Dental Traumatology

Dental Traumatology 2012; **28**: 452–456; doi: 10.1111/j.1600-9657.2011.01096.x

Rat tissue reaction to MTA FILLAPEX®

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Accepted 6 November, 2011

Abstract – The aim of this study was to evaluate the rat subcutaneous tissue reaction to implanted polyethylene tubes filled with mineral trioxide aggregate (MTA) FILLAPEX® compared to the reaction to tubes filled with Sealapex® or Angelus MTA®. These materials were placed in polyethylene tubes and implanted into the dorsal connective tissue of Wistar rats for 7, 15, 30, 60, and 90 days. The specimens were stained with hematoxylin and eosin or Von Kossa or left unstained for examination under polarized light. Qualitative and quantitative evaluations of the reaction were performed. All materials caused moderate reactions after 7 days, which decreased with time. The reactions were moderate and similar to that evoked by the control and Sealapex® on the 15th day. MTA FILLAPEX® and Angelus MTA caused mild reactions beginning after 15 days. Mineralization and granulation birefringent to polarized light were observed with all materials. It was concluded that MTA FILLAPEX® was biocompatible and stimulated mineralization.

Generally the main goal of root canal therapy is the proper cleaning and shaping of the root canal system followed by filling of the canal with gutta-percha and sealer. Ideally, sealers should have favorable physical and chemical properties (1). In addition, it is highly desirable for sealers to be biocompatible because they can come in direct contact with the periodontal tissues through the apical foramen and accessory communications. Because they could delay wound healing, it is important to study the reaction of tissues to these sealers before their clinical use (2).

The presence and release of substances from sealers may generate different reactions when in contact with tissues. The reaction varies according to the substance, the amount released, and the resorption speed. Sealapex® (SybronEndo, Glendora, CA, USA) is a sealer that contains calcium oxide (CaO), which in contact with water forms calcium hydroxide ($Ca(OH)_2$) (3, 4). Sealapex® has been shown to induce only a mild inflammatory reaction when it contacts the periapical tissues (2, 3, 5).

Mineral trioxide aggregate (MTA) has been extensively studied. It was designed to be used in pathologic or iatrogenic root perforations and in root-end cavities (6, 7). Studies have shown that MTA promotes favorable tissue reactions characterized by the absence of severe inflammatory reactions, the presence of a fibrous capsule, and the induction of mineralized repair tissue (8, 9). However, despite its favorable characteristics, MTA does not exhibit the physical properties needed to be used as a sealer, owing to its working time, setting time, and difficult handling (1, 10).

An MTA-based sealer (Angelus®; Londrina, Paraná, Brazil) was recently introduced to the market. It is a paste–paste sealer whose composition is a trade secret. However, it is known that synthetic Portland Cement clinkers, which are dark gray nodular materials made by heating ground limestone and clay at a temperature of about 1400–1500°C, and disalicylate are the basic components and form an ionic polymer. According to the manufacturer, it has the following physical properties: working time, 35 min; flow capacity, 27.66 mm; setting time, 130 min; optical density, 77%; and solubility, 0.1%. Moreover, it is easily manipulated. However, no study has evaluated its biological characteristics. Thus, the aim of this study was to compare the tissue reactions of MTA FILLAPEX, Sealapex and Angelus MTA in the subcutaneous connective tissues of the rat, including their ability to stimulate mineralization.

