
Radiopacity and histological assessment of Portland cement plus bismuth oxide

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Objective. The present study evaluated the subcutaneous connective tissue reactions and the radiopacity of MTA, Portland cement (PC), and Portland cement plus bismuth oxide (BO).

Study design. Forty rats were divided into 5 groups (n = 8 per group): A1: Control (empty capsule); A2: Pro-Root MTA; A3: PC; A4: PC + BO 1:1; and A5: PC + BO 2:1. Polyethylene tubes were filled with the test materials and standardized radiographic images were taken. Histological evaluation was done after 7 and 60 days. Student t test and Fisher's test were used in the statistical analysis ($P < .05$).

Results. The radiopacity of the materials were in decreasing order: A2 > A4 > A5 > A3. No differences were found for the tissue response in the 2 experimental periods. A positive correlation between BO concentration and radiopacity of PC was determined.

Conclusion. The histological evaluation suggests that all studied materials were biocompatible at 7 and 60 days. (**Oral Surg Oral Med Oral Pathol Oral Radiol Endod** 2008;106:e69-e77)

Mineral trioxide aggregate (MTA) has excellent physical and biological properties such as biocompatibility and sealing ability, and the capacity of promoting pulp and periradicular tissue repair encourage its use.¹ MTA may be an ideal material because it consistently induced the regeneration of periodontal ligament tissues, the apposition of a cementumlike material, and formation of bone.² MTA has been reported to be biocompatible in many in vivo and in vitro studies.^{3,4}

Portland cement is the main chemical component of MTA. However, as it is intended for purposes other than biological, many studies have been done to compare the physical and biological properties of Portland cement with those of MTA. Analysis of the chemical components of both materials showed the same composition, with the exception that MTA also contains bismuth, which confers radiopacity.⁵ MTA and Portland cement were reported to be similar macroscopi-

cally, microscopically, and when analyzed by X-ray diffraction.⁶ Furthermore, osteoblastlike cells had similar growth and matrix formation when in contact with either set MTA or Portland cement.⁶ MTA and Portland cement have comparable antibacterial properties.⁵ De-Deus et al.⁷ reported that 2 brands of MTA and Portland cement initially showed similar elevated cytotoxic effects that decreased gradually over time allowing the cell culture to reestablish. Saidon et al.⁸ reported that MTA and Portland cement have similar biological properties, whereas MTA is expensive and Portland cement is a rather cheap material.

One of the factors that might limit the use of Portland cement in endodontics is the lack of radiopacity. Addition of radiopaque substances to Portland cement that do not alter its properties may enhance its use in endodontics, suggesting a possible substitute for MTA in endodontic procedures. The purpose of this study was to evaluate the tissue reaction and radiopacity of Portland cement when bismuth oxide is added.

MATERIALS AND METHODS

Animals and sample grouping

This study was approved by the Ethics Committee in Animal Research of the Institute of Biology Roberto Alcântara Gomes of Rio de Janeiro State University, Rio de Janeiro, Brazil. The procedures followed existing guidelines on animal experimentation. Connective tissue reaction in rats⁹ and the standardized methods to

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Table I. Experimental design

Material and experimental period	Initial	Final	Losses
	n	n	
Portland cement + BO 1:1 (7 days)	8	6	2
Portland cement + BO 2:1 (7 days)	8	6	2
MTA (7 days)	8	8	0
Pure Portland (7 days)	8	8	0
Control (7 days)	8	5	3
Portland cement + BO 1:1 (60 days)	8	8	0
Portland cement + BO 2:1 (60 days)	8	6	2
Pure Portland (60 days)	8	8	0
Control (60 days)	8	8	0
MTA (60 days)	8	6	2
TOTAL	80	69	11

evaluate the biological reactions recommended by the Federation Dentaire Internationale¹⁰ and American Dental Association¹¹ were used.

Forty albino rats (*Rattus norvegicus*), adult males and in good health, approximately 90 days of age and 300 g of body weight were selected. The animals were obtained from the Animal Laboratory of Experimental Surgery of the College of Medical Sciences of Rio de Janeiro State University. The animals were placed in appropriate cages, with a maximum of 8 animals per cage, and received appropriate granular food (Nuvilab CR1, Nuvital Nutrientes Ltda., Colombo, PR, Brazil) and water ad libitum.

