Fig. 1a, c). Both materials, after storage

Table 2 Fluid transport measures multiple comparisons between different material-storage combinations for each time point

	t_1 (1 h)	t_2 (3 h)	t_3 (6 h)	t_4 (24 h)	t_5 (1 month)	t_6 (3 months)
Biodentine-saline vs. MTA-saline	$p < 0.002$	$p < 0.035$	$p < 0.009$	NS	NS	$p < 0.0001$
Biodentine-saline vs. biodentine-citric acid	NS	NS	NS	NS	NS	NS
MTA-saline vs. MTA-citric acid	NS	NS	NS	NS	NS	NS
Biodentine-citric acid vs. MTA-citric acid	$p < 0.005$	$p < 0.015$	NS	NS	$p < 0.023$	NS

Kruskal-Wallis test, post hoc Mann-Whitney *U* tests, $p < 0.05$

NS not significant difference

Table 3 Fluid transport repeated measures multiple comparisons between time points for each material-storage combination

	Biodentine-saline	MTA-saline	Biodentine-citric acid	MTA-citric acid
t_4 (24 h) vs. t_5 (1 month)	$p<0.023$	NS	$p<0.023$	NS
t_4 (24 h) vs. t_6 (3 month)	$p<0.008$	$p<0.015$	NS	NS
t_5 (1 month) vs. t_6 (3 month)	NS	NS	$p<0.023$	NS

Friedman's test, post hoc Wilcoxon signed rank tests, $p<0.05$

NS not significant difference

in saline, showed an uneven crystalline surface with similar hexagonal crystals (Fig. 1b, e). MTA stored in citric acid showed similar microstructure as when stored in saline (Fig. 1c). However, Biodentine stored in citric acid showed a relatively smooth surface, which consisted of spheroidal crystals without hexagonal plates. The crystallized structure, which formed after exposure of Biodentine to citric acid, presented a cluster of globular crystalline with round-shaped structure (Fig. 1f).

Discussion

The purpose of this study was to investigate the sealing ability, as well as the microstructure of Biodentine, in comparison to MTA, in two different environments that could mimic clinical situations. Under certain clinical applications, these materials are placed in an environment in which inflammation may be present and the surface of the unset material may be exposed to a low pH [34]. The materials were exposed to citric acid no longer than 24 h, simulating the situation in which the

initiating factors of an inflammatory process are removed by proper treatment.

The methodology used in the present study was the fluid transport model, which has been shown to give reliable quantitative results for the assessment of the sealing ability of calcium silicate-based materials [2, 18]. Dentin disks from anterior bovine teeth were used, as they could be considered an appropriate substitute for human teeth in analogous studies [35]. Although no direct comparison should be made about the sealing ability of the materials in clinical conditions, the fluid transport tests may provide pertinent information about their properties.

After hydration of MTA powder, hydroxyapatite crystals are developed and a hybrid layer between dentin and MTA is formed [36]. The morphology of the hydroxyapatite crystals is related to various factors including the environmental pH [37]. Literature has indicated that lower pH environments could affect various physical and chemical properties of MTA [28–31, 38]. It has been shown that the low pH of the surrounding microenvironment affects the hydration reaction of MTA [39] and that the more acidic the MTA solution during the setting process, the more extensive is its porosity [30].

