Journal of Dental Sciences (2017) 12, 107–111

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jds.com

Journal of Dental Sciences

ORIGINAL ARTICLE

# Hard tissue reaction to mineral trioxide aggregate and experimental root-end filling material in guinea pig mandibles

**Ali Akhavan [a], Peter Parashos [b], Sayed Mohammad Razavi [c], Amin Davoudi [d]*, Elham Shadmehr [a,e]**

[a] *Torabinejad Dental Materials Research Center and Department of Endodontics, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran*
[b] *Melbourne Dental School, University of Melbourne, Melbourne, VIC, Australia*
[c] *Dental Implants Research Center and Department of Oral and Maxillofacial Pathology, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran*
[d] *Dental Implants Research Center and Resident of Prosthodontics, Department of Prosthodontics, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran*
[e] *Postgraduate Endodontic Resident, UB Dental School, Buffalo, NY, USA*

Received 6 September 2015; Final revision received 31 August 2016
Available online 7 March 2017

**KEYWORDS**
cold ceramic;
inflammation;
mineral trioxide aggregate;
root-end filling material

**Abstract** *Background/purpose:* Root-end filling materials are used to fill and seal the root apex during periradicular surgery. Mineral trioxide aggregate (MTA) is a widely-used material because of its particular characteristics. Cold ceramic (CC) is an experimental material that has been recently introduced. The purpose of this study was to compare bone tissue response to CC and MTA in an animal model.
*Materials and methods:* Forty-five male guinea pigs (weighing 750–850 g) were anesthetized with 10 mg/kg ketamine HCL and 12 mg/kg xylazine. A triangular incision of around 15 mm was prepared in the posterior site along the symphysis in both right and left sides of the mandible. A 3 mm $\times$ 3 mm diameter cylindrical hole was prepared in each side using a trephine. Two Teflon cylindrical tube applicators were filled with white MTA and CC and inserted into the defects separately. Histopathological evaluation of the specimens was completed after 2 weeks and 12 weeks. The extent of inflammation was recorded and analyzed using the Mann–Whitney *U* test and SPSS software version 12 at a significance level of 0.05.
*Results:* MTA and CC produced moderate and mild hard tissue responses respectively after 2 weeks and 12 weeks. No significant differences were found in the distribution of the responses between the two groups at either time point.

* Corresponding author. Hezarjarib Street, School of Dentistry, Isfahan University of Medical Sciences, Isfahan 8169743493, Iran.
*E-mail address:* Amindvi@yahoo.com (A. Davoudi).

http://dx.doi.org/10.1016/j.jds.2016.11.003

*Conclusion:* Both CC and MTA demonstrated biocompatibility with minor adverse impact on hard tissue and healing recovery after 12 weeks.


## Introduction

Root-end surgery as an approach to produce periradicular tissue healing is generally the final option after failure of conventional root canal therapy and nonsurgical retreatment to retain a tooth. Root-end filling materials are used to fill and seal the root-end cavity after resection of the root apex in periradicular surgery.[1] Desirable features for a root-end filling material are: (1) sealing ability; (2) bonding to dentin; (3) biocompatibility; (4) ability to stimulate healing of the periradicular tissues; (5) easy manipulation; (6) radiopacity; (7) moisture tolerance; and (8) dimensional stability.[2,3]

Although many root-end filling materials have been used to date, none of them are ideal because of some undesirable features.[4] Among them, mineral trioxide aggregate (MTA) is popular due to its particular characteristics such as good sealing ability, biocompatibility, and inducing hard tissue regeneration.[5–7] However, the literature reports negative opinions toward MTA and its physical properties such as not being easy to handle, taking a long time to set, color change, and cost.[8–10]

Cold ceramic (CC) is an experimental material for root-end filling[11–13] with a principal component of calcium oxide (Table 1) and it can be sterilized using dry heat.[11] As with MTA, CC needs moisture for setting[11] and has an initial setting time of 10 minutes (4 hours for MTA) and finally sets by 24 hours.[11] This fast set reduces the risk of dislodgement and blood contamination when used as a root-end filling material.[14] The sealing ability of CC appears promising[13] and compared with MTA it may provide a more favorable seal in a blood contaminated root-end cavity.[11] Furthermore, the cytotoxicity of CC appears comparable to MTA and less than intermediate restorative material.[13] Another study observed tissue reactions after implantation of CC and MTA in the subcutaneous tissues of rats and reported no significant difference in inflammation after 7 days and 30 days.[15]

Therefore, CC shows promise as a root-end filling material, and because literature concerning CC is sparse, this study aimed to expand the knowledge base and evaluate bone response to CC in comparison with MTA in an animal model.

