Biocompatibility and setting time of gray Portland cement clinker with or without calcium sulfate

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ABSTRACT

Objective: To evaluate the biocompatibility of Grey Portland Cement Clinker without and with 2% and 5% calcium sulfate. **Methods:** Twenty-four mice received subcutaneously polyethylene tubes filled with grey Portland Cement Clinker without or with 2% or 5% calcium sulfate. After 15, 30 and 60 days of implantation, the animals were killed and specimens were prepared for microscopic analysis. Setting times of each material were also evaluated according to the ASTM specification # C266-08. ANOVA and Tukey's test for setting time and Kruskal Wallis and Dum test for biocompatility at 5% significance level were used. **Results:** Histologic observation showed no statistical difference among the materials in the subcutaneous tissues. **Conclusion:** Clinker without calcium sulfate showed 5 min for initial setting time and 55 min for final setting time, followed by clinker with 2% sulfate calcium (8/95 min) and clinker with 5% sulfate calcium (10/110 min).

Keywords: Mineral Trioxide Aggregate. Clinker. Portland cement.

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Introduction

Mineral Trioxide Aggregate (MTA) is currently used for endodontic therapy such as root-end filling, perforation repair, resorptive defect repair, pulpotomy and apexification,[5] due to its good marginal adaptation, sealing ability, antimicrobial activity and biocompatibility.[6-10] MTA is basically Portland cement type 1 with bismuth oxide added for radiopacity.[6,11,12] Portland cement is manufactured by a clinkering process of these unprocessed materials. The components of Portland cement produce calcium hydroxide and a silicate hydrate gel during hydration responsible of the biocompatibility of the cement.[13-15] The biocompatibility of MTA and Portland cement has previously been tested in implantation tests,[8,16,17] human cells test[9] and in human and animal direct pulp capping procedures.[4,18] These studies showed similar results for Portland cement and MTA.

In cement industry, the gypsum is added to the Portland cement in amounts of 3-6% to retard the setting time and this was confirmed by Camilieri[15] that showed decrease of the setting time of Portland cement excluding the gypsum in the final stage of manufacturing. Significant reduction in setting time is helpful during clinical procedures because of MTA exhibits a longer setting time.[19-26] Clinker cement is the main component of Portland cement and has an adequate setting time, alkaline pH and calcium release.

In 2003, in Argentina, was launched Endo CPM sealer, the first root canal filling material MTA-based.

The aim of this study was to evaluate the subcutaneous tissue response to implantation of polyethylene tubes containing grey Portland cement clinker without /with 2% and 5% calcium sulfate and Endo CPM sealer. Setting times of each material were also evaluated.

Material and methods

The research protocol was approved by the Ethics Committee for Research on Animals # 02/2006. The materials used in this study were: grey Portland cement clinker without calcium sulfate (Fancesa, Sucre, Bolivia), grey Portland cement clinker with 2% or 5% calcium sulfate (Carlo Erba Reagents, Italia) and Endo CPM sealer (EGEO SRL, Argentina). Grey Portland cement clinker was served under laboratory conditions to a particle size of 0.062 mm (Bronzinox, Brasil).

Twenty four adult male Wistar albinos rats (Rattus norvegicus albinos), weighing between 200-250 grams were used for this experiments. Animals were anesthetized by intramuscular dose of 25 mg/kg cloridrate of ketamine and 10 mg/kg cloridrate of xylazine. In each animal, two anterior and two posterior incisions were made through the dorsal skin using a nº 15 scalpel blade. A blunt dissecting instrument was used to create a 20 mm deep pocket in the subcutaneous tissue to receive the implants. Polyethylene tubes (1 mm diameter and 10 mm length) were filled either with Portland cement clinker without calcium sulfate, Portland cement clinker with 2% of calcium sulfate, Portland cement clinker with 5% of calcium sulfate and Endo CPM sealer in power/liquid ratio 3:1. Other side of the tube was sealed with gutta-percha and served as control. Each animal received four implants. The wounds were sutured, with removal of sutures after 7 days.

After 15, 30 and 60 days of implantation, the animals were killed by overdose of Ketamine. The connective tissue containing the implant were excised and kept in 10% formalin. A section parallel to the long axis of the tube was made with 5 µm-thick and stained with hematoxylin and eosin. The tissue reactions at the end of the tubes in direct contact with the cements were evaluated and scored as: 0 – without inflammation cells (no reaction); 1:<25 inflammatory cells (mild reaction); 2:25-125 inflammatory cells (moderate reaction); 3:>125 inflammatory cells (severe reaction). A Kruskal-Wallis test was carried out to determine the significant difference for the inflammatory response of the test materials ($p < 0.05$).

