
Cell and tissue reactions to mineral trioxide aggregate and Portland cement

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Objective. Mineral trioxide aggregate (MTA) is being widely used for root-end fillings, pulp capping, perforation repairs, and other endodontic procedures. MTA and Portland cement (PC) have many similar physical, chemical, and biologic properties. PC cement has shown promising potential as an endodontic material in several studies in vitro and in vivo. The purpose of this study was to compare the cytotoxic effect in vitro and the tissue reaction of MTA and Portland cement in bone implantation in the mandibles of guinea pigs.

Study design. Millipore culture plate inserts with freshly mixed or set material were placed into the culture plates with already attached L929 cells. After an incubation period of 3 days, the cell morphology and cell counts were studied. Adult male guinea pigs under strict asepsis were anesthetized, during which a submandibular incision was made to expose the symphysis of the mandible. Bilateral bone cavities were prepared and Teflon applicators with freshly mixed materials were inserted into the bone cavities. Each animal received 2 implants, one filled with ProRoot and 1 with PC. The animals were killed after 2 or 12 weeks, and the tissues were processed for histologic evaluation by means of light microscopy.

Results. There was no difference in cell reactions in vitro. Bone healing and minimal inflammatory response adjacent to ProRoot and PC implants were observed in both experimental periods, suggesting that both materials are well tolerated.

Conclusions. MTA and PC show comparative biocompatibility when evaluated in vitro and in vivo. The results suggest that PC has the potential to be used as a less expensive root-end filling material.

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Mineral trioxide aggregate (MTA) was developed as a root-end filling material for periapical surgery and for the sealing of communications between the root canal system and the surrounding tissues.¹ MTA was shown to be superior to other commonly used root-end filling materials such as amalgam, IRM, and Super-EBA in studies of marginal adaptation and leakage.²⁻⁵ MTA was reported to be biocompatible in many studies in vitro and in vivo. In 1998, Koh et al^{6,7} studied the cytomorphology of osteoblasts and cytokine production in the presence of MTA. They reported that MTA offers a biologically active substrate for bone cells and stimulates interleukin production. Mitchell et al⁸ reported that MTA is biocompatible and

suitable for clinical trials. Zhu et al⁹ reported that osteoblasts have a favorable response to MTA as compared with IRM and amalgam. Several reports have also indicated that MTA has an antimicrobial effect.^{10,11}

At present, MTA is widely used in endodontic therapy. It has shown good results when used in the treatment of immature apices, for repairs of perforations, and for direct pulp capping as in root-end filling.¹²

Recently, studies have compared MTA with Portland cement (PC) and the findings suggest that PC has major ingredients in common with MTA. Some of these ingredients are calcium phosphate, calcium oxide, and silica. MTA also contains bismuth oxide, which increases its radiopacity; this is absent in PC.^{11,13} MTA and PC have almost identical properties macroscopically, microscopically, and by x-ray diffraction analysis.¹⁴ It was also shown that PC and MTA have a similar effect on pulpal cells when used as a direct pulp-capping material in rats.¹⁴ Furthermore, osteoblast-like cells had similar growth and matrix formation when grown on either set MTA or PC.¹⁴ Both MTA and PC allowed for dentin bridge formation after pulpotomy was performed on dogs.¹⁵ MTA and PC formed calcite crystal granulations when placed in dentin tubes that were implanted subcutaneously in rats, suggesting that they are similar materials.¹⁶ MTA and PC also have comparable antibacterial activity.¹¹

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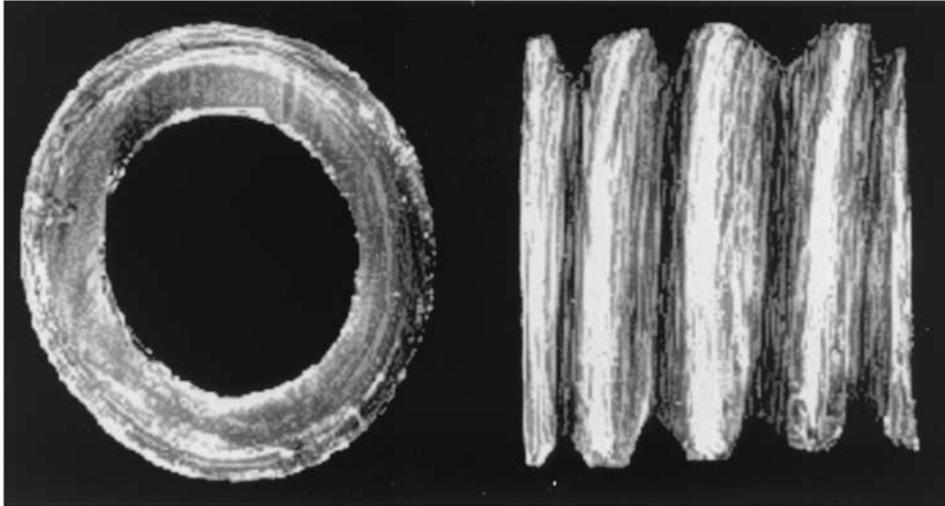