The specimens were randomly divided into 5 groups (n = 8 per group), according to the test material and the experimental period (Table I):

- A1, Negative control: empty capsule
- A2, Positive control: Pro-Root MTA
- A3, Pure Portland: Type V Portland
- A4, Portland cement + Bismuth oxide (Portland cement + BO 1:1)
- A5, Portland cement + Bismuth oxide (Portland cement + BO 2:1)

Sample preparation

The Pro-Root MTA (Pro Root MTA, Dentsply Tulsa Dental, Tulsa, OK) was mixed according to the manufacturer instructions. One gram of high initial resistance (HIR), also known as type V Portland cement (Irajazinho, Votorantim Cimentos, Rio Branco, São Paulo, Brazil), and bismuth oxide (Reagentes Analíticos Dinâmica, São Paulo, Brazil) were mixed with 0.35 mL of distilled water to produce a homogeneous paste⁷; the Portland cement was mixed to a consistency similar to the MTA and it was sterilized with ethylene oxide.⁷

Polyethylene tubes (5 mm in length and 1.5 mm of internal diameter) were made from Scalp (Jinan

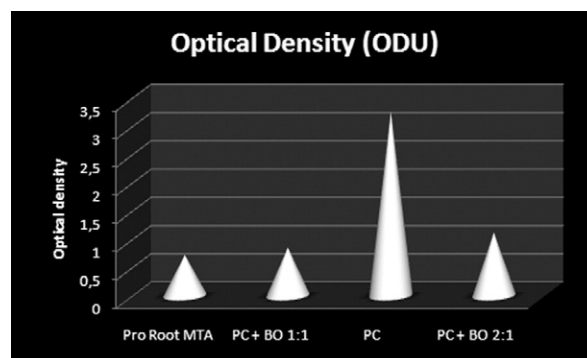


Fig. 1. Optical density of the tested materials.

Dragon Crown Medical Apparatus Ltd., Xu Lin, China) intravenous infusion apparatus and sterilized in an autoclave. The sterilized tubes were filled with the test materials with the aid of endodontic condensers. After preparation, the tubes were filled with the materials. Each animal received 2 implants of the same material.

Analysis of sample radiopacity

Ten samples for each tested material were used for the x-ray radiopacity assessment. Standardized radiographic images of the polyethylene tubes filled with the test materials were taken before implantation in the animals. Images were taken with a conventional x-ray device (Espectro 70X electronic, Dabi Atlante, Ribeirão Preto, SP, Brazil) using 0.7 seconds for exposure, at 60 kVP and 10 mA. A distance of 10 mm was standardized as the distance from the cylinder to the periapical film in all samples (Kodak série E – P21, São José dos Campos, SP, Brazil). Film processing was handled in an automatic processor (SFR 800, J Morita Corporation, Osaka, Japan) at 30° C for 4 minutes. The automatic processor was cleaned and the solutions changed before film processing.

Samples were then sent to the Brazilian Film Industry (IBF, Rio de Janeiro, RJ, Brazil) where the optical density of each material was evaluated in a photodensitometer (OE330, J Morita Corporation, Osaka, Japan), with a 0 to 0.4 reflex measuring scale; 0.3 points of measures, at 230V and 0.5 mA, and dimensions of 200 × 305 × 280 mm. The coefficient of linear attenuation of each material was calculated using the amount of light emerging from the radiograph. Three sites were randomly picked by the device, of which the optical density was measured and the arithmetic mean taken for determination of the coefficient of linear attenuation.

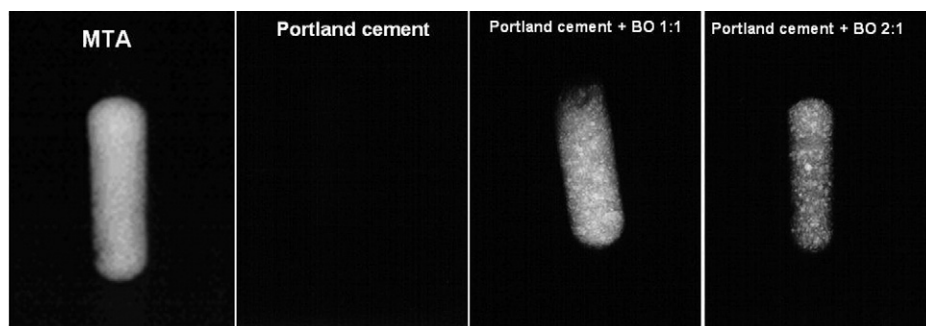


Fig. 2. Radiopacity of the tested materials.