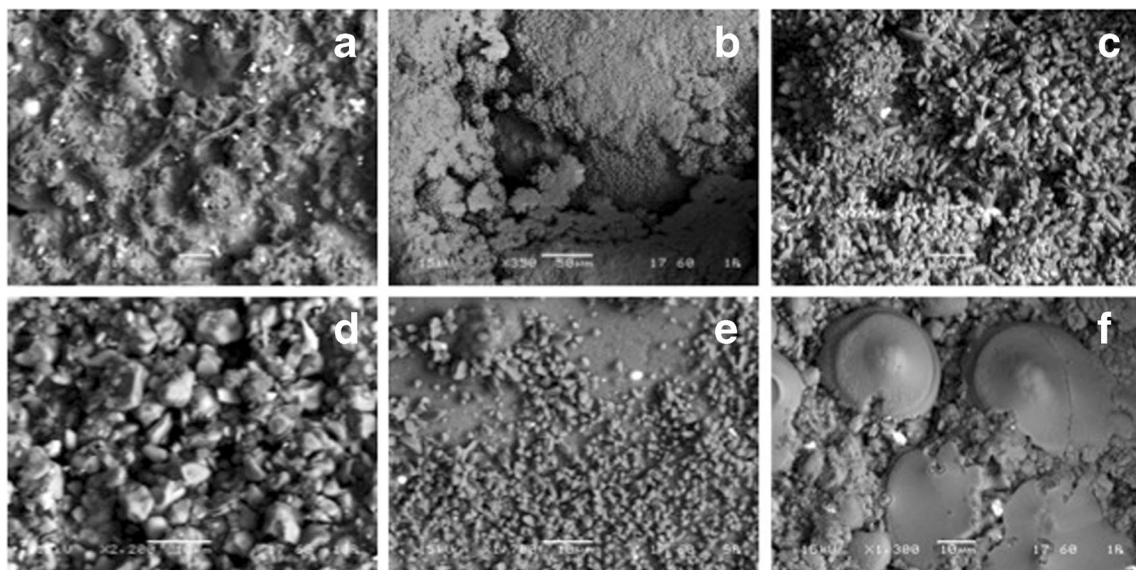


Fig. 1 Representative scanning electron micrographs of materials after storage in different environments. **a** Freshly mixed MTA, **b** MTA stored in saline, **c** MTA stored in citric acid, **d** freshly mixed Biodentine, **e** Biodentine stored in saline, and **f** Biodentine stored in citric acid

Furthermore, Saghiri et al. [31] reported that the time needed for leakage to occur was significantly shorter in samples stored at lower pH values. In both studies [30, 31], specimens were exposed to butyric acid with pH values of 4.4, 5.4, 6.4, and 7.4 for 4 and 3 days, respectively. Lee et al. [39] also reported that MTA crystals could dissolve in an environment of pH 5 and result in an unstable structure. In the present study, the materials were exposed for no longer than 24 h to citric acid buffered at pH 5.4, which probably did not affect the hydration behavior of MTA long term. In addition, various types of acid may have different effects on the physical and chemical properties of cements [40]. Lee et al. [39] did not state the type of acid, and this lack of information could also be one of the reasons for the different findings.

At the 3-month measurement, the MTA stored in saline showed statistically significant greater ability to prevent fluid movement than Biodentine in the same storage ($p < 0.0001$). However, when materials were stored in an acidic environment, no statistical significant difference was found at the 3-month measurement (Table 2). The exposure of Biodentine to the acidic environment enhanced the ability to prevent fluid movement over time (Table 2). These results could be caused by the alterations in the microstructure of Biodentine that were also observed after the exposure of the material to the citric acid (Fig. 1f). Although the precise explanation for these morphologic differences is unknown, the acid etching seems to result in morphologic changes of Biodentine in a different manner than MTA, which are in agreement with the results of Elnaghy [32]. These alterations of the properties of Biodentine after storage in acidic environment should be further studied before advocating the clinical application of Biodentine successively in lower pH values. These in vitro results are an indication about their potential capacity under these experimental conditions.

Conclusion

The exposure to an acidic environment seems to result in morphological changes of Biodentine in a different manner than MTA. MTA shows good ability to prevent fluid movement over time, in both environments. The ability of Biodentine to prevent fluid movement over time was enhanced in the acidic environment. Based on the results of the present study, it is not yet possible to make a clear recommendation about the material to be used in an acidic environment. These in vitro results are an indication about their potential behavior, and further research should be undertaken to establish a correlation between the exposure to an acidic environment and the clinical performance of these materials.