Fig 1. Cylindrical applicator for the controlled implantation of materials. The hollow applicator is made of Teflon (diameter, 2 mm; length, 2 mm).

However, MTA is a costly material. Given the low cost and apparently similar properties of PC (in comparison with MTA), it is reasonable to consider PC as a possible substitute for MTA in endodontic applications.

MTA has favorable bone reaction when implanted in the tibia and mandible of guinea pigs.¹⁷ However, there have not been any reports that compared the bone reaction to MTA with the reaction to PC. In addition, no studies have been conducted *in vitro* to compare freshly mixed PC with MTA. Therefore, the purpose of this study was to evaluate the cytotoxic response to freshly prepared MTA and PC and to compare the tissue reaction to MTA and PC in bone when implanted in the mandible of the guinea pig.

MATERIAL AND METHODS

Experiment *in vitro*

L929 mouse fibroblasts were obtained from the American Type Culture Collection (Manassas, Va). Cells were grown in Eagle's minimum essential medium supplemented with 10% fetal bovine serum (Hyclone Laboratories Inc, Logan, Utah) and 1% antibiotic/antimycotic cocktail (300 units/mL of penicillin, 300 µg/mL of streptomycin, 5 µg/mL of amphotericin B; Gibco BRL, Gaithersburg, Md) under standard cell culture conditions (ie, 37°C, 100% humidity, 95% air, and 5% CO₂).

The materials used in this study were MTA (ProRoot, Dentsply Tulsa Dental, Tulsa, Okla) and PC (Quikrete, Columbus, Ohio). The ProRoot was mixed according to the manufacturer's instructions. The PC was sterilized with ethylene oxide and mixed to a

consistency similar to ProRoot. In 1 set of experiments, fresh pellets (1 × 1 × 3 mm³) of the tested materials were used. In another set of experiments, the pellets of the tested material were allowed to set for 2 weeks in cell culture medium at 37°C. The medium was changed every day during this time.

Cells were seeded into 6-well cell culture plates (Sigma, St Louis, Mo) at an initial density of 2×10^5 cells/well with 2 mL of medium. The cells were incubated for 24 hours to allow adhesion. Culture plate inserts (30 mm in diameter, 0.4 µm pore size; Millipore Corp, Bedford, Mass), each with 1 pellet of material, were placed into culture plates. Cells cultured with only a culture plate insert served as controls. These experiments were undertaken with freshly prepared or set materials. Each group was studied in triplicate experiments. After an incubation period of 3 days, the cell morphology was examined under a phase-contrast microscope (CK40 Culture Microscope; Olympus American Inc, Melville, NY) and photographed. Subsequently, cells were detached with 0.25% trypsin and 1 mmol EDTA in Hanks' balanced salt solution (Gibco BRL). The detached cells were electronically counted (Coulter Electronics, Hialeah, Fla). The data were statistically analyzed by using 1-way analysis of variance to compare total cell numbers per well within the groups of fresh or the set material.

Experiment *in vivo*

The experimental protocol was approved by the Animal Care Committee at the University of Connecticut Health Center.

The materials were implanted by using Teflon applicators in a manner previously described.¹⁸ Teflon is a

