Evaluation of tissue response to MTA and Portland cement with iodoform

Carlos Alberto Herrero de Morais, PhD,[a] Norberti Bernardineli, PhD,[b] Roberto B. Garcia, PhD,[b] Marco A.H. Duarte, PhD, and [c] Danilo M.Z. Guerisoli, MSc,[d] São Paulo, Brazil
MARINGÁ STATE UNIVERSITY AND UNIVERSITY OF SÃO PAULO

Objective. The aim of this study was to evaluate the biocompatibility of Portland cement with the addition of iodoform, compared to MTA (ProRoot).
Study design. Eighteen Wistar albino rats were divided into 3 groups of 6 animals each. Polyethylene tubes were filled either with freshly mixed MTA or Portland cement mixed with iodoform (20% wt/wt) and implanted subcutaneously. An empty tube served as control. After 7, 30, or 60 days, the implants together with the surrounding tissues were removed in blocks. Sections were evaluated for the presence and thickness of a fibrous capsule, presence of granulation tissue, and the severity of inflammatory response. Data were submitted to nonparametric statistical analysis with individual comparisons between groups at a significance level of $P < 0.05$.
Results. There were no differences between inflammatory responses at 7 and 30 days. After 60 days from surgical removal, there was significantly more tissue reaction to the MTA and Portland cement compared to the control group.
Conclusion. There were no significant differences regarding inflammatory responses between MTA and Portland cement with iodoform after 7, 30, or 60 days. After 60 days, the fibrous capsule around the Portland cement appeared more organized than tissue surrounding MTA implants. After 60 days, there was still a significantly increased tissue reaction to the 2 cements compared to the empty polyethylene tubes.
(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;102:417-21)

Mineral trioxide aggregate (MTA) was developed to repair furcal perforations and as a material for root-end fillings in periapical surgeries.[1,2] Zinc oxide-eugenol based cements, Super EBA, amalgam, and other materials were used as root-end filling materials with a relative success rate[3,4]; however, MTA presented better marginal adaptation when compared to these materials.[5]

MTA offers a biologically active substrate for bone cells and permits cementoblast attachment, growth, and the production of mineralized matrix gene and osteocalcin expression.[6,7] The biocompatibility of MTA is also reported in many studies.[8-10]

The chemical composition of MTA is similar to Portland cement, excepting the bismuth oxide, which is present in MTA.[11-14] Portland cement and MTA also present the same antimicrobial behavior,[12] inflammatory tissue response,[8-10] and prostaglandin (PGE_2) inhibitory effect on monocytes.[15] Due to its biocompatibility and potential to promote bone healing, Portland cement may become the base of a viable dental restorative material and possibly a material for orthopedics.[16]

Whereas the composition and the biological properties of Portland cement were proven to be similar to MTA, the low radiopacity of the Portland cement makes it difficult to visualize on dental radiographs. The ISO 6876/2001 establishes that root canal sealers should be at least as radiopaque as 3 mm of aluminum thickness,[17] whereas the ANSI/ADA specification 57 determines that the difference in radiopacity between sealers and dentine or bone must be equivalent to at least 2 mm of aluminum.[18]

The addition of bismuth oxide as a radiopaque agent to the basic components of the cement seems to be the main formulation of a commercially available mineral trioxide aggregate (ProRoot, Dentsply, Tulsa Dental, Ballaigues, Switzerland).[11-14] However, adding bismuth oxide to Portland cement as a radiopaque agent would be unlikely, since it is not readily available to the regular clinician.

Iodoform (CHI_3) has a high molecular weight

[a]Professor of Endodontics, Maringá State University (UEM), Maringá, PR, Brazil, Professor of Endodontics, Centro de Ensino Superior de Maringá (CESUMAR), Maringá, PR, Brazil.
[b]Department of Endodontics, Bauru School of Dentistry, University of São Paulo, Bauru, SP, Brazil.
[c]Professor of Endodontics, Universidade Sagrado Coração, Bauru, SP, Brazil.
[d]Postgraduate student (PhD), Ribeirão Preto School of Dentistry, University of São Paulo, Ribeirão Preto, SP, Brazil.
Received for publication June 16, 2005; returned for revision Aug 24, 2005; accepted for publication Sep 26, 2005.
1079-2104/$ - see front matter

doi:10.1016/j.tripleo.2005.09.028

(393.71), thus presenting good radiopacity. The mixture of calcium hydroxide and iodoform as a paste has, for a long time, been successfully used for controlling the infection of necrotic root canals and as a coadjutant in periapical healing, including root perforations.[19-24] In addition, it does not increase the levels of chromosome aberrations.[25] These characteristics, as well as the availability to dentists, make it suitable as a radiopaque agent to be added to Portland cement.