**Table 1** The main compositions of mineral trioxide aggregate (MTA) and cold ceramic (CC) based on weight (%).

| Components | CaO | BaO | $SiO_2$ | $Bi_2O_3$ | $H_2O$ and $CO_2$ | $SO_3$ | $Al_2O_3$ |
|---|---|---|---|---|---|---|---|
| MTA | 44.23 | — | 21.20 | 16.13 | 14.49 | 0.53 | 1.92 |
| CC | 48.12 | 18.61 | 16.19 | — | — | 10.15 | 3.68 |

*Note*. From "Mineral trioxide aggregate material use in endodontic treatment: a review of the literature," by H.W. Roberts, J.M. Toth, D.W. Berzins, D.G. Charlton, 2008, *Dental Materials*, 24, p. 149–64. Copyright 4021360300098, Elsevier. Adapted with permission

## Materials and methods

Ethical approval was granted by the Torabinejad Dental Research Center, Isfahan University of Medical Sciences, Isfahan, Iran with identification number 287233. All procedures were conducted strictly in accordance with ethical standards and with the last update of the Helsinki Declaration.[16] The maintenance and care of the animals complied with the ethical guidelines of the Torabinejad Dental Research Center.

Forty-five male guinea pigs (English short hair breed weighing 750–850 g, derived from Pasteur Institute of Iran, Tehran, Iran) were involved in this interventional and experimental study. Each animal was anesthetized initially with 10 mg/kg ketamine HCL (Alfasan, Woerden, The Netherlands) and 12 mg/kg xylazine (Alfasan, Woerden, The Netherlands) under supervision of a veterinarian in the Torabinejad Dental Research Center. General anaesthesia was maintained using 5% halothane (Nicholas Piramal India Limited, Mumbai, India) and $N_2O$. Local anaesthesia was also provided in the mucobuccal fold with 3.6-mL lidocain (Daroo Pakhsh Pharmaceutical Co., Tehran, Iran). The submandibular area was shaved and the skin was disinfected with a 5% tincture of iodine. A triangular incision of about 15 mm was made between the incisor and the caudal side of the symphysis joining the two halves of the mandible on both right and left sides. The mucoperiostal flap was raised using a periosteal elevator and a 3 mm × 3 mm diameter cylindrical hole was prepared in each side using a size #3 trephine (ACE Surgical Supply Co., Brockton, MA, USA) under sterile saline irrigation.

Because Teflon causes no significant irritation to tissues,[17] two one-sided open cylindrical tube applicators (inner diameter = 1 mm; outer diameter = 2 mm; length = 2 mm) which corresponded to the defects were filled with white MTA (ProRoot, Dentsply Tulsa Dental, Tulsa, OK, USA) and CC (Shahid Sadoughi University of Medical Sciences, Yazd, Iran) under sterile conditions, separately.

The MTA tube was inserted into the left side defect so that the test material was placed adjacent to the bone. The CC tube filled the right defect in the same way. The mucoperiostal flap was replaced over the tubes and the incision was sutured with 3-0 black silk. The observation periods were 2 weeks and 12 weeks according to a study by Torabinejad et al.[18] The guinea pigs were euthanized in each time interval (24 specimens after 2 weeks and 21 specimens after 12 weeks). The mandibles were dissected out and the bone adjacent to the tubes, *in situ*, was cut into 10-mm

**Table 2** Ranking of the hard tissue response based on the histopathological observations.

| A (No inflammation) | B (Mild) | C (Moderate) | D (Severe) |
|---|---|---|---|
| No signs of inflammatory cells | Low infiltration of chronic inflammatory cells | Moderate infiltration of chronic inflammatory cells and PMNs | High infiltration of chronic inflammatory cells and PMNs |
| Bone regeneration | Low vasodilation | Moderate vasodilation | Abscess |
| Presence of fibroblasts and fibrotic tissue | Bone regeneration | Low-to-moderate destruction of bone tissue | Extensive destruction of bone tissue |
| | Fibrotic tissue | Granulation tissue | Signs of necrosis |

PMN = polymorphonuclear leukocytes.

blocks. The samples were immersed in 10% neutral buffered formalin. They were then demineralized in EDTA, dehydrated in alcohol, embedded in paraffin, serially sectioned at 6 μm and prepared for routine histological observation. The slides were stained with hematoxylin–eosin.