Acknowledgments The authors would like to greatly acknowledge Dr. Hagay Shemesh (Chair, Division of Endodontology, Academic Centre for

Dentistry Amsterdam (ACTA), the Netherlands) for his critical comments that greatly improved the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

1. Parirokh M, Torabinejad M (2010) Mineral trioxide aggregate: a comprehensive literature review—part I: chemical, physical, and antibacterial properties. *J Endod* 36:16–27
2. Torabinejad M, Parirokh M (2010) Mineral trioxide aggregate: a comprehensive literature review—part II: leakage and biocompatibility investigations. *J Endod* 36:190–202
3. Lee SJ, Monsef M, Torabinejad M (1993) Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. *J Endod* 19:541–544
4. Aeinehchi M, Eslami B, Ghanbariha M, Saffar AS (2003) Mineral trioxide aggregate (MTA) and calcium hydroxide as pulp-capping agents in human teeth: a preliminary report. *Int Endod J* 36:225–231
5. Nair PN, Duncan HF, Pitt Ford TR, Luder HU (2008) Histological, ultrastructural and quantitative investigations on the response of healthy human pulps to experimental capping with mineral trioxide aggregate: a randomized controlled trial. *Int Endod J* 41:128–150
6. Torabinejad M, Watson TF, Pitt Ford TR (1993) Sealing ability of a mineral trioxide aggregate when used as a root end filling material. *J Endod* 19:591–595
7. Pace R, Giuliani V, Pini Prato L, Baccetti T, Pagavino G (2007) Apical plug technique using mineral trioxide aggregate: results from a case series. *Int Endod J* 40:478–484
8. Simon S, Rilliard F, Berdal A, Machtou P (2007) The use of mineral trioxide aggregate in one-visit apexification treatment: a prospective study. *Int Endod J* 40:186–197
9. Gandolfi MG, Iacono F, Pirani C, Prati C (2012) The use of calcium-silicate cements to reduce dentine permeability. *Arch Oral Biol* 57:1054–1061
10. Huang GT (2009) Apexification: the beginning of its end. *Int Endod J* 42:855–866
11. Kontakiotis EG, Filippatos CG, Tzanetakakis GN, Agrafioti A (2015) Regenerative endodontic therapy: a data analysis of clinical protocols. *J Endod* 41:146–154
12. Ber BS, Hatton JF, Stewart GP (2007) Chemical modification of ProRoot MTA to improve handling characteristics and decrease setting time. *J Endod* 33:1231–1234
13. Prati C, Gandolfi MG (2015) Calcium silicate bioactive cements: biological perspectives and clinical applications. *Dent Mater* 31:351–370
14. Laurent P, Camps J, De Méo M, Déjou J, About I (2008) Induction of specific cell responses to a Ca(3)SiO(5)-based posterior restorative material. *Dent Mater* 24:1486–1494
15. Ng YL, Mann V, Rahbaran S, Lewsey J, Gulabivala K (2008) Outcome of primary root canal treatment: systematic review of the literature—part 2. Influence of clinical factors. *Int Endod J* 41:6–31