Table II. Initial and final numbers of implants

Materials	Experimental periods, days	No. implants per group
Portland cement + BO 1:1	7	16
	60	
Portland cement + BO 2:1	7	
	60	
Pro-Root MTA (positive control)	7	
	60	
Pure Portland	7	
	60	
Empty capsule (negative control)	7	
	60	

Surgical procedures

The animals were anesthetized intraperitoneally with 0.3 mL ketamine (100 mg mL⁻¹) containing acepromazine (0.2 mg mL⁻¹) in all surgical periods (Fluothane, Zeneca Farmacêutica do Brazil Ltd., Cotia, SP, Brazil). Trichotomies in the back region of the animals were followed by aseptic procedures with povidine (Povidet, Tecnofarma Indústria e Comércio Ltd., Campinas, SP, Brazil) and placement of fenestrated surgical covering. Two small incisions of approximately 20 mm in length were made in the right and left sides of the back of the animal and the subcutaneous tissue dissected with rounded scissors to 10 mm in depth to create 2 surgical pouches. The polyethylene tubes filled with the test materials were then introduced, 1 in each side, parallel to the incisions. Incisions were sutured with mononylon 4.0. All surgical procedures were performed under supervision of a veterinarian of the Animal Laboratory.

Immediately after implantation, the animals were observed until recuperation of their physical activities and placed in the cages with no feeding restrictions. The animals were seen every day for a period of 60 days.

At 7 (for 20 animals, n = 8 per group) and 60 days (for 20 animals, n = 8 per group) the animals were

anesthetized again for excisional biopsy of the implants and surrounding tissues. The animals were killed by cervical dislocation while still under the effect of anesthesia.

Histological analysis

Biopsies were placed in 10% buffered formalin until histologically processed. Tissues were embedded in paraffin wax (Isofar Chemical products, Rio de Janeiro, RJ, Brazil) and longitudinal 4-µm sections (including the open ends of the tubes) were obtained with a microtome to allow examination of the tissues in contact with the test materials. Sections were stained with hematoxylin and eosin and viewed under light microscopy (Axiscoppe, Carl Zeiss Vision GmbH, Hallbergmoos, Germany) at ×40, ×100, and ×400 magnification. Twenty sections were examined for each tube and the interface at the opening of the polyethylene tubes, between the material and the tissue, was examined and evaluated for the intensity of inflammation. For each section, the tissue response to the different test materials was evaluated in 5 microscopic fields, which were randomly chosen with the aid of a computer algorithm (<http://www.random.org>). Two independent, blinded, and experienced pathologists were used to evaluate the tissue reactions. The degree of inflammation was determined according to the type and number of predominant cells. The presence or absence of neutrophils, inflammatory infiltrate, giant cells, and macrophages was recorded. For each of these elements, the following numerical scores were attributed: 0, absent; 1, mild (present in small numbers or in small groups); 2, moderate (present in large numbers, yet not predominantly in the microscopic field); 3, intense (present as infiltrate, predominantly in the microscopic field). Because of the qualitative character of the histological evaluation, always the higher degree of both examiners was chosen.

Table III. Histological evaluations of tested materials at 7 and 60 days (values in scores)

<i>Material and experimental period</i>	<i>Acute inflammation</i>	<i>Chronic inflammation</i>	<i>Giant cells</i>	<i>Macrophages</i>
Portland cement + BO 1:1 (7 days)	2.17	0.83	0.33	0.33
Portland cement + BO 2:1 (7 days)	1.83	0.67	0	0
Pro-Root MTA (7 days)	1.63	0.88	0.25	0.13
Pure Portland (7 days)	2.10	0.60	0	0
Negative Control (7 days)	1.60	1.00	0	0
Portland cement + BO 1:1 (60 days)	0.38	1.63	0.25	0.75
Portland cement + BO 2:1 (60 days)	0.50	1.83	1.00	1.00
Pure Portland (60 days)	0.52	1.60	1.00	1.00
Pro-Root MTA (60 days)	0.33	1.80	1.00	1.1
Negative control (60 days)	0.13	1.63	0.38	0.63

