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Biocompatibility and biomineralization assessment of a new root canal sealer and root-end filling material

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Abstract – *Objective:* This study investigated the short-term subcutaneous tissue reaction and biomineralization ability of two epoxy-based root canal sealers containing calcium hydroxide (MBP and MBPc) and ProRoot MTA. *Materials and methods:* Polyethylene tubes containing the materials were implanted into the dorsal connective tissue of Wistar rats (n = 52) for 7 or 30 days; empty implanted tubes served as controls. Specimens were stained with hematoxylin–eosin and von Kossa stain or left unstained for observation under polarized light. Qualitative and quantitative evaluations of all tissue reactions were performed. One-way ANOVA and the Kruskal–Wallis test were used for statistical analysis ($P < 0.05$). *Results:* No significant differences were observed among the groups. All three materials induced mild-to-moderate tissue reactions at 7 days, which decreased over time. Dystrophic mineralization and birefringent structures were observed only in the ProRoot MTA® group. *Conclusion:* Both MBP and MBPc appear to be biocompatible but do not stimulate biomineralization.

An ideal root-end filling material and/or root canal sealer should adhere to the dentin surface, maintain a sufficient seal, and be insoluble in tissue fluids, dimensionally stable, non-resorbable, radiopaque, and biocompatible if not bioactive (1, 2). Historically, several materials have been used for root-end filling and perforation repair, such as amalgam, zinc oxide–eugenol cement, composite resin, and glass ionomer cement (1–3).

Mineral trioxide aggregate (MTA) reportedly has adequate biocompatibility, good sealing ability, and low cytotoxicity when compared with the other materials currently used in endodontic therapy (4). The main components of MTA are tricalcium oxide, tricalcium silicate, bismuth oxide, tricalcium aluminate, tetracalcium aluminoferrite, and silicate oxide. It was introduced for repairing pathological or iatrogenic root perforations and root-end filling (4–7). In addition, *in vivo* studies have shown that MTA induces the formation of mineralized tissue such as dentin- and cementum-like tissue (8–13). Its biomineralization ability induces the formation of a layer with tag-like structures at the cementum–dentin interface, which positively influences the push-out bond strength (13). Its clinical effectiveness has been observed in various endodontic procedures including vital pulp therapy, root-end closure, root perforation repair, periapical surgery, and treatment of pulpal and periodontal healing complications following dental trauma (14–18).

Calcium ions released from MTA probably react with phosphates in the tissue fluid to form hydroxyapatite, a property that would be directly related to the sealing ability and dentinogenic activity of MTA (8, 19). In addition to trioxides, mineral oxides such as bismuth oxide (added to MTA for radiopacity) would be responsible for the physicochemical properties of this material (8).

The use of MTA for root canal filling should be explored because, so far, none of the available endodontic sealers have ideal biocompatibility characteristics (1, 2). However, the physical characteristics of this material make both manipulation and filling of the root canal system difficult (4, 12, 20).

In 1984, a calcium hydroxide-containing epoxy resin-based root canal sealer (MBPc) was developed as an option for root perforation repair (8). MBPc is

packaged in glass vials as a hydrophobic paste, mixed in a 4:1 (base-to-catalyst) ratio, has a 4-h setting time, and is radiopaque in accordance with the ISO/DIS 6876 recommendation (8, 21, 22). The sealing ability and marginal adaptability of this material were found to be excellent (23). Further, MBPc and MTA had similar biocompatibility in rat alveolar sockets; however, MBPc did not show biomineralization ability (8).

A new formulation of MBPc, termed MBP, has been developed as a root canal sealer. MBP comprises paste A containing calcium hydroxide, bismuth subnitrate, and resin bisphenol A ether and paste B containing barium sulfate, bismuth subnitrate, resin hardener, and castor bean (*Ricinus communis*) polymer. The material information sheet states that the chemical composition of MBP is similar to that of MBPc but with reduced epoxy resin content to improve the consistency.