Thus, the objective of this study was to evaluate the biocompatibility of Portland cement mixed with iodoform, compared with the MTA (ProRoot).

METHODS

Eighteen adult, male Wistar albino rats (*Rattus norvegicus albinus*), weighing between 200-250 grams, were used for this experiment. Specimens were distributed in 3 groups of 6 animals each, to be examined after 7, 30, and 60 days from the surgical procedure.

After the animals had been anesthetized by intraperitoneal administration of sodium pentobarbital (0.03 g/kg), the dorsal skin was shaved and disinfected. Three incisions were made through the skin using a No. 15 scalpel blade, and 2 cm pockets were prepared by blunt dissection of the incisions. Polyethylene tubes (1.3 mm inner diameter and 7 mm length) were previously autoclaved and filled either with MTA (ProRoot, Dentsply) or Portland cement (Votorantin, São Paulo, Brazil; 80% mixed with iodoform, Probem, Catanduva, Brazil, 20% wt/wt).

The powder/liquid ratio used for both groups was 3:1, and the polyethylene tubes were implanted immediately after loaded with the tested materials. Each implant was carefully placed in a pocket, and the third incision received an empty sterilized tube to serve as control.

The wounds were sutured, with removal of sutures after 7 days. After the experimental periods, all animals from the group were again anesthetized, the dorsal skin was shaved and disinfected, and the implants together with their surrounding tissues were removed in blocks. Animals were killed by an overdose of anesthetic immediately after removal of tissue samples.

After histologic processing, the tissue was serially sectioned longitudinally with a microtome setting at 6 μm. The samples were stained with hematoxylin and eosin. These sections were evaluated microscopically for the occurrence and thickness of a fibrous capsule, presence of granulation tissue, and inflammatory response according to previously established scores (0, no reaction; 1, mild reaction; 2, moderate reaction; 3, severe reaction).

Data were submitted to nonparametric statistical analysis (Kruskal-Wallis) with individual comparisons between groups (Miller) at a significance level of $P < 0.05$.

Table. Mean scores and standard deviation of inflammatory response after 7, 30, and 60 days from surgical procedure

	7 days	*30 days*	*60 days*
Control	2.8 ± 0.4	1.8 ± 0.7	1.1 ± 0.4
MTA	3 ± 0	2.1 ± 0.4	2.6 ± 0.5
Portland cement with iodoform	2.6 ± 0.5	2.6 ± 0.5	2.6 ± 0.5

Scores: 0, no reaction; 1, mild reaction; 2, moderate reaction; 3, severe reaction.

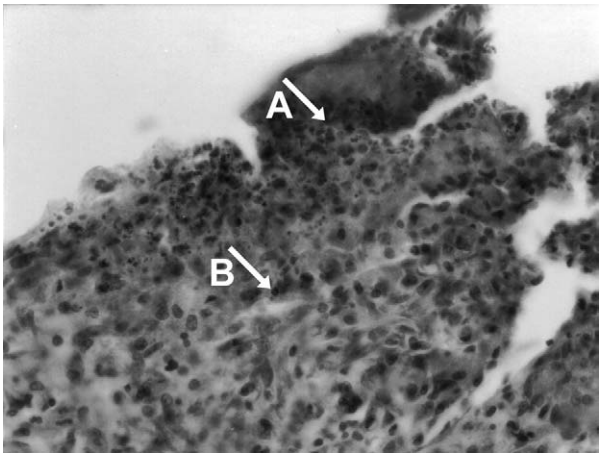

Fig. 1. MTA implants after 7 days. *Arrows* indicate: A) necrotic tissue with prevalence of B) macrophages (hematoxylin-eosin stain, ×40 original magnification).

RESULTS

The Table presents the mean scores observed for the experimental groups after 7, 30, and 60 days from the surgical procedure. The most expressive inflammatory reactions at 7, 30, and 60 days are presented in Figs. 1 to 6.

Statistical analysis of the degree of inflammatory reaction was evaluated, and significant differences were found (Kruskal-Wallis, $P < 0.05$). Individual comparison between groups (Miller test, $P < 0.05$) revealed that there were no differences between inflammatory responses at 7 and 30 days.

However, at 60 days significant differences between MTA, Portland cement, and control groups were found. The Portland cement group presented a more organized fibrous capsule than MTA.