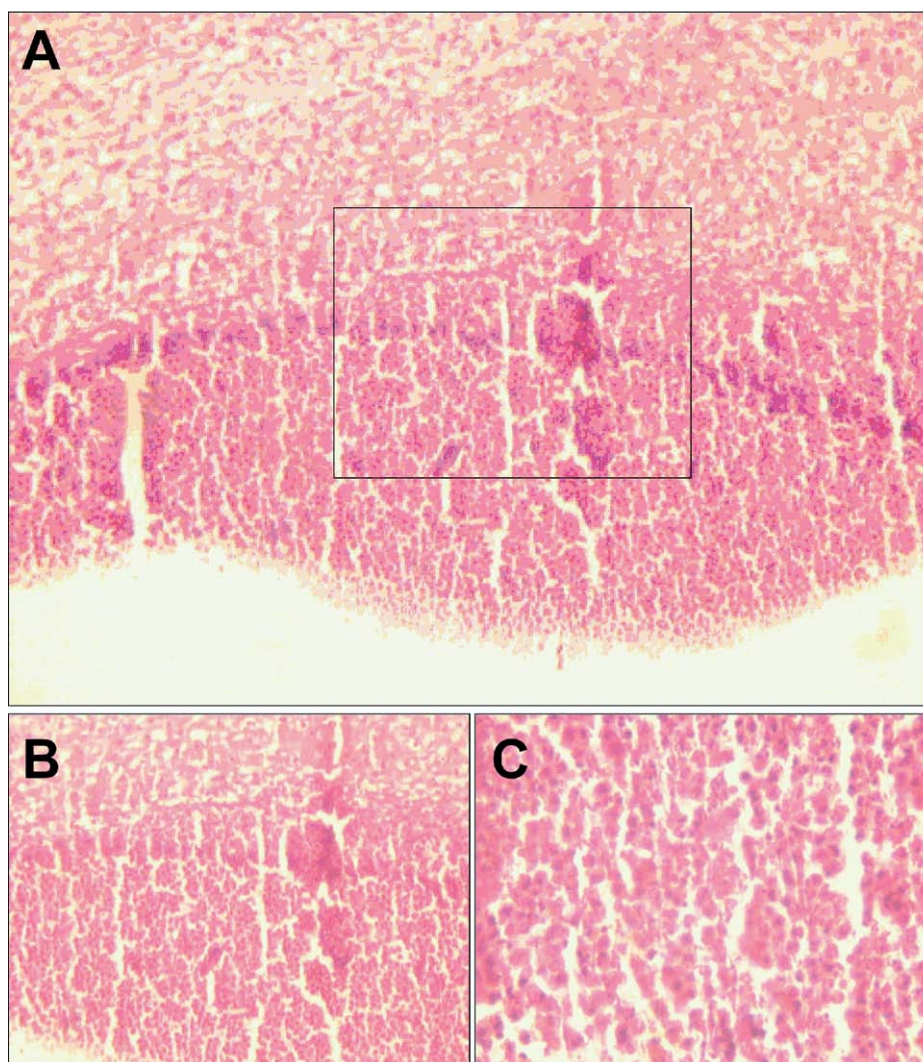


Fig. 3. Portland cement 7 days. **A** and **B**, Intense acute inflammatory infiltrate was observed in the Portland cement sample (hematoxylin and eosin [H&E], original magnification $\times 100$). **C**, Neutrophils ($\times 400$).

Statistical analysis

The preliminary analysis of the raw pooled radiopacity data did not show a normal distribution (Kolmogorov-

Smirnov test). Further statistical analysis was performed using nonparametric test methods: Kruskal-Wallis H-test and Dunn's multiple comparison pos-hoc tests.