Although MBP apparently possesses advantages that make it suitable for use as a root canal sealer, its tissue reaction has not been examined to date. The aim of this study was to investigate the short-term subcutaneous tissue reactions and biomineralization ability of MBP and MBPc in comparison with an MTA-based root-end filling material (ProRoot MTA®; Dentsply Tulsa Dental Specialties, Tulsa, OK, USA).

Materials and methods

Fifty-two male 4- to 6-month-old Wistar albino rats, weighing 250–280 g, were used in the study. The animals were housed in temperature-controlled rooms and received water and food *ad libitum*. The experimental protocol was approved by and conducted in accordance with the guidelines of the institutional ethical committee.

Forty-two polyethylene tubes (Abbott Laboratories of Brazil, Sao Paulo, SP, Brazil) with a 1.0-mm internal diameter, 1.6-mm external diameter, and 10.0-mm length were filled with the experimental materials. MBP, MBPc, and ProRoot MTA® were prepared according to the manufacturers' recommendations and inserted into the tubes with a lentulo spiral (Dentsply Maillefer, Tulsa, OK, USA). Ten empty polyethylene tubes were used as controls.

The rats were anesthetized with ketamine (87 mg kg^{-1} Francotar; Virbac do Brasil Ind e Com Ltda, Roseira, SP, Brazil) and xylazine (13 mg kg^{-1} Rompum; Bayer S.A., São Paulo, Brazil) injections intramuscularly. After antisepsis with 5% iodine solution, the back of each rat was shaved and a 2.0-cm incision was made in a head–tail orientation with a number 15 Bard-ParkerTM blade (BD, Franklin Lakes, NJ, USA). The skin was reflected to create a pocket on the right side of the incision. A polyethylene tube was implanted into the space created by blunt dissection, and the skin was closed with 4-0 silk sutures.

At 7 or 30 days after implantation, the animals were sacrificed with an overdose of the anesthetic solution, and the polyethylene tubes together with the surrounding tissues were removed and fixed in 10% buffered formalin at pH 7.0. The tubes were then cut longitudinally with a sharp blade (24, 25). The specimens were embedded in paraffin, cut into 6-μm sections, and stained with hematoxylin–eosin and von Kossa stain or left unstained for observation under polarized light. The von Kossa staining technique was used to observe biomineralization, because mineralized structures stain darkly. The polarized light technique was used to demonstrate birefringent structures related to calcium carbonate crystals originating from the combination of calcium ions from the material and carbonic gas from the tissue (10, 11). Tissue reactions at the open end of the tubes were scored according to previous studies (10, 11, 25), as follows: 0, no or few inflammatory cells and no reaction; 1, fewer than 25 cells and mild reaction; 2, between 25 and 125 cells and moderate reaction; and 3, 125 or more cells and severe reaction. Fibrous capsules were considered thin when <150 μm and thick when ≥ 150 μm. Necrosis and calcification were recorded as present or absent. The average number of cells in each experimental group was calculated from 10 separate areas (400× magnification).

Data were analyzed by a single calibrated operator in a blinded manner. One-way ANOVA and the Kruskal–Wallis test were used for statistical analysis; $P < 0.05$ was considered significant.

Results

Control group

At 7 days, moderate inflammatory cell infiltration consisting of lymphocytes and macrophages was present in the fibrous capsule (Fig. 1a,b). On day 30, the connective tissue was well organized, and infiltration of a few chronic inflammatory cells was observed (Fig. 1c,d). The empty tubes did not show von Kossa staining, and no birefringent structures were observed under polarized light at both time points (Fig. 2a–d).

ProRoot MTA® group

On day 7, a superficial layer of irregular thickness, coagulation necrosis, and nuclear fragmentation in close contact with the material were observed (Fig. 1e, f). However, on day 30, irregular basophilic areas could be seen, which could probably serve as a matrix for mineralization (Fig. 1g,h). Birefringent structures and positive von Kossa staining were observed near the tube opening at both time points (Fig. 2e–h).