DISCUSSION

This study followed the recommended standard practices for biological evaluation of dental materials of the Fédération Dentaire Internationale.[26]

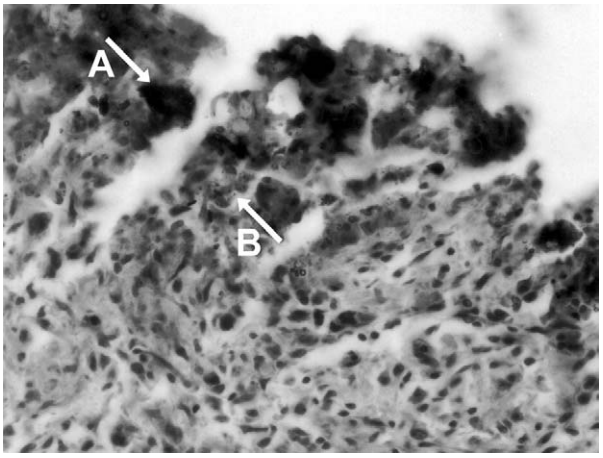

Fig. 2. Portland cement with iodoform implants after 7 days. *Arrows* indicate: A) necrotic tissue and fragments of material (in black) engulfed by B) macrophages (hematoxylin-eosin stain, ×40 original magnification).

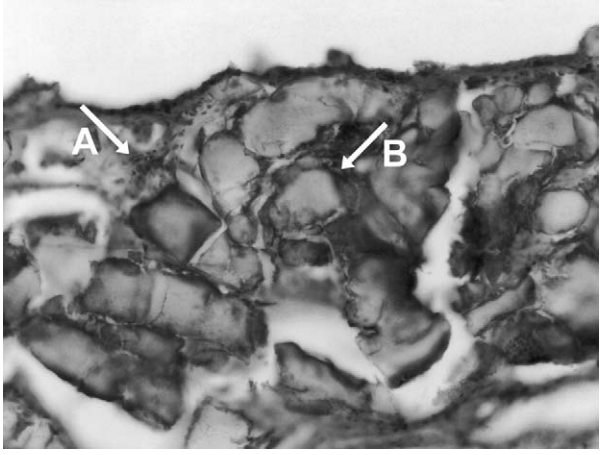

Fig. 3. MTA implants after 30 days. *Arrows* indicate: A) conglomerates of hyaline aspect corpuscles, suggesting B) dystrophic calcification (hematoxylin-eosin stain, ×40 original magnification).

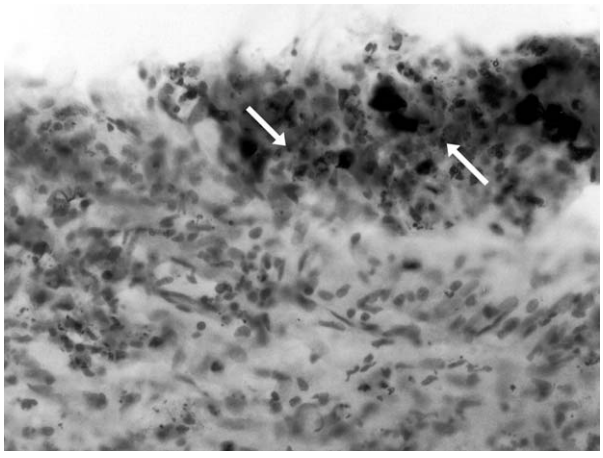

Fig. 4. Portland cement with iodoform implants after 30 days. *Arrows* indicate fragments of dark brown material engulfed by macrophages (hematoxylin-eosin stain, ×40 original magnification).

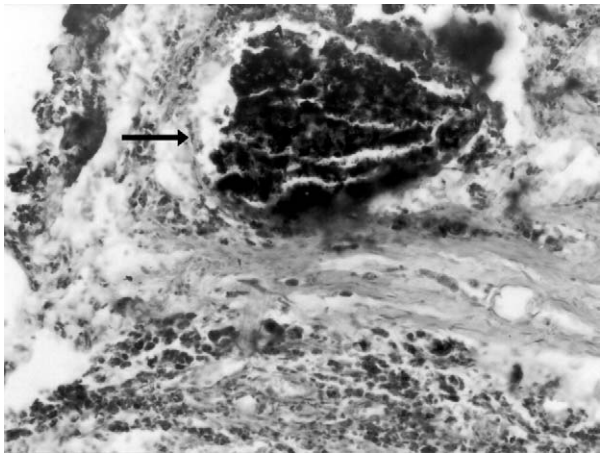

Fig. 5. MTA implants after 60 days. *Arrow* indicates fragments of material (in black) engulfed by macrophages in connective tissue undergoing a maturing process (hematoxylin-eosin stain, ×40 original magnification).