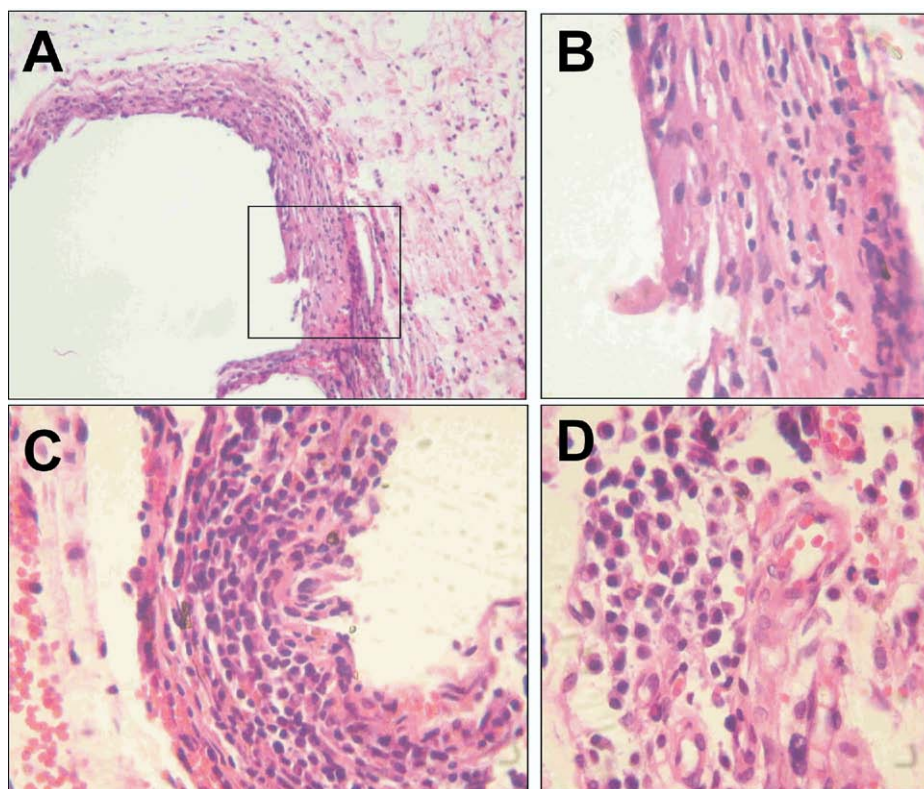


Fig. 4. Biopsy after 60 days. **A**, Overview of the implant area in Portland cement sample (H&E, original magnification $\times 50$). **B**, A higher magnification of the area outlined in **A** showing a discrete chronic inflammatory infiltrate was observed ($\times 100$). **C**, Intense chronic inflammatory infiltrate was observed in the MTA sample ($\times 200$). **D**, A higher magnification of the central area in **C** showing a chronic inflammatory infiltrate with lymphocyte prevalence (H&E, original magnification $\times 400$).

Histological comparison between groups was evaluated through Fisher's test.

In the 2 tests, the alpha-type error was set at 0.05. Statistical Package for Social Sciences (SPSS 11.0, SPSS Inc., Chicago, IL) and Origin 6.0 (Microcal Software, Inc., Northampton, MA) were used as analytical tools.

RESULTS

Analysis of sample radiopacity

There is a direct correlation between material radiopacity and optical density, or coefficient of linear attenuation. Thus, according to this methodology, if the values are closer to zero, the more radiopaque is the material. The results are expressed in Figs. 1 and 2.

The radiopacity of the materials were in decreasing order: Portland cement + BO 1:1 > Portland cement + BO 2:1. No significant statistical differences were observed between the MTA and Portland cement + BO 1:1. Portland cement + BO 2:1 was statistically different from groups MTA and Portland cement + BO 1:1 presenting lowest radiopacities.

Nevertheless, Portland cement + BO 2:1 presented values almost 3 times greater than pure Portland cement. Pure Portland cement was significantly different from the other materials ($P < .01$), presenting the lowest radiopacity of all groups.

Histopathological analysis

The initial and final numbers of implants are presented in Table II. Of a total of 64 implants initially, 53 remained to be evaluated. Eleven implants from animals belonging to different groups were lost during postoperative periods, resulting in some incomplete groups. The histological evaluations of the materials at 7 and 60 days are summarized in Table III and illustrated in Figs. 3 to 6.

Acute inflammatory response

At 7 days, no significant statistical differences were observed ($P > .05$) in the number of neutrophils among the groups (see Fig. 3). Negative control group presented a moderate number of neutrophils per field, significantly lower than the other groups ($P < .03$),