MBPc group

MBPc induced a tissue reaction similar to that of ProRoot MTA®. On day 7, a moderate inflammatory cell infiltrate composed of macrophages and lymphocytes in the connective tissue, with a few young fibroblasts and new blood vessels, was observed (Fig. 1i,j). On day 30, a mild inflammatory cell infiltrate, with a few chronic inflammatory cells, was observed (Fig. 1k,l). However, neither von Kossa-stained areas nor birefringent structures were observed at both time points (Fig. 2i–l).

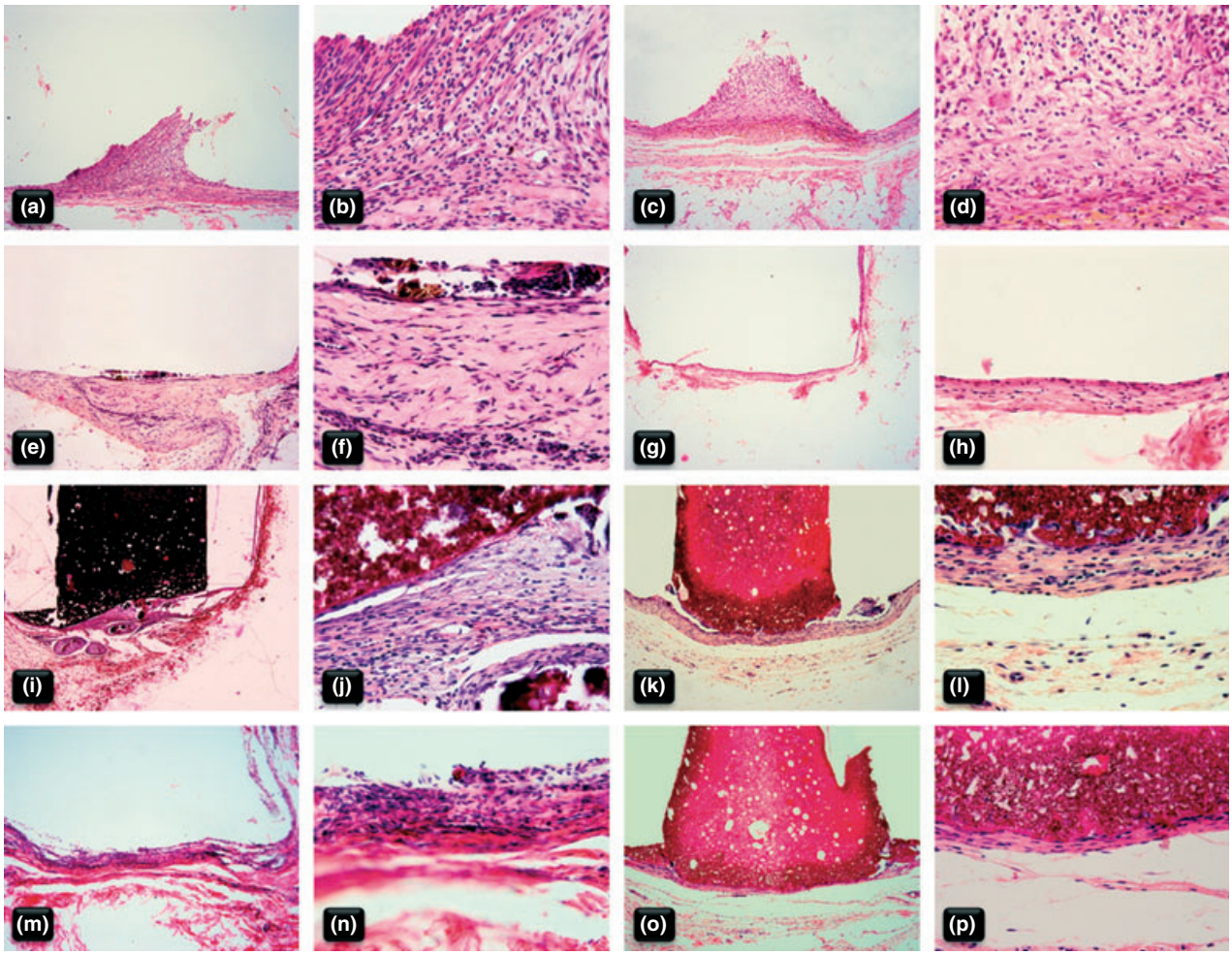

Fig. 1. Subcutaneous tissue reactions in the experimental groups. Control group: (a, b) thick fibrous capsule formation and moderate inflammatory cell infiltration after 7 days; (c, d) thin fibrous capsule formation and mild inflammatory cell infiltration after 30 days. ProRoot MTA®: (e, f) moderate chronic inflammatory cell infiltration and thick fibrous capsule formation after 7 days; (g, h) reduced fibrous capsule thickness and inflammatory cell infiltration after 30 days. MBPc: (i, j) thick fibrous capsule formation and moderate inflammatory cell infiltration after 7 days; (k, l) mild chronic inflammatory cell infiltration and thin fibrous capsule formation after 30 days. MBP: (m, n) thick fibrous capsule formation and moderate inflammatory cell infiltration, comprising lymphocytes and macrophages, after 7 days; (o, p) thin fibrous capsule surrounding the tube with few chronic inflammatory cells after 30 days. Hematoxylin and eosin staining, 10× and 40× magnification.