16. Bates CF, Carnes DL, del Rio CE (1996) Longitudinal sealing ability of mineral trioxide aggregate as a root-end filling material. *J Endod* 22:575–578
17. Yatsushiro JD, Baumgartner JC, Tinkle JS (1998) Longitudinal study of the microleakage of two root-end filling materials using a fluid conductive system. *J Endod* 24:716–719
18. Wu MK, Kontakiotis EG, Wesselink PR (1998) Long-term seal provided by some root-end filling materials. *J Endod* 24:557–560
19. Aqrabawi J (2000) Sealing ability of amalgam, super EBA cement, and MTA when used as retrograde filling materials. *Braz Dent J* 188:266–268
20. Roy CO, Heanson BG, Gerrets TF (2010) Effect of an acid environment on leakage of root-end filling materials. *J Endod* 27:7–8
21. Davis JL, Jeanson BG, Davenport WD, Gardiner D (2003) The effect of irrigation with doxycycline or citric acid on leakage and osseous wound healing. *J Endod* 29:31–35
22. Tay KC, Loushine BA, Oxford C, Kapur R, Primus CM, Gutmann JL, Loushine RJ, Pashley DH, Tay FR (2007) In vitro evaluation of a ceramicrorete-based root-end filling material. *J Endod* 33:1438–1443
23. Yildirim T, Oruçoğlu H, Cobankara FK (2008) Long-term evaluation of the influence of smear layer on the apical sealing ability of MTA. *J Endod* 34:1537–1540
24. Camilleri J, Gandolfi MG, Siboni F, Prati C (2011) Dynamic sealing ability of MTA root canal sealer. *Int Endod J* 44:9–20
25. Francisconi LF, Honório HM, Rios D, Magalhães AC, Machado MA, Buzalaf MA (2008) Effect of erosive pH cycling on different restorative materials and on enamel restored with these materials. *Oper Dent* 33:203–208
26. Formosa LM, Mallia B, Bull T, Camilleri J (2012) The microstructure and surface morphology of radiopaque tricalcium silicate cement exposed to different curing conditions. *Dent Mater* 28:584–595
27. Hashem AA, Wanees Amin SA (2012) The effect of acidity on dislodgment resistance of mineral trioxide aggregate and bioaggregate in furcation perforations: an in vitro comparative study. *J Endod* 38:245–249
28. Camilleri J, Pitt Ford TR (2006) Mineral trioxide aggregate: a review of the constituents and biological properties of the material. *Int Endod J* 39:747–754
29. Watts JD, Holt DM, Beeson TJ, Kirkpatrick TC, Rutledge RE (2007) Effects of pH and mixing agents on the temporal setting of tooth-colored and gray mineral trioxide aggregate. *J Endod* 33:970–973
30. Namazikhah MS, Nekoofar MH, Sheykhezadeh MS et al (2008) The effect of pH on surface hardness and microstructure of mineral trioxide aggregate. *Int Endod J* 41:108–116
31. Saghiri MA, Lotfi M, Saghiri AM et al (2008) Effect of pH on sealing ability of white mineral trioxide aggregate as a root-end filling material. *J Endod* 34:1226–1229
32. Smith JB, Loushine RJ, Weller RN et al (2007) Metrologic evaluation of the surface of white MTA after the use of two endodontic irrigants. *J Endod* 33:463–467
33. Elnaghy AM (2014) Influence of acidic environment on properties of biodentine and white mineral trioxide aggregate: a comparative study. *J Endod* 40:953–957
34. Malamed SF (2004) Handbook of local anesthesia, 5th edn. Mosby, St. Louis
35. Reis AF, Giannini M, Kavaguchi A, Soares CJ, Line SR (2004) Comparison of microtensile bond strength to enamel and dentin of human, bovine, and porcine teeth. *J Adhes Dent* 2:117–121
36. Sarkar NK, Caicedo R, Ritwik P et al (2005) Physicochemical basis of the biologic properties of mineral trioxide aggregate. *J Endod* 31:97–100
37. Qu H, Wei M (2008) The effect of temperature and initial pH on biomimetic apatite coating. *J Biomed Mater Res B Appl Biomater* 87:204–212
38. Shokouhinejad N, Nekoofar MH, Irvani A, Kharrazifard MJ, Dummer PM (2010) Effect of acidic environment on the push-out bond strength of mineral trioxide aggregate. *J Endod* 36:871–874
39. Lee YL, Lee BS, Lin FH, Yun Lin A, Lan WH, Lin CP (2004) Effects of physiological environments on the hydration behavior of mineral trioxide aggregate. *Biomaterials* 25:787–793
40. Taylor HFW (1997) Cement chemistry, 2nd edn. Thomas Telford Ltd, London