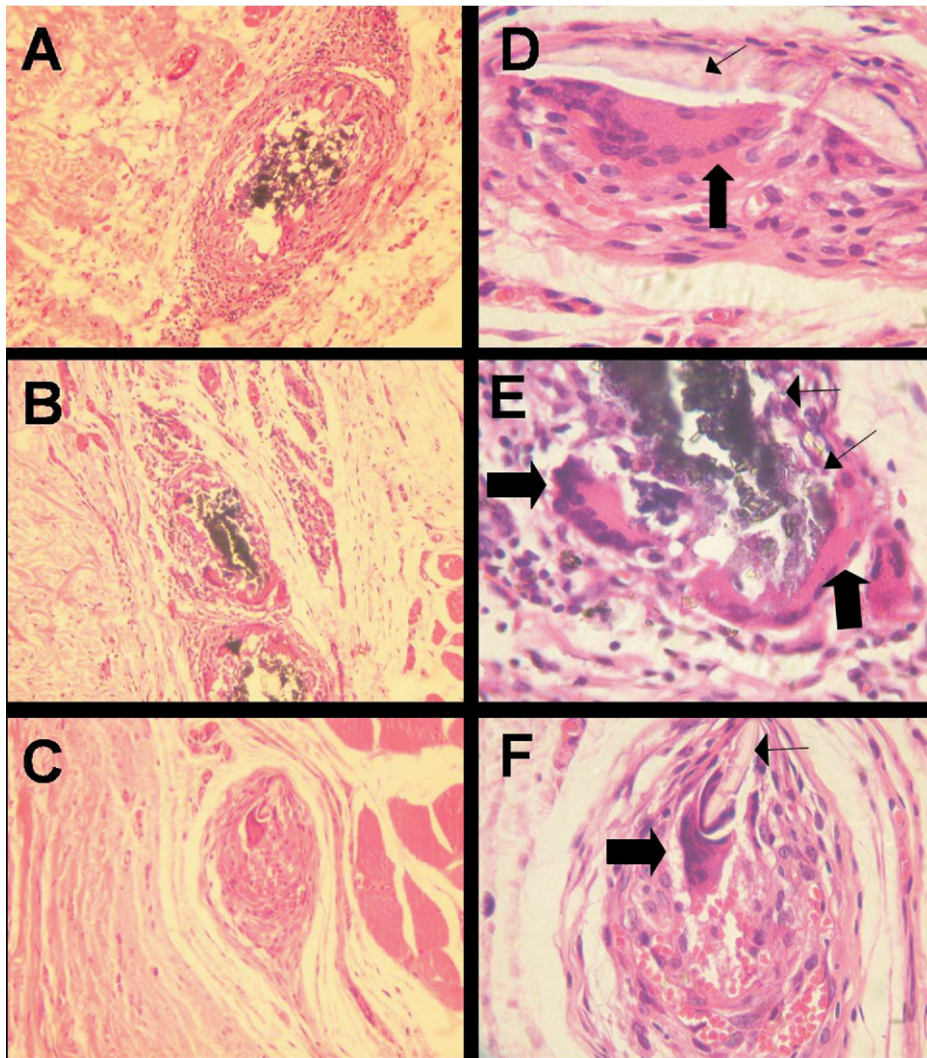


Fig. 5. Biopsy after 60 days. **A, B, and C**, Foreign body-type granuloma surround Portland cement particle (H&E, original magnification $\times 100$). **D, E, and F**, Body giant cells (*thick arrows*) involve the Portland cement particles (*thin arrows*) ($\times 400$).

demonstrating a less intense inflammatory reaction. No difference was found among the experimental groups and positive control group ($P > .2$).

At 60 days, no significant differences were observed among the experimental groups or when compared to the control groups ($P > .2$). However, there was a significant reduction ($P < .0001$) in acute inflammation in all groups when compared with the experimental period of 7 days.

Chronic inflammatory response

A discrete inflammatory infiltrate was observed at 7 days with no statistical differences between experimental and control groups ($P > .4$).

A significant increase in chronic inflammation was seen at 60 days when compared with the same group at

7 days ($P < .001$) (see Fig. 4). Average quantification of mononuclear infiltrate showed no significant differences among the groups tested ($P > .4$).

Giant cells

The presence of giant cells was significantly greater in the group of 60 days ($P < .001$), usually as part of strange body-type granulomas observed in those samples (see Fig. 5). No statistical differences were found between the groups ($P > .06$). Negative control samples at 7 days did not present any giant cells.