Material and methods

Thirty 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

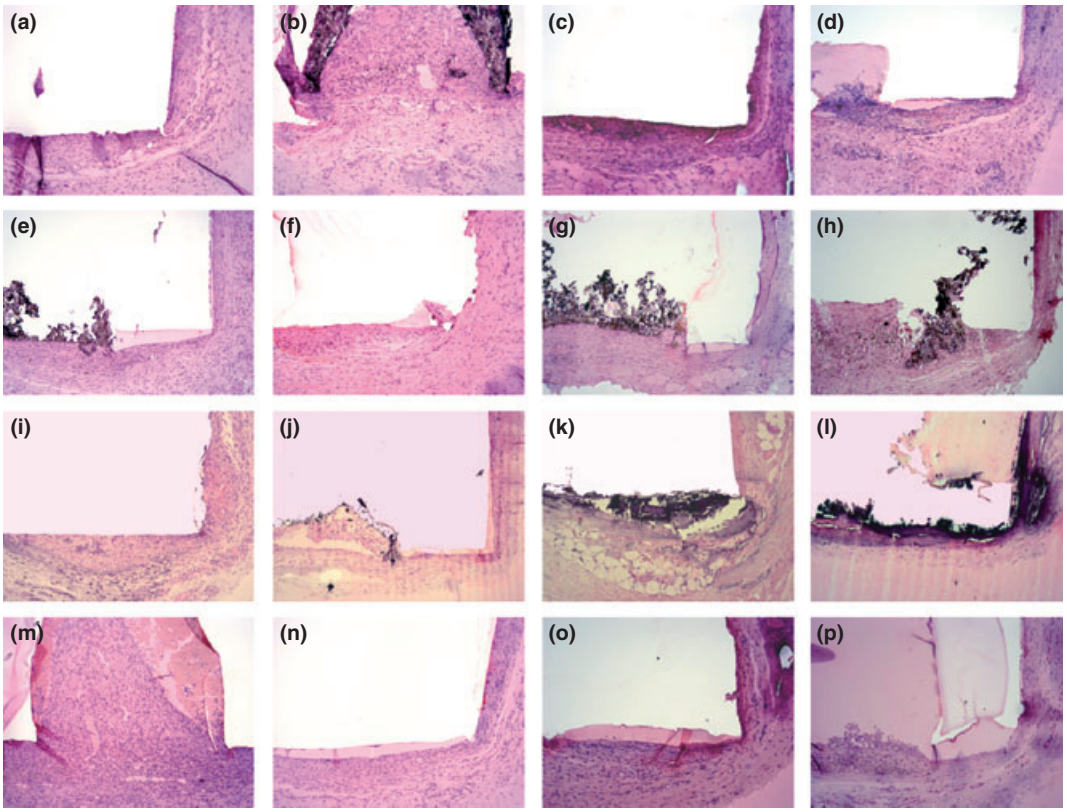

Fig. 1. After 7 days, thick fibrous capsule formation and moderate inflammatory cell infiltration were observed with Sealapex® (a), FILLAPEX® (e), Angelus MTA® (i), and Control (m). After 15 days, note that fibrous capsule remained thick with moderate inflammatory cell infiltration with Sealapex® (b) but it was thin with mild inflammatory cell infiltration with FILLAPEX® (f), Angelus MTA® (j), and Control (n). After 60 and 90 days, the thickness of fibrous capsule and the numbers of inflammatory cells reduced near the tube infiltration site with Sealapex® (c,d, respectively), FILLAPEX® (g,h), Angelus MTA® (k,l), and Control (o,p). Hematoxilin and eosin 100×.

received water and food *ad libitum*. The care of the animals was performed according to the Araçatuba School of Dentistry-UNESP Ethical Committee, which approved the project before the beginning of the experiment.

Ninety polyethylene tubes (Abbott Labs of Brazil, Sao Paulo, Brazil) with a 1.0-mm internal diameter, 1.6-mm external diameter, and 10.0-mm length were filled with the test materials. Sealapex®, MTA FILLAPEX®, and Angelus MTA® were prepared according to the manufacturer's recommendations and inserted into the tubes with a lentulo spiral (Maillefer Dentsply, Tulsa, OK, USA). Thirty polyethylene tubes remained empty to be used as controls.

The animals were disinfected with 5% iodine solution, after that they were shaved under xylazine (10 mg kg^{-1}) and ketamine (25 mg kg^{-1}) anesthesia. A 2-cm incision was made in a head–tail orientation on the shaved back of each animal with a number 15 Bard-Parker™ blade (Franklin Lakes, NJ, USA). The skin was reflected to create two pockets 6 cm apart on each side of the incision, one in the cranial portion and another in the caudal portion. After the tubes were implanted into the ockets, the skin was closed with 4/0 silk sutures.

After 7, 15, 30, 60, and 90 days from the implantation time, six animals were killed by overdose of an anesthetic solution. The tubes with surrounding tissues were removed and fixed in 10% buffered formalin at pH 7.0 (11, 12). The tubes were then bisected transversely. Both halves were cut again longitudinally with a sharp blade to allow the surfaces to be readily kept in contact with the processing solutions. The specimens were processed for glycol methacrylate embedding, serially sectioned into 3-μm slices, and stained with hematoxylin-eosin (13). The 10-μm slices were stained according to the Von Kossa technique or remained unstained for observation under polarized light. The technique was used to stain mineralized structures. The polarized light technique demonstrated birefringent structures related to calcium carbonate crystals originating from the combination of calcium ions from the material and carbonic gas from the tissue (14).