Several studies attest to the similarities between ProRoot and Portland cement, either regarding physical, biological, or microbiological aspects of these materials.[8-16] Lack of acceptable radiopacity is a major concern when considering the use of Portland cement as a substitute to ProRoot, because visual differentiation of the material from the surrounding tissues is needed in radiographs.

In this study, the choice of iodoform as a radiopaque agent to be added to the Portland cement is due to its good radiopacity, prompt availability to the clinician, and previous reports as harmless to the pulp and periapical tissues.[19-25,27]

Seven days after the surgical procedure, the Portland cement implants showed less inflammation compared to the other implant groups (Table). The incorporation of 20% (wt/wt) iodoform as a radiopaque agent to the Portland cement may be the reason for this. A previous study reports that when iodoform is added to calcium hydroxide dressings in induced root perforations in dogs, its antimicrobial action does not interfere with tissue calcification.[27]

After 30 days of implantation, the MTA group presented areas of hyaline, slightly basophilic tissue, suggesting dystrophic calcification (Fig. 3). Such findings

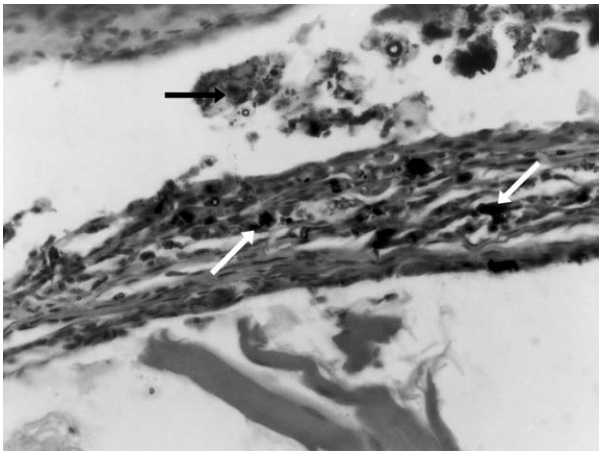

Fig. 6. Portland cement with iodoform implants after 60 days. *Arrows* indicate fragments of material (in black) engulfed by macrophages in capsular tissue (hematoxylin-eosin stain, ×40 original magnification).

have been observed in other studies.[8,28] It is believed that the mechanism involved in deposition of hard tissue that occurs with calcium hydroxide is the same as MTA and Portland cement, because calcium oxide is formed when in contact with living tissue.[8] The same was observed when MTA was used in the apical foramina of dog's teeth.[9]

Sixty days after surgical removal, the control group presented a dense, fibrous connective tissue rich in collagen fibers. The connective tissue was maturing around the tubes filled with MTA, but a moderate inflammatory reaction was still present (Fig. 5). Portland cement with iodoform produced similar tissue reaction, but the fibrous capsule appeared more organized than around the MTA implants (Fig. 6). The formation of such fibrous capsules surrounding polyethylene implants has also been reported in previous studies.[28-33] The inflammatory response observed in the experimental groups is probably due to the extrusion of the materials from the implanted tubes, favoring the maintenance of mononuclear inflammatory cells in the area.

CONCLUSIONS

There were no significant differences in inflammatory responses between MTA and Portland cement with iodoform after 7, 30, or 60 days. After 60 days, a more organized fibrous capsule was found surrounding Portland cement with iodoform when compared to MTA.

The authors would like to thank Ana Claudia Bergamaschi and Catarina de Lima from Dentsply Brazil for supplying the ProRoot used in this research.