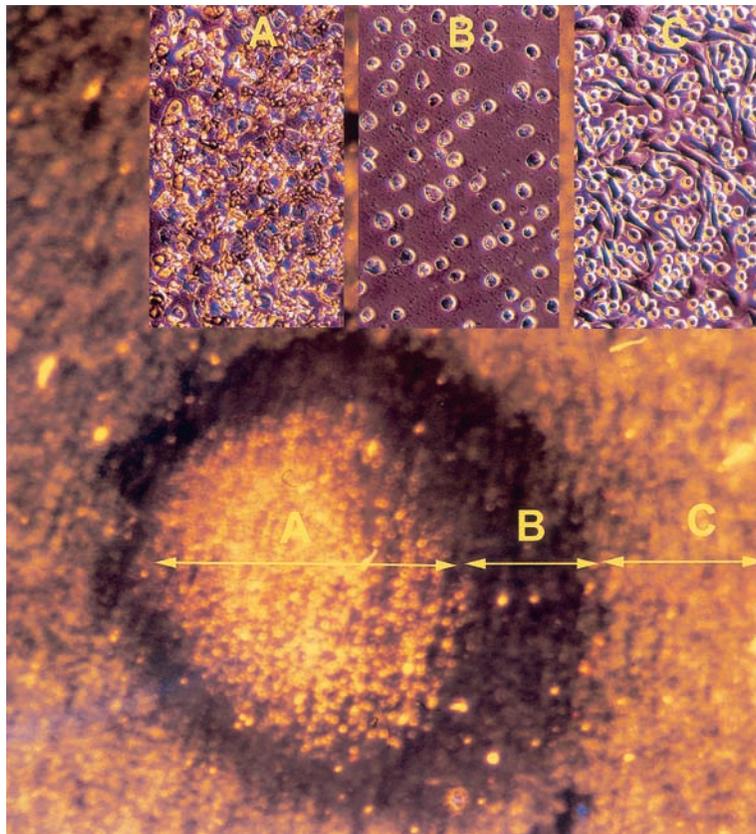


Fig 2. Cell reaction to freshly prepared ProRoot and Portland cement (L929 cells). **A**, Area under freshly prepared material. The cells and medium proteins are coagulated. **B**, The cells are lysed in an area surrounding the coagulated zone directly under the material. **C**, At a distance from the freshly prepared material, the cells grow normally.

biocompatible polymer and, as a solid, causes no tissue reaction. Therefore, the surface of a Teflon applicator, when used as a carrier of the experimental material, can serve as the negative control. The cylindrical Teflon applicator has a diameter of 2 mm and a length of 2 mm (Fig 1). It has an opening at 1 end, where the experimental material is inserted. The outer surface of the Teflon applicator has a spiral ridge to anchor it firmly after insertion in the bone.¹⁸ The applicators were autoclaved before use.

Twenty-eight male guinea pigs, each weighing 750 to 850 g, were used in this study. Each animal was anesthetized by an intramuscular injection of ketamine (40 mg/kg; Fort Dodge Animal Health, Fort Dodge, Iowa), and xylazine (5 mg/kg; Lloyd Laboratories, Shenandoah, Iowa).

The submandibular surgical site of each animal was shaved and disinfected with 5% tincture of iodine. Local anesthesia was administered by infiltration with 0.25 mL of 3% Carbocaine (Abbott Laboratories, North Chicago, Ill). After an extraoral skin incision in the midline over the mandible was made, the symphysis

Table I. Total cell number per well of mouse fibroblasts after 3-day incubation with MTA or Portland cement

	Control (SD)	ProRoot (SD)	Portland cement (SD)
Fresh ($\times 1000$)	960.05 (31.33)	881.27 (50.83)	825.24 (36.23)
Set	960.05 (31.33)	980.68 (63.73)	937.73 (16.36)

Values are means.
 MTA, Mineral trioxide aggregate.

was located. In conjunction with the use of copious irrigation with saline solution, the bilateral bone cavities were prepared at 2 to 3000 rpm by using a series of 3 burs of increasing size. The last bur created a cavity fitting the applicator exactly. Both ProRoot and PC were mixed with sterile saline solution to achieve the desired consistency and were packed freshly into the Teflon applicators. Each animal received 1 ProRoot implant and 1 PC implant. The implants were immediately inserted into the bone cavity with the open end in contact with the bone. After secured placement of the