Two blinded oral and maxillofacial pathologists examined the bone tissue reactions under ×100 magnification (Zeiss, Jena, Germany). The amount of hard tissue response was ranked according to Shahi et al[19] which is summarized on Table 2. The recorded data were analyzed using the Mann–Whitney *U* test and SPSS software version 12 (SPSS Inc., Chicago, IL, USA) at a significance level of 0.05.

## Results

Statistical analysis of the results of the responses to both materials did not show any significant differences in the distribution of hard tissue responses between and within the two groups after 2 weeks and 12 weeks (Table 3). Figure 1 illustrates the histopathological hard tissue responses to the both tested materials at both intervals. However, histological examination revealed a mild response to CC after 2 weeks (Figure 1A) and a less inflammatory response to CC after 12 weeks (Figure 1C). Hence, more bone formation and less inflammatory cell infiltration were observed in the CC group.

## Discussion

*In vivo* studies on root-end filling materials and their biological characteristics require a number of delicate considerations such as the site of implantation, amount of material, powder and liquid ratios, providing conditions for the interaction of the materials with the biological system, environment temperature, and working time.[19] Although such *in vivo* studies contribute to the understanding of the biologic responses to a material, they cannot affirm biocompatibility with 100% certainty.[20] The encountered limitations of the present study included the sample size, the physiological sensitivity of guinea pigs to even minor unstable situations, and providing just two intervals with a long intervening period for histological analysis.

**Table 3** Hard tissue response to both mineral trioxide aggregate (MTA) and cold ceramic (CC) materials after 2 weeks and 12 weeks.

| Intervals | Grade | MTA | CC | Pe |
|---|---|---|---|---|
| After 2 wk | A | 4 | 4 | 0.50 |
| | B | 8 | 11 | |
| | C | 12 | 9 | |
| | D | 0 | 0 | |
| After 12 wk | A | 1 | 3 | 0.06 |
| | B | 9 | 12 | |
| | C | 9 | 6 | |
| | D | 2 | 0 | |
| P | | 0.42 | 0.70 | — |

Placement of an experimental material into a polyethylene tube (Teflon) prevents the diffusion of the material into the connective tissue and it also simulates the situation of the root canal and root-end filling.[17] The results confirmed that MTA is a biocompatible material and appropriate for root-end filling, and also that the response to bone tissue for CC was similar. The predominant elements in MTA are calcium, silicon, and bismuth (oxides), respectively,[9,21,22] while the predominant elements in CC are calcium, barium, and silicon, respectively (Table 1). Barium oxide (BaO), one of the components of CC, is a radiopacifier[23] and its biocompatibility has been reported in tibial defects of rats.[24]

Oxide forms of late transitional elements (like Fe, Mn, and Cu) produce strong colors because their d-electrons can be excited by light in the visible spectrum.[25] However, the oxide forms of other elements (Ca, Si, Al, Mg, and S), with much less excitable electrons, are colorless or white. The $SO_3$ content is comparably much higher in CC than MTA (Table 1), which, because it is white, decreases the possibility of color changes.[26]

Calcium hydroxide is the main product of CC and so the mild infiltration of inflammatory cells may be due to the high initial pH of CC.[26] Calcium in its hydroxide form is the main chemical compound released by both MTA[27] and CC when mixed with water. However, the longer-term adverse effects of a material are more important than initial effects.[28] It has been found that calcium hydroxide can induce wider and thicker hyaline formations than MTA.[29] The cementogenic activity of MTA relies on its released calcium ions, which interacts with phosphate groups in the surrounding tissue fluid to form hydroxyapatite on the surface of white and gray MTA.[30] This phenomenon may also explain the favorable response of hard tissue to CC because of its calcium ion content.