The setting time of the cements tested were carried out under controlled temperature and humidity (37+/-1ºC and 95+/-5% relative humidity) according to the International Stands Organization (ISO) 6876 specification and the ASTM C266-03 standard test. The material were mixed and inserted in metallic ring mold (10 mm in diameter and 2 mm thickness). Three specimens were fabricated for each cement. After 180 seconds, each specimen was indented with the 113.4g Gilmore needle for determining initial setting time. After the initial setting time a 456.5g Gilmore needle was used to determine the final setting time. The data were submitted to statistical analysis using the ANOVA and Tukey test for multiple comparisons ($p < 0.05$).

Results

Microscopic analysis

In the period of 15 days was observed a fibrous capsule formation outlining the polyethylene tube. The capsule was thicker, with collagen fibers arranged around the cement and a large number of fibroblasts, showing high synthetic activity. This area also presented small blood vessels and few inflammatory cells. Endo-CPM showed a greater inflammatory response with statistical significant differences among the materials ($p < 0.05$). Clincker with 2%, 5% and without calcium sulfate showed similar results, with no statistical significant difference among the materials ($p < 0.05$) (Fig 1).

In 30 days the tissue response was similar to the 15 days period. However, more organized capsule was found with collagen fibers disposed parallel to the cement containing few fibroblasts and inflammatory cells; predominantly, macrophages. Collagen fibers, in this period were thicker, blood vessels less numerous and there were smaller empty spaces than the 15 day period indicating a larger degree of tissue organization. There was no statistical significant difference among the materials ($p < 0.05$) (Fig 2).

In 60 days the tissue showed a thick fiber structure parallel positioned to the cement surface. There were few fibroblasts, little visualization of other structures,

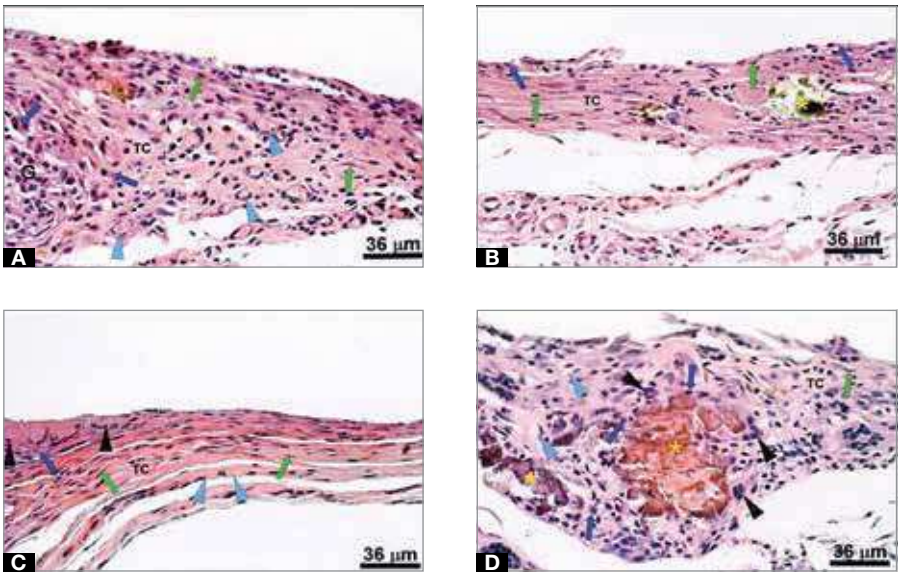

Figure 1. 15 days: **A**) Clincker without calcium sulfate; **B**) Clicker with 2% calcium sulfate; **C**) Clincker with 5% calcium sulfate; **D**) CPM sealer.(blue arrow: macrophage; blue arrow head: blood vessels; black arrow head: giant cells; CT: connective tissue; green arrow: fibroblasts; asterisk: extruded material). HE 40x