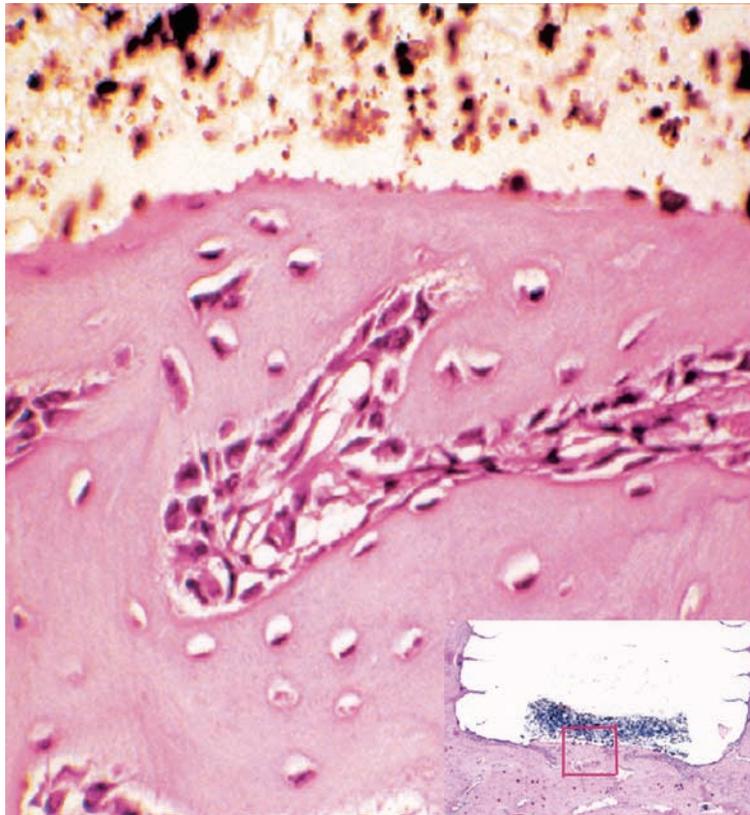


Fig 3. The ProRoot implant at the 2-week observation period. Type I response. *Insert* shows remnants of ProRoot (*black area*). The *square* in the *insert* indicates the enlarged area. The bone has healed in direct contact with the implanted material (hematoxylin-eosin, original magnification $\times 250$).

implants, the muscle layer and skin were repositioned and sutured with absorbable gut sutures. Fourteen guinea pigs were euthanized after 2 weeks, and the remaining 14 were euthanized after 12 weeks. The animals were euthanized by a CO₂ overdose. The mandibles were dissected free of the soft tissue and prefixed in 10% formalin. Within an hour, the mandible was trimmed back to a final specimen size of approximately 5 mm and fixed in formalin at 4°C for 24 hours. The specimens were decalcified with Cal-Ex (Fisher Diagnostics, Fair Lawn, NJ). The samples were dehydrated in alcohol, embedded in paraffin, and serially sectioned with a microtome set at 5 μ m. Sections were stained with hematoxylin and eosin. Histologic slides of the tissue adjacent to the implanted materials were evaluated under a light microscope by 2 examiners (J.S., L.S.W.S.) who were blinded to the type of material was used.

Tissue reactions to the materials were unremarkable and very similar. Therefore, the presence of inflammatory cells and the degree of hard and soft tissue healing were the only criteria considered. The hard and soft tissue criteria used were as follow:

- Type I reaction: New bone apposition in direct contact with the material.
- Type II reaction: Newly formed bone separated from the material by a thin layer of fibrous connective tissue.

The outcomes of the implants were recorded. The results were subjected to χ^2 analyses. Differences were considered significant at $P < .05$.

RESULTS

Experiment in vitro

In the control groups, the L929 mouse fibroblasts grew and formed a confluent monolayer. The effects of the fresh ProRoot and PC on cells were similar. There were denatured medium proteins and dead cells in the area just beneath the inserts with the materials (Fig 2, A). Around this area of dead cells a zone of lysed cells (Fig 2, B) was observed. Normal cell growth was seen beyond the lysed zone (Fig 2, C). The set ProRoot and PC had no effect on cell appearance.

Table I shows the total cell numbers after a 3-day incubation with ProRoot or PC. The total cell number in the fresh ProRoot and the fresh PC groups was significantly lower ($P < .01$) than that in the control