Importantly, there are currently no studies assessing molecular expressions, inductions, or inhibitions of CC. A previous study using subcutaneously implanted materials in

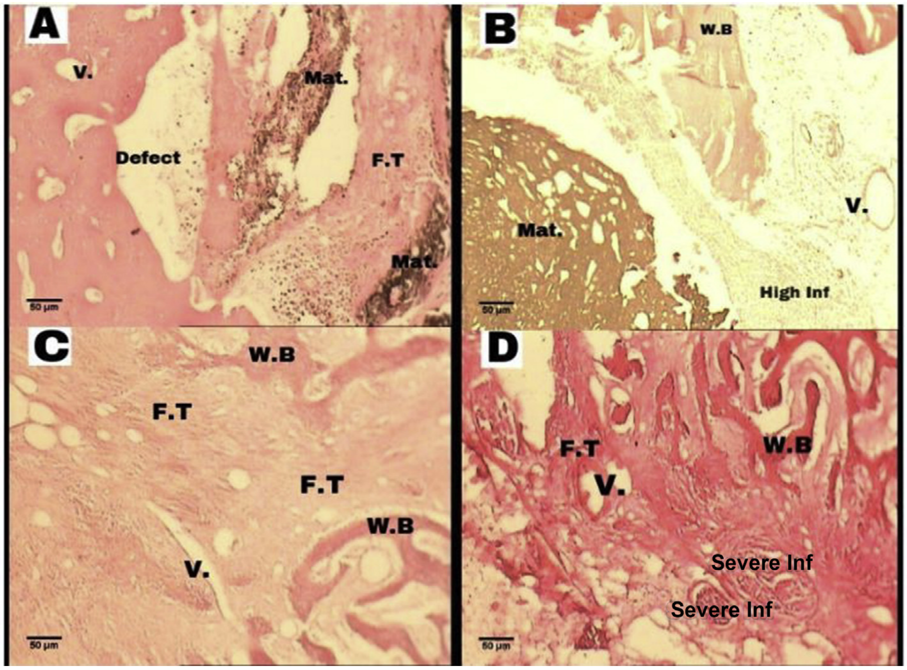


**Figure 1** The histopathological observation of hard tissue response to the both tested materials under ×100 magnification; (A) mild response to cold ceramic after 2 weeks; (B) moderate response to mineral trioxide aggregate after 2 weeks; (C) no inflammatory response to cold ceramic after 12 weeks; and (D) severe response to mineral trioxide aggregate after 12 weeks. Woven bone (W.B), vasodilation (V), inflammation (Inf.), tested material (MAT.), and fibrotic tissue (F.T) were observed in histological analysis.

rats examined the biological responses to MTA and CC and reported similar findings to the present study with both materials not showing any significant differences. However, the number of inflammatory cells was higher in the CC group after 7 days but reverse findings were reported after 30 days.[15] Furthermore, their ranking criteria were different from the present study which may explain the differences.[15] Similarly, Mozayeni et al[13] found that CC and MTA had similar cell viability and suggested that CC may be a suitable option as a biocompatible root-end filling material.

Within the limitations of this study, both CC and MTA demonstrated no significant differences in biocompatibility. Both materials revealed appropriate hard tissue response with a minor adverse impact on hard tissue and healing recovery after 12 weeks. This, together with the advantages of rapid setting and cost, indicate that CC could be a valuable material for root-end fillings. Further research on other properties of CC is indicated, especially *in vivo* studies in humans, before the material can be recommended for this purpose.

## Conflicts of interest

The authors have nothing to disclose.

## References

1. von Arx T, Gerber C, Hardt N. Periradicular surgery of molars: a prospective clinical study with a 1-year follow-up. *Int Endod J* 2001;34:520–5.
2. Gartner AH, Dorn SO. Advances in endodontic surgery. *Dent Clin North Am* 1992;36:357–78.
3. Bodrumlu E. Biocompatibility of retrograde root filling materials: a review. *Aust Endod J* 2008;34:30–5.
4. Saxena P, Gupta SK, Newaskar V. Biocompatibility of root-end filling materials: recent update. *Restor Dent Endod* 2013;38:119–27.
5. Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review—part II: leakage and biocompatibility investigations. *J Endod* 2010;36:190–202.
6. Apaydin ES, Shabahang S, Torabinejad M. Hard-tissue healing after application of fresh or set MTA as root-end-filling material. *J Endod* 2004;30:21–4.
7. Torabinejad M, Hong CU, Lee SJ, Monsef M, Pitt Ford TR. Investigation of mineral trioxide aggregate for root-end filling in dogs. *J Endod* 1995;21:603–8.
8. Lee YL, Lin FH, Wang WH, Ritchie HH, Lan WH, Lin CP. Effects of EDTA on the hydration mechanism of mineral trioxide aggregate. *J Dent Res* 2007;86:534–8.
9. Roberts HW, Toth JM, Berzins DW, Charlton DG. Mineral trioxide aggregate material use in endodontic treatment: a review of the literature. *Dent Mater* 2008;24:149–64.
10. von Arx T. Failed root canals: the case for apicoectomy (periradicular surgery). *J Oral Maxillofac Surg* 2005;63:832–7.
11. Hasheminia SM, Nejad SL, Dianat O, Modaresi J, Mahjour F. Comparing the sealing properties of mineral trioxide aggregate and an experimental ceramic based root-end filling material in different environments. *Indian J Dent Res* 2013;24:474–7.
12. Modaresi J, Aghili H. Sealing ability of a new experimental "cold ceramic" material compared to glass ionomer. *J Clin Dent* 2006;17:64–6.
13. Mozayeni MA, Salem Milani A, Alim Marvasti L, Mashadi Abbas F, Modaresi SJ. Cytotoxicity of cold ceramic compared with MTA and IRM. *Iran Endod J* 2009;4:106–11.