MBP group

The MBP group showed a similar tissue reaction as the other groups at 7 days, including a moderate chronic inflammatory cell infiltrate consisting of lymphocytes and macrophages within a fibrous capsule (Fig. 1m,n). On day 30, the tissues demonstrated a mild inflammatory reaction, with a few chronic inflammatory cells and thin fibrous capsule (Fig. 1o, p). As in the case of the MBPc group, von Kossa staining and birefringent structures were not observed (Fig. 2m–p).

Comparison among the groups

The data were compared at each time point and are presented in Tables 1 and 2. Hematoxylin and eosin staining revealed a similar tissue response of the inflammatory reaction among the groups (Fig. 1a–p). There was no statistically significant difference between the scores of the different groups on day 7 or day 30 (median score 1) ($P > 0.05$) (Table 1). Only the ProRoot MTA® group was positive for Von Kossa stain and the presence of birefringent structures under polarized light (Fig. 2a–p) (Table 2). Data related to the percentage of samples in each group categorized according to the presence of fibrous capsules, biomineralization ability, and necrosis are shown in Table 2.

Discussion

Several *in vitro* and *in vivo* tests are used to evaluate the biocompatibility of dental materials, such as testing the cytotoxicity of materials in a cell culture, implantation tests, and animal studies (26). Implantation in the subcutaneous tissue of rats is one of the most appropriate