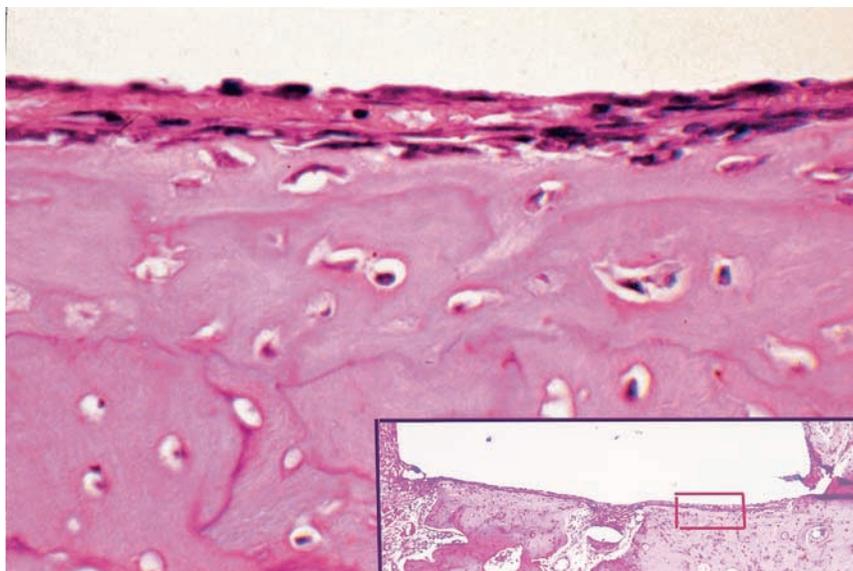


Fig 4. The ProRoot implant at the 2-week observation period. Type II response. The *square* in the *insert* indicates the enlarged area. The ProRoot material was lost during histologic preparation. The bone tissue has healed well, but a thin layer of fibrous connective tissue separates the bone tissue from direct contact with the implanted material. No inflammatory cells were present (hematoxylin-eosin, original magnification $\times 250$).

group. There was no statistically significant difference in cell numbers between ProRoot and PC ($P > .05$). In the set material group, there was no statistically cell number difference either between the control group and the tested material groups ($P > .05$) or between the set ProRoot and the PC groups ($P > .05$).

Experiment in vivo

Two animals died of unrelated causes, leaving 13 animals in each group for evaluation. The surgical sites healed with no objective signs of infection.

Two-week results. No implant had a noticeable accumulation of inflammatory cells. In the PC group, 7 implants had a type I reaction and 6 had a type II reaction. In the ProRoot group, 8 implants had a type I reaction (Fig. 3) and 5 implants showed type II reaction (Fig 4). There was no statistical difference in the distribution between the 2 groups (Table II).

Twelve-week results. In the PC group, 10 implants had a type I reaction (Fig 5) and 3 implants had a type II reaction. In the ProRoot group, 7 implants had a type I reaction and 6 implants had a type II reaction. No statistically significant difference was seen between the groups (Table III).

Although no chronic inflammatory cells were observed in any of the 12-week implants, 2 of the ProRoot implants had a dense accumulation of macrophages (Fig 6).

Table II. Bone implants in the guinea pig (2-week results)

Material	Type I reaction	Type II reaction	Total
ProRoot	8	5	13
Portland cement	7	6	13
Total	15	11	26

$\chi^2 = 0.1576$, not significantly different at the 0.05 level.

Table III. Bone implants in the guinea pig (12-week results)

Material	Type I reaction	Type II reaction	Total
ProRoot	7	6	13
Portland cement	10	3	13
Total	17	9	26

$\chi^2 = 1.529$, not significantly different at the 0.05 level.

DISCUSSION

The pellets of the 2 materials were placed into the cell culture wells with culture inserts to eliminate the material particles from the cells, which were electronically counted. Preliminary results showed that placing the material pellets directly into the cell culture wells and placing the material pellets through the culture inserts resulted in no significant differences. The size of the material pellets was chosen on the basis of the approximate amount used clinically.