REFERENCES

1. Bernabé PFE, Holland R, editors. Odontologia: Arte e Conhecimento. São Paulo: Artes Médicas: 2003; p. 225-64.
2. Lee S, Monsef M, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J Endod 1993;19:541-44.
3. Pitt Ford TR. Effect of super-EBA as a root end filling on healing after replantation. J Endod 1995;21:13-5.
4. Pitt Ford TR. Effect of various zinc oxide materials as root-end fillings on healing after replantation. Int Endod J 1995;28:273-8.
5. 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;21:295-9.
6. Koh ET, McDonald F, Pitt Ford TR, Torabinejad M. Cellular response to mineral trioxide aggregate. J Endod 1998;24:543-7.
7. Thomson TS, Berry JE, Somerman MJ, Kirkwood KL. Cementoblasts maintain expression of osteocalcin in the presence of mineral trioxide aggregate. J Endod 2003;29:407-12.
8. Holland R, Souza V, Nery MJ, et al. Reaction of rat connective tissue implanted dentin tube filled with mineral trioxide aggregate, Portland cement or calcium hydroxide. Braz Dent J 2001;12:3-8.
9. Holland R, Souza V, Nery MJ, Otoboni Filho JA, Bernabé PFE, Dezan E. Reaction of dog's teeth to root canal filling with mineral trioxide aggregate or a glass ionomer sealer. J Endod 1999;25:728-30.
10. Saidon J, Jianing H, Zhu Q, Safavi K, Spangberg L. Cell and tissue reactions to mineral aggregate and Portland cement. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;95:483-9.
11. Wucherpfennig AL, Green DB. Mineral trioxide vs. Portland cement: two biocompatible filling materials. J Endod 1999;25: 308, abstract.
12. Estrela C, Bammann LL, Estrela CRA, Silva RS, Pécora JD. Antimicrobial and chemical study of MTA, Portland cement, calcium hydroxide paste, Sealapex and Dycal. Braz Dent J 2000;11:3-9.
13. Funteas UR, Wallace JA, Fochtman EW. A comparative analysis of mineral trioxide aggregate and Portland cement. Aust Endod J 2003;29:43-4.
14. Camilleri J, Montesin FE, Brady K, Sweeney R, Curtis RV, Ford TR. The constitution of mineral trioxide aggregate. Dent Mater 2005;21:297-303.
15. Safavi K, Nichols FC. Secretion of PGE_2 from monocytes to MTA or Portland cement [abstract]. J Endod 2000;26:540.
16. Abdullah D, Ford TR, Papaioannon S, Nicholson J, McDonald F. An evaluation of accelerated Portland cement as a restorative material. Biomaterials 2002;23:4001-10.
17. International Organization for Standardization ISO 6876/2001: Dental Root Sealing Materials.
18. American Dental Association. Specification #57 for endodontic filling materials. J Am Dent Assoc 1984;108:88.
19. Castagnola W, Wirz J. The use of iodoform paste (Walkhoff method) in modern endodontic therapy. Quintessence Dent Technol 1976;7:19-23.
20. Garcia-Godoy F. Evaluation of an iodoform paste in root canal therapy for infected primary teeth. J Dent Child 1987;54:30-4.
21. Thomas AM, Chandra S, Pandey RK. Elimination of infection in pulpectomized deciduous teeth: a short-term study using iodoform paste. J Endod 1994;20:233-5.
22. Bramante CM, Berbert A. Influence of time of calcium hydroxide iodoform paste replacement in the treatment of root perforations. Braz Dent J 1994;5:45-51.
23. Thomas AM, Chandra S, Chandra S, Pandey RK. Elimination of

infection in pulpectomized deciduous teeth: a short-term study using iodoform paste. J Endod 1994;20:233-5.
24. Nurko C, Garcia-Godoy F. Evaluation of a calcium hydroxide/iodoform paste (Vitapex) in root canal therapy for primary teeth. J Clin Pediatr Dent 1999;23:289-94.
25. Hikiba H, Watanabe E, Barrett JC, Tsutsui T. Ability of fourteen chemical agents used in dental practice to induce chromosome aberrations in Syrian hamster embryo cells. J Pharmacol Sci 2005;97:146-52.
26. Stanford JW. Fédération Dentaire Internationale – Recommended standard practices for biological evaluation of dental materials. Int Dent J 1980;9:140-88.
27. Bramante CM, Neto CB, Lia RCC, Laund F, Esberard RM. Tratamento de perfurações radiculares com pastas de hidróxido de cálcio e iodofórmio: Emprego de diferentes veículos - Estudo histológico em dentes de cães. Rev Bras Odontol 1986;4:20-30.
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. Phillips JP. Rat connective tissue response to hollow polyethylene tube implants. J Canad Dent Ass 1967;33:59-64.
30. Torneck CD. Reaction of rat connective tissue to polyethylene tube implants, part I. Oral Surg Oral Med Oral Pathol 1966;21:379-87.
31. Torneck CD. Reaction of rat connective tissue to polyethylene tube implants, part II. Oral Surg Oral Med Oral Pathol 1967;24:674-83.
32. Langeland K, Guttuso J, Langeland L, Tobon G. Methods in the study of biologic responses to endodontic materials. Oral Surg Oral Med Oral Pathol 1969;27:522-42.
33. Olsson B, Sliwkowski A, Langeland K. Subcutaneous implantation for the biological evaluation of endodontic materials. J Endod 1981;7:355-69.

Reprint requests:

Carlos Alberto Herrero Morais, PhD
Professor of Endodontics
Maringá State University (UEM)
Maringá, PR, Brazil
Professor of Endodontics
Centro de Ensino Superior de Maringá (CESUMAR)
Maringá, PR, Brazil
caherrero@uol.com.br