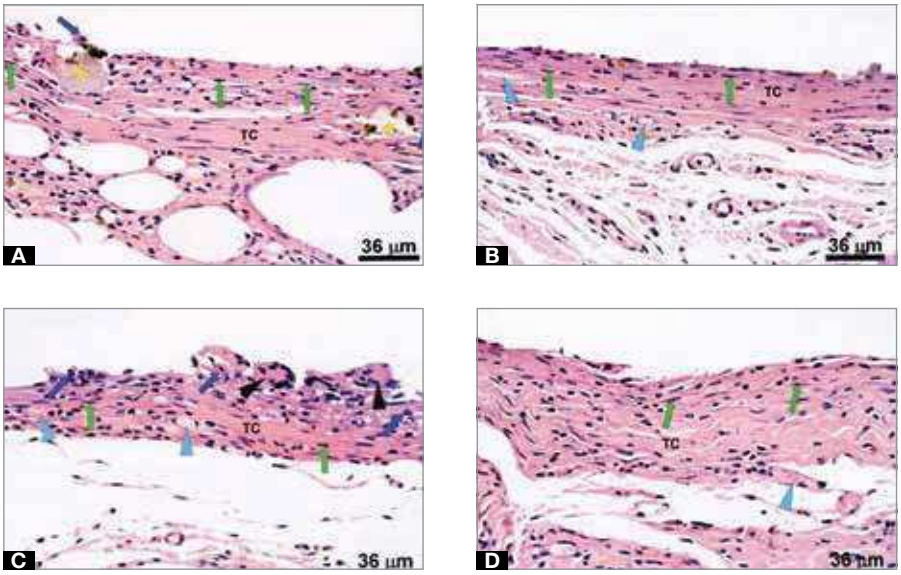

Figure 2. 30 days: **A**) Clincker without calcium sulfate; **B**) Clicker with 2% calcium sulfate; **C**) Clincker with 5% calcium sulfate; **D**) CPM sealer. (blue arrow: macrophage; blue arrow head: blood vessels; black arrow head: giant cells; CT: connective tissue; green arrow: fibloblasts; asterisk-extruded material). HE 40x

decreasing in quantity. There were few blood vessels and absence of inflammatory cells, with no statistical significant difference among materials ($p < 0.05$) (Fig 3).

In the three experimental periods the clinker without calcium sulfate showed very little inflammatory response.

In control (gutta-percha) the tissue response was similar to the 15, 30 and 60 days period, showed a thick fiber structure parallel positioned to the material. There was no statistical significant difference among the different periods ($p < 0.05$) (Fig 4).

Figure 1 to 4 shows tissue response for grey Portland cement clinker without calcium sulfate, grey Portland cement clinkler with 2% or 5% calcium sulfate, Endo CPM sealer and control.

Table 1 show mean scores attributed to the inflammatory cells, adjacent to implanted material surface at 15, 30 and 60 days.

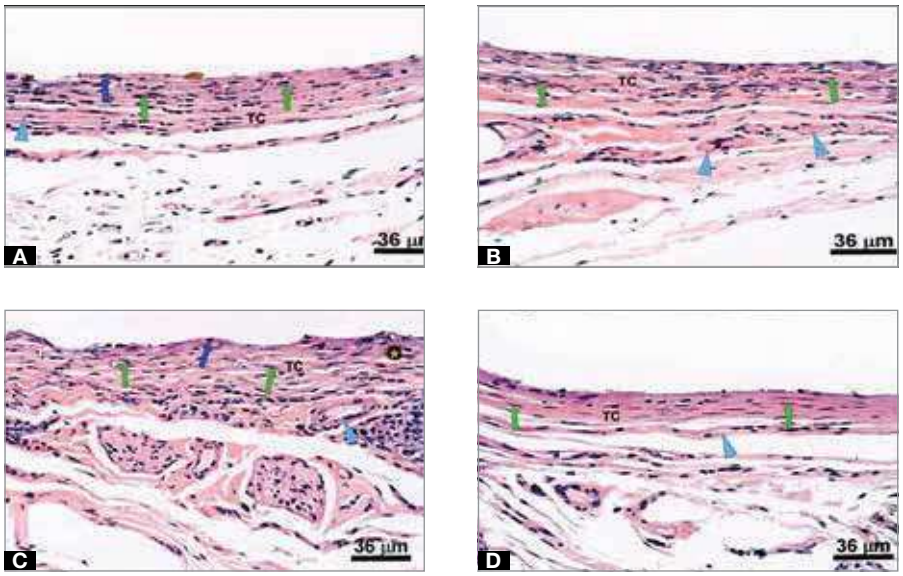

Figure 3. 60 days: **A**) Clincker without calcium sulfate; **B**) Clicker with 2% calcium sulfate; **C**) Clincker with 5% calcium sulfate; **D**) CPM sealer.(blue arrow: macrophage; blue arrow head: blood vessels; CT: connective tissue; green arrow: fibroblasts). HE 40x