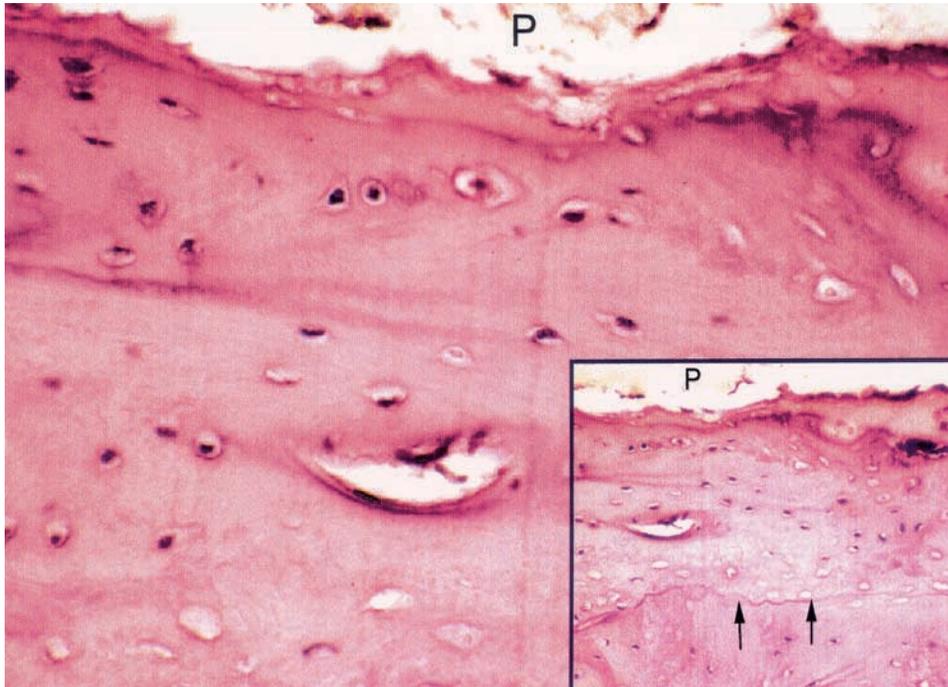


Fig 5. The Portland cement implant at the 12-week observation period. Type I response. The Portland cement, which was lost during histologic preparation, was located at *P*. The floor of the originally prepared bone cavity is indicated with *arrows*. The newly formed bone is healthy with direct implant contact (hematoxylin-eosin, original magnification $\times 250$).

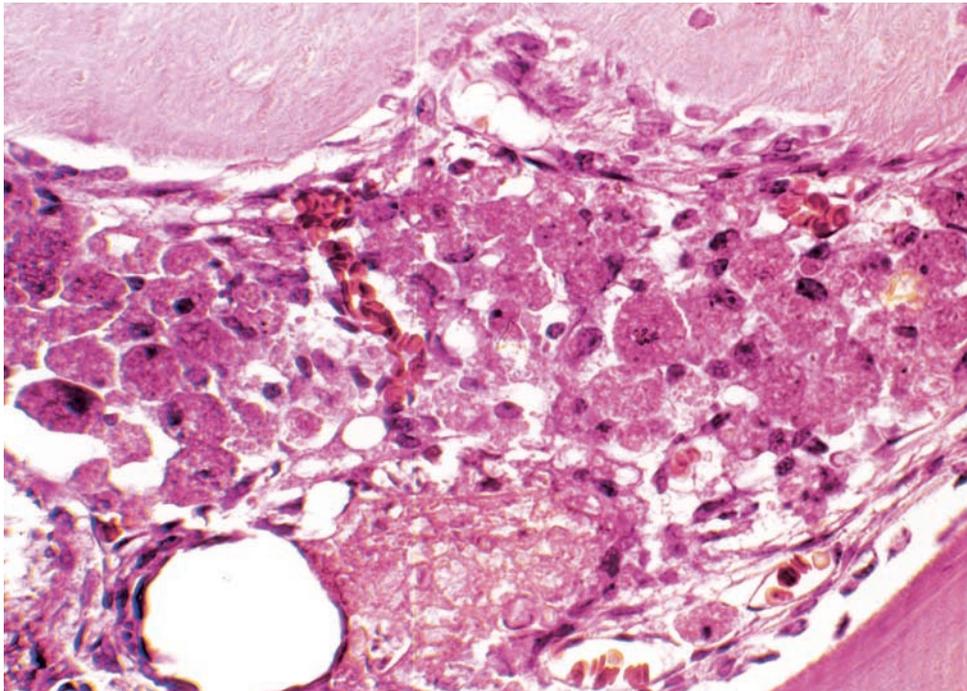


Fig 6. The ProRoot implant at the 12-week observation period. A significant accumulation of macrophages is seen in tissue between the healing bone and the implant material. The macrophages are filled with particles (hematoxylin-eosin, original magnification $\times 250$).

Because of the high surface pH, especially when freshly mixed, ProRoot and PC caused denaturation of adjacent cells and medium proteins. As the materials set, the pH changes and the cell injuries subside. The reaction patterns and cell reactions were identical for PC and ProRoot.

Tissue reactions associated with ProRoot and PC implants were comparable, with a large number of implants having direct bone integration. There were no inflammatory processes associated with most of the implants, suggesting that both materials are equally biocompatible.

An assumption that the particle size of ProRoot is smaller than that of PC may explain the distinct accumulation of macrophages in the ProRoot implants. Although an important finding, the number of cases with macrophages is too small to draw any definite conclusion. This matter should be studied further because the significant accumulation of macrophages in these 2 implants is of concern.

Our results are comparable with those of other published studies in which the tissue reaction to PC was compared with the tissue reaction to ProRoot.¹³⁻¹⁶

The results from our study support the idea that PC has the potential to be used in clinical situations similar to those in which MTA (ProRoot) is being used. Although the results are very encouraging, more studies in human beings need to be conducted before unlimited clinical use can be recommended.

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