Inflammatory reactions in the tissue in contact with the material on the open end of the tube were scored according to previous studies (12, 13, 15, 16) as follows: 0, none or few inflammatory cells and no reaction; 1, <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 it is <150 μm and considered thick at ≥ 150 μm. Necrosis and calcification were recorded in μm^2 according to Leica Qwin software (Leica Microsystems, Wetzlar, Germany). An

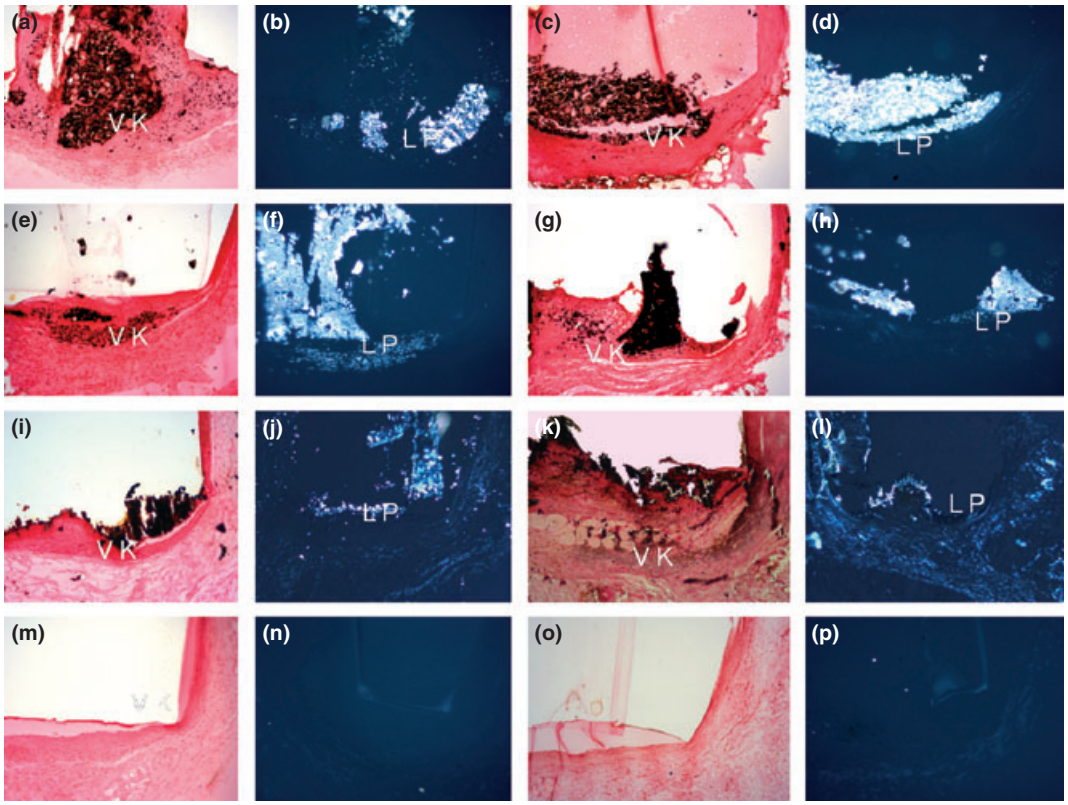

Fig. 2. After 30 and 90 days, note the presence of dystrophic calcification on the tube opening with Sealapex® (a,c, respectively), FILLAPEX® (e,g), Angelus MTA® (i,k), but not with Control (m,o). Von Kossa 100×. After 30 and 90 days, observe the presence of birefringent structures to polarized light, confirming the mineralization induction with Sealapex® (b,d, respectively), FILLAPEX® (f,h), Angelus MTA® (j,l), but not with Control (n,p). Polarized light 100×.

average number of cells for each group was obtained from 10 separate areas (400× magnification). Analyses were performed by a single calibrated operator in a blinded manner. Results were statistically analyzed by one-way ANOVA and Kruskal–Wallis tests.