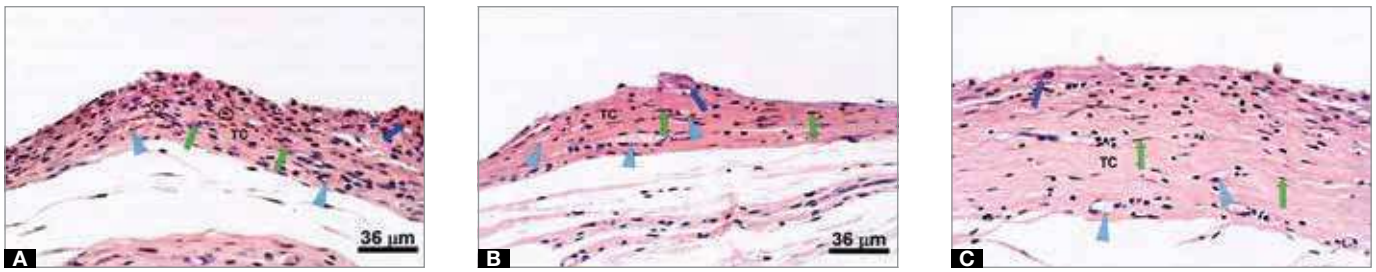

Figure 4. Control: gutta-percha **A**) 15 days; **B**) 30 days; **C**) 60 days. (blue arrow: macrophage; blue arrow head: blood vessels; CT: connective tissue; green arrow: fibroblasts). HE 40x

Table 1. Mean scores attributed to the inflammatory cells, adjacent to implanted material surface at 15, 30 and 60 days period.

	15 d	30 d	60 d
Endo CPM Sealer	2	1	1
Clinker	0	0	0
Clinker + 2% $CaSO_4$	1	1	1
Clinker + 5% $CaSO_4$	1	2	1

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

Table 2. Mean of initial and final setting times (values expressed in minutes).

Material	Initial setting Time	Final setting Time
Endo CPM Sealer	6	22
Clinker	5	55
Clinker + 2% $CaSO_4$	8	95
Clinker + 5% $CaSO_4$	10	110

Setting time

The initial and final setting time for all material are shown in Table 2. Endo CPM sealer show shortened initial and final setting times (6/22 min); Clinker without calcium sulfate show initial setting time 5 min and final setting time 55 min, followed by clinker with 2% sulfate calcium (8/95 min), clinker with 5% sulfate calcium (10/110 min).

Discussion

Several studies examined the similarities in the physical, biologic and microbiologic aspect of MTA and Portland cement.[4,6,7,9,10,12,18,24]

The present study shows that all the tested materials presented a similar behavior with small differences regarding to the evaluated periods. During the literature review, there were no found *in vivo* biocompatibility studies about the gray Portland cement clinker. Consequently, a comparison among the materials was performed since they have the same composition of Portland cement clinker. The good biocompatibility of MTA and Portland cement was verified in several studies and is basically attributed to calcium hydroxide formation after hydration.[4,13,14]

In 15 days period, Portland cement clinker without calcium sulfate exhibited the smallest amount of inflammatory cells in comparison to another cement without statistical significant differences. Few specimens showed overfilled cement. Collagen fibers density, in 30 and 60 days periods, increased providing a more organized and dense fibrous capsule with decrease of the fibroblast number and blood vessels decreased when compared to shorter periods, demonstrating a better organization and tissue maturation with time. A thin fibrous capsule, with few chronic inflammatory cells (macrophages, lymphocytes, and multinucleated giant cells) circumscribed the material, in the 15 days period. These cells diminished in the 30 and 60 days periods.

Regarding to materials setting time studies show an initial setting time of 40 minutes for MTA ProRoot and 12 minutes for MTA-Angelus[11,20,25] MTA final setting times reported are 140-170[11,20,24] and Portland cement initial and final setting times have been 70 minutes and 170 minutes [20,25] respectively. In our study we observed that clinker Portland cement without calcium sulfate exhibited the smallest initial setting time (6.18 minutes), followed by Portland cement clinker with 2% calcium sulfate (9.22 minutes), and Portland cement clinker with 5% calcium sulfate (10.06 minutes). The results of setting time of Portland cement clinker are similar to the results reported by Camilleri[15] that reported initial and final setting times of 6 and 12 minutes.

Conclusion

The inflammatory response of grey clinker Portland cement without or with 2% or 5% calcium sulfate is similar when they were implanted in mice's subcutaneous tissue for 15, 30, and 60 days. Endo CPM sealer show the smallest initial and final setting time following by grey clinker Portland cement without calcium sulfate, grey clinker Portland cement with 2% and 5% calcium sulfate. Calcium sulfate delayed the grey Portland cement clinker setting time.

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