Macrophages

A significant increase in the number of macrophages was observed after 60 days when compared with the

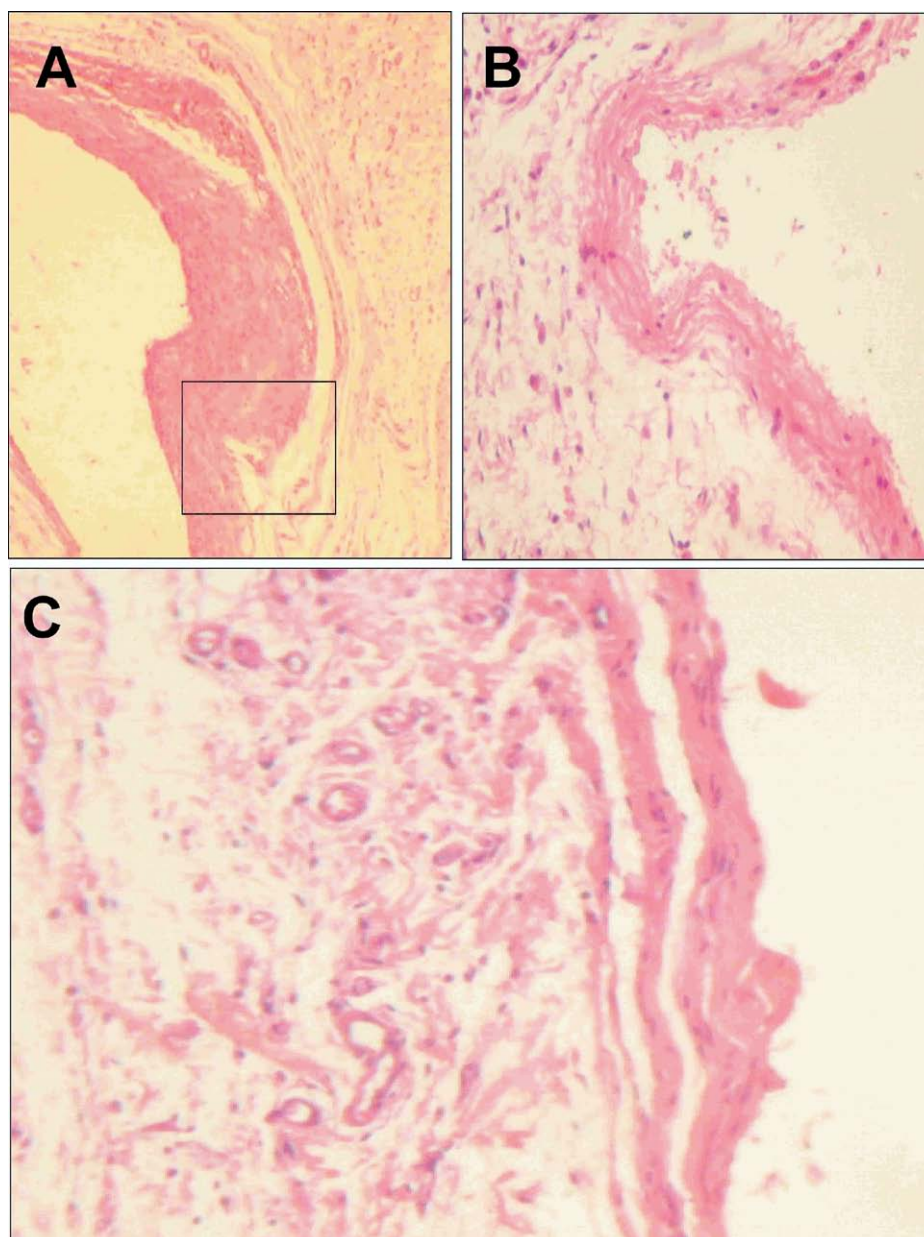


Fig. 6. Biopsy after 60 days. Formation of a thick (A), moderate (B), and thin (C) fibrous capsule in Portland cement samples (H&E, original magnification $\times 100$).

period of 7 days ($P < .01$). No statistical differences were observed among the groups in both experimental periods.

Fibrosis and calcification

Formation of a fibrous capsule of moderate thickness was detected in all samples, including controls, at 60 days (see Fig. 6). In the same group, approximately 20% of the samples presented isolated sites of dystrophic calcification (see Fig. 7).