14. Camilleri J. The physical properties of accelerated Portland cement for endodontic use. *Int Endod J* 2008;41:151—7.
15. Modaresi J, Yavari AS, Dianat SO, Shahrabi S. A comparison of tissue reaction to MTA and an experimental root-end restorative material in rats. *Aust Endod J* 2005;31:69—72.
16. Carlson RV, van Ginneken NH, Pettigrew LM, Davies A, Boyd KM, Webb DJ. The three official language versions of the Declaration of Helsinki: what's lost in translation? *J Med Ethics* 2007; 33:545—8.
17. Torneck CD. Reaction of rat connective tissue to polyethylene tube implants. *I Oral Surg Oral Med Oral Pathol* 1966;21: 379—87.
18. Torabinejad M, Ford TR, Abedi HR, Kariyawasam SP, Tang HM. Tissue reaction to implanted root-end filling materials in the tibia and mandible of guinea pigs. *J Endod* 1998;24:468—71.
19. Shahi S, Rahimi S, Yavari HR, et al. Effect of mineral trioxide aggregates and Portland cements on inflammatory cells. *J Endod* 2010;36:899—903.
20. Wataha JC. Principles of biocompatibility for dental practitioners. *J Prosthet Dent* 2001;86:203—9.
21. Asgary S, Parirokh M, Eghbal MJ, Brink F. Chemical differences between white and gray mineral trioxide aggregate. *J Endod* 2005;31:101—3.
22. Asgary S, Parirokh M, Eghbal MJ, Brink F. A comparative study of white mineral trioxide aggregate and white Portland cements using X-ray microanalysis. *Aust Endod J* 2004;30:89—92.
23. Alaçam T, Görgül G, Omürlü H. Evaluation of diagnostic radiopaque contrast materials used with calcium hydroxide. *J Endod* 1990;16:365—8.
24. Acarturk O, Lehmicke M, Aberman H, Toms D, Hollinger JO, Fulmer M. Bone healing response to an injectable calcium phosphate cement with enhanced radiopacity. *J Biomed Mater Res B Appl Biomater* 2008;86:56—62.
25. Asgary S, Eghbal MJ, Parirokh M, Ghoddusi J, Kheirieh S, Brink F. Comparison of mineral trioxide aggregate's composition with Portland cements and a new endodontic cement. *J Endod* 2009;35:243—50.
26. Kolokouris I, Economides N, Beltes P, Vlemmas I. *In vivo* comparison of the biocompatibility of two root canal sealers implanted into the subcutaneous connective tissue of rats. *J Endod* 1998;24:82—5.
27. Fridland M, Rosado R. Mineral trioxide aggregate (MTA), solubility, and porosity with different water-to-powder ratios. *J Endod* 2003;19:814—7.
28. Yaltirik M, Ozbas H, Bilgic B, Issever H. Reactions of connective tissue to mineral trioxide aggregate and amalgam. *J Endod* 2004;30:95—9.
29. Cintra LT, de Moraes IG, Estrada BP, et al. Evaluation of the tissue response to MTA and MBPC: Microscopic analysis of implants in alveolar bone of rats. *J Endod* 2006;32:556—9.
30. Sarkar NK, Caicedo R, Ritwik P, Moiseyeva R, Kawashima I. Physicochemical basis of the biologic properties of mineral trioxide aggregate. *J Endod* 2005;31:97—100.