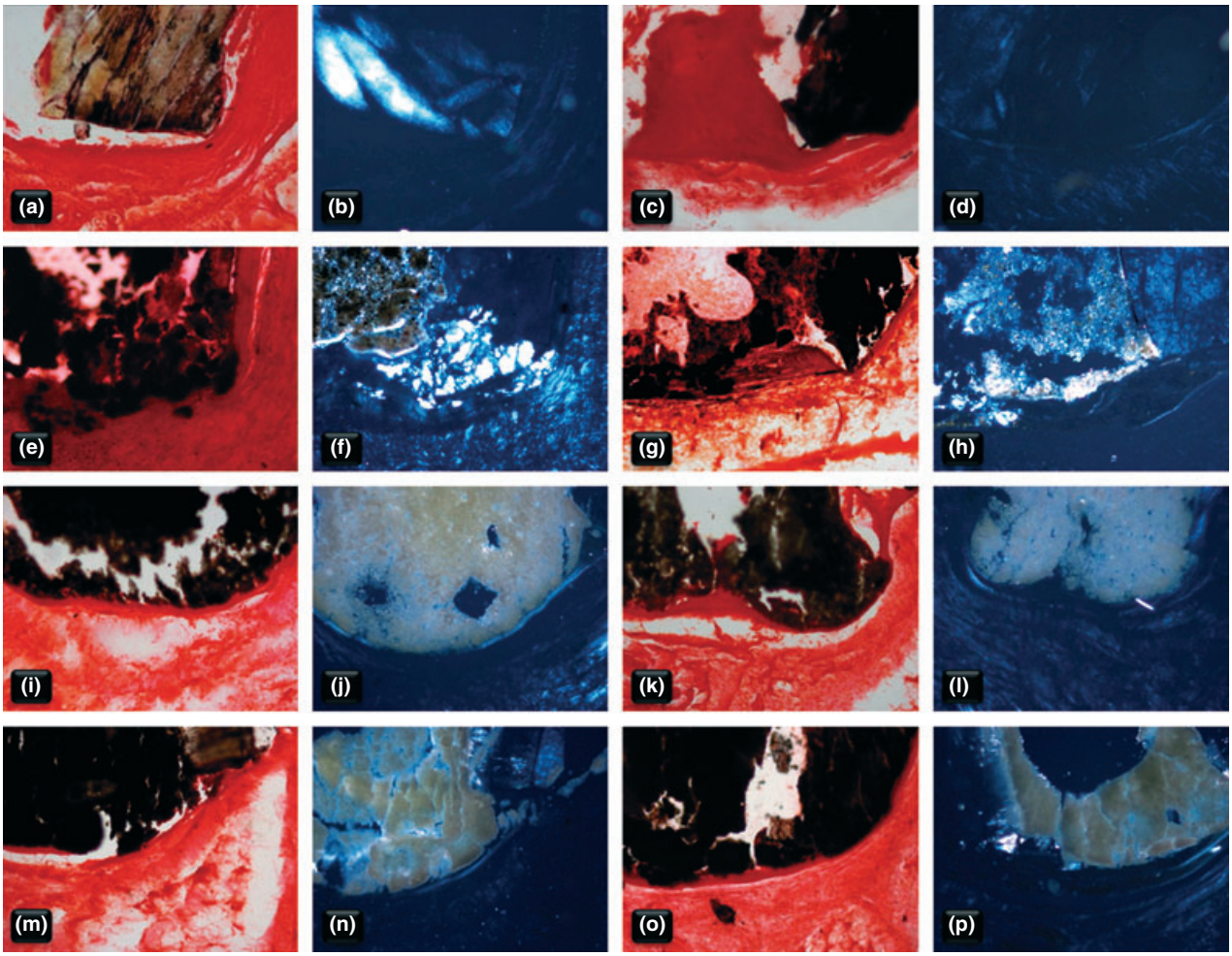

Fig. 2. Biomineralization in the experimental groups. Control group: absence of (a, c) dystrophic calcification at the tube opening and (b, d) birefringent structures after 7 and 30 days. ProRoot MTA®: presence of (e, g) dystrophic calcification at the tube opening and (f, h) birefringent structures after 7 and 30 days. MBPc: absence of (i, k) dystrophic calcification at the tube opening and (j, l) birefringent structures after 7 and 30 days. MBP: absence of (m, o) dystrophic calcification at the tube opening and (n, p) birefringent structures after 7 and 30 days. (a, c, e, g, i, k, m, o) von Kossa staining, 10× magnification; (b, d, f, h, j, l, n, p) polarized light, 10× magnification.

tests to determine the local effects of the potential new biomaterials (27–29).

In the present investigation, the empty tubes used in the control group promoted few or no reactions in the subcutaneous tissue, and normal repair tissues were formed, similar to the results previously reported (11, 25). At the 7-day interval, all the experimental groups evoked mild-to-moderate inflammatory reactions, but the response decreased and the fibrous capsule became thinner over time.