DISCUSSION

Biocompatibility is one of the most important properties of an endodontic material because it will be in contact permanently with living tissues in the periapical region. Materials used in root-end filling, furcal perforation, and as apical barrier besides the necessary preliminary tests, must have their biocompatibility characteristics investigated.¹² Among the methods recommended for evaluation of dental materials are preliminary tests

Fig. 7. Dystrophic calcification (H&E, original magnification $\times 400$).

that determine a general toxicity profile of the material, a secondary test that evaluates local toxicity, and a test of the tissue response in animal experimentation. Following this sequence seems logical as it results in the elimination of inadequate materials, reduction of indiscriminate use of animal research, and reduction of the possibility of use of incompatible substances in humans.¹³

Several *ex vivo* and *in vivo* investigations have proved that MTA presents many ideal and beneficial properties.^{9,14,15} Such studies, which started as simple laboratory tests and nowadays have involved studies in humans, demonstrated that MTA provides excellent sealing in retrograde fillings¹⁶; is capable of stimulating pulp¹⁷ and periapical regeneration¹⁵; and besides being biocompatible,^{7,18} presents good antimicrobial effect.¹⁹ These findings regarding MTA properties have triggered the development of several studies, as for example, comparison of gray and white MTA²; *in vivo* sealing ability of MTA in endodontically treated teeth with or without an apical plug of MTA²⁰; comparison of the effect of fresh and set MTA in the repair process²¹; adaptation of MTA to root canals with different application techniques²²; and effect of time and humidity regarding setting time, adaptation, and retention of MTA.²³

More recently, many studies have associated MTA with Portland cement. These studies have demonstrated the similarities in composition, mechanism of action, and cytotoxicity of both materials.^{1,5,9,14,7,18} The only exception is the lack of radiopacity of Portland cement.⁵ MTA and Portland cement have the same physical, chemical, and biological properties.⁸ MTA is composed of tricalcium and dicalcium silicate, which on hydration produce calcium silicate hydrate gel and calcium hydroxide.²⁴

In histopathological studies, tissue reactions are affected by shape and size of material particle. Some studies support the direct implantation of the material in the subcutaneous tissue, whereas others support implantation of polyethylene,²⁵ teflon,⁸ or dentin tubes filled with the test materials.⁹ This latter technique simulates a clinical condition, as it retains the material inside the tube providing a pattern for the area of tissue-material interaction. The methodology used in the present study regarding implantation of the material and subsequent contact with the connective tissue followed previous studies. Yaltirik et al.²⁵ observed signs of inflammation in the connective tissue adjacent to empty polyethylene tubes for a period of 15 days after implantation as a result of the trauma caused by tube implantation. The inflammatory infiltrate reduced progressively after the third week. The present work also showed an acute inflammatory reaction in both test and control samples at 7 days, suggesting that the inflammatory reaction observed was a result of the surgical intervention instead of a reaction to the test materials. No infection was present in sutures.

Real regeneration of periapical region requires a mutual effort of osteoblasts, fibroblasts, and cementoblasts to guarantee the repair of bone, periodontal ligament, and cement, respectively.²⁶ Many endodontic materials routinely used as root-end fillings do not provide complete periodontal regeneration.²⁷ MTA induces the formation of cement by allowing gene and protein expression related to the process of cement formation.^{25,26}

The number and quality of the cells in contact with root-end filling materials may be used as a criterion for evaluation of material toxicity.²⁸ In addition to cell morphology, cell growth and cytokine expression were also used by Mitchel et al.²⁹ to study biocompatibility. The biocompatibility of a material is evidenced by new bone and cement formation in the affected region.^{9,14,16} In studies that involve only connective tissue, as this present work, biocompatibility is demonstrated suggestively by the formation of a thin fibrous capsule around the material. Indeed, the formation of a foreign body-type granuloma represents a limiting chronic inflammatory response indicating that the organism was capable of isolating the material from other tissues.

In general, the cellular events and inflammatory response observed in the present study were similar in both experimental periods. No difference was observed between MTA and Portland cement and the addition of bismuth oxide did not interfere with the biocompatibility of the cements. A similar result was reported in a study using MTA, pure Portland cement, and Portland cement plus 20% and 30% bismuth oxide.³⁰ The purpose of the present study was also to analyze if the addition of a

radiopaque substance to Portland cement would affect the tissue response to the material. Both works showed that the biocompatibility of Portland cement was maintained. These results suggest that bismuth oxide may be chemically inert, as reported before.³¹

Trindade et al.³⁰ suggested to manufacturers of MTA to increase the amount of radiopaque substances to enhance radiographic visualization of the materials. Portland cement plus bismuth oxide (1:1) presented similar radiopacity to MTA and was biocompatible in tissue response. This is an interesting observation regarding the lack of radiopacity of Portland cement and how to overcome it. Nevertheless, other studies with different methodological models, increased sample number, and experimental periods are necessary to guarantee clinical applicability of Portland cement and determination of an adequate proportion between Portland cement and bismuth oxide.

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