ProRoot MTA® evoked a moderate chronic inflammatory response, but this reduced over time and was contained within a thin fibrous connective tissue capsule surrounding the tube end. In both time periods, positive areas were observed for calcium by Von Kossa staining. These findings were similar to the results previously reported (10, 11). MTA contains calcium oxide and calcium phosphate in its formulation. Calcium oxide may react with water or tissue fluids to form calcium hydroxide. In contact with water, dissociates into calcium ions and hydroxyl ions. The calcium ions can then react with carbon dioxide in the tissues to form calcium carbonate granulations, observed as calcite crystals birefringent to polarized light. These crystals

Table 1. Inflammatory scores of the treatment groups

	Control group		ProRoot MTA		MBPc		MBP	
Time	7 days	30 days	7 days	30 days	7 days	30 days	7 days	30 days
Inflammatory response								
0 – Absent	0/5	3/5	0/7	5/7	0/7	5/7	0/7	6/7
1 – Mild	3/5	2/5	5/7	2/7	5/7	2/7	5/7	1/7
2 – Moderate	2/5	0/5	2/7	0/7	2/7	0/7	2/7	0/7
3 – Severe	0/5	0/5	0/7	0/7	0/7	0/7	0/7	0/7

Table 2. Percentage of samples in each group categorized according to the presence of fibrous capsules, biomineralization ability, and necrosis

	Control group (%)		ProRoot MTA (%)		MBPc (%)		MBP (%)	
Time	7 days	30 days	7 days	30 days	7 days	30 days	7 days	30 days
Fibrous capsules								
<150 μm	40	60	57.1	71.4	42.9	71.4	71.4	85.7
>150 μm	60	40	42.9	28.6	57.1	28.6	28.6	14.3
Biomineralization ability	0	0	100	100	0	0	0	0
Necrosis/presence	0	0	0	0	0	0	0	0

could serve as nucleation sites, stimulating the deposition of hard tissues (30).

In this study, MBP and MBPc sealers evoked a moderate chronic inflammatory response at the seventh day, which reduced with time, was observed in a thin fibrous connective tissue capsule surrounding the tube. However, were not observed positive areas for calcium by Von Kossa staining. It was previously shown that both MBPc and ProRoot MTA® released calcium ions into their local surroundings, decreasing this release with time (31).

In the present study, it was not possible to detect the same biomineralization ability as has been seen with MTA. However, further studies should be conducted to confirm the present results, such as the dental alveolar implantation method using bone markers (12). In the present study, the short experimental period could be a key reason why some biological effects of the test materials (including biomineralization) were not observed. These effects may be observable in longer experimental periods (e.g. 60 or 90 days).

It is known that the presence of specific agents in the composition of a dental material does not imply their dissociation and release after setting, because the curing reaction and presence of another agent can inhibit the release of these ions (31). Therefore, although the *in vitro* methodology has indicated calcium release (31), calcium ions were not detected in the subcutaneous tissue of the MBP and MBPc groups. This result confirms that the presence of calcium hydroxide in a root canal sealer does not guarantee its release as expected, at least in a short observational period.

MBP and MBPc will be bioactive only if calcium and hydroxyl ions are released over time under biological conditions (32).

Calcium ions are also important for the activation of calcium-dependant adenosine (33), cell migration and differentiation (34), and formation of calcium carbonate crystals, which enable mineralization (32, 33). These biological activities can explain the good clinical results observed with the use of some root canal sealers, which stimulate foramen closure by means of mineralization, determining biological sealing ability (9, 30). Despite their biocompatibility, calcium hydroxide-containing epoxy resin-based root canal sealers MBP and MBPc failed to stimulate biomineralization; therefore, they are unlikely to replace the currently used materials.

Conclusion

In the rat model, MBP, MBPc, and ProRoot MTA® produced similar subcutaneous tissue reactions, but only ProRoot MTA® stimulated dystrophic mineralization, indicating its biomineralization ability.

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Conflict of interest

The authors disclose no conflicts of interest.

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