Results

Test materials

On the 7th day, moderate inflammatory cell infiltration consisting of lymphocytes and macrophages was observed in the fibrous capsules for all materials tested (Fig. 1a,e,i). The intensity of inflammation was reduced on days 15, 30, 60, and 90 with thin fibrous capsules near the tubes and almost no inflammatory cells for all materials tested except for Sealpex®, which had the intensity of inflammation reduced from the 30th day on (Fig. 1b–d,f–h,j–l). Granulations birefringent to polarized light and Von Kossa positivity were observed near the tube openings for all materials tested (Fig. 2).

Control (empty tubes)

On days 7 and 15, moderate chronic inflammatory cell infiltration consisting of lymphocytes and macrophages was observed in the fibrous capsules (Fig. 1m,n). The fibrous capsules surrounding the tubes were thin with few chronic inflammatory cells after 30, 60, and 90 days (Fig. 1o,p). No Von Kossa positivity or birefringent structures were observed (Fig. 2m–p).

Comparisons among the groups

The data were compared for each time point as shown in Table 1. After 7 days, there were no statistically significant differences among the scores of the different groups (median score 2) except for Sealapex®. Sealapex® caused more necrosis and calcifications ($P < 0.001$). After 15 days, there were no statistical differences in the inflammatory cell numbers between the MTA FILLAPEX® and Angelus MTA® groups and between the Sealapex® and control groups. However, the median inflammatory cell scores for the MTA FILLAPEX® and Angelus MTA® (median score of 1) groups were lower than those of the other groups (median score of 2) ($P < 0.001$). Sealapex® and Angelus MTA® induced more calcification than MTA FILLAPEX®. After 30, 60, 90 days, there were no statistically significant differences among the inflammation scores for the different groups (median score 1). There were, however, significantly more areas of mineralization in the Sealapex® group than in the other groups ($P < 0.001$).

Table 1. Percentage of samples in each group categorized according to the inflammatory score, presence of necrosis, and thickness of fibrous capsule

	Score						
Material	0	1	2	3	Calcification	Necrosis	Capsule
7 days							
Control	0	0	100	0	0^{a1}	0^{a1}	Thick
Sealapex	0	0	100	0	186968.7^{b3}	266519.7^{d4}	Thick
Fill Apex	0	0	100	0	64849.6^{c2}	90327.4^{b2}	Thick
MTA	0	0	100	0	55464.0^{c2}	176535.0^{c2}	Thick
15 days							
Control	0	0	100	0	0^{a1}	0^{a1}	Thick
Sealapex	0	0	100	0	191671.5^{b3}	201183.7^{b3}	Thick
Fill Apex	0	100	0	0	20007.1^{c2}	0^{a1}	Thin
MTA	0	100	0	0	172246.8^{b3}	0^{a1}	Thin
30 days							
Control	0	100	0	0	0^{a1}	0^{a1}	Thin
Sealapex	0	100	0	0	342533.8^{b4}	0^{a1}	Thin
Fill Apex	0	100	0	0	189488.0^{c3}	0^{a1}	Thin
MTA	0	100	0	0	153184.8^{c3}	0^{a1}	Thin
60 days							
Control	0	100	0	0	0^{a1}	0^{a1}	Thin
Sealapex	0	100	0	0	341080.5^{b4}	0^{a1}	Thin
Fill Apex	0	100	0	0	121466.7^{c3}	0^{a1}	Thin
MTA	0	100	0	0	106930.8^{c3}	0^{a1}	Thin
90 days							
Control	0	100	0	0	0^{a1}	0^{a1}	Thin
Sealapex	0	100	0	0	$240491.3b^{a4}$	0^{a1}	Thin
Fill Apex	0	100	0	0	121963.1^{c3}	0^{a1}	Thin
MTA	0	100	0	0	110484.7^{c}	0^{a1}	Thin

MTA, mineral trioxide aggregate.

Score: 0 – none or few inflammatory cells and no reaction; 1 – <25 cells and mild reaction; 2 – between 25 and 125 cells and moderate reaction; 3 – 125 or more cells and severe reaction.

Same letters indicate no statistical difference among the materials and same numbers indicate no statistical difference of each material in different experimental periods of time. The areas of calcification and necrosis were measured in μm^2.

Discussion

The empty tubes in this study caused few reactions in subcutaneous connective tissues, similar to previously reported findings (2, 12, 17).

In this study, MTA FILLAPEX® evoked a moderate chronic inflammatory reaction on the 7th day that was reduced in a short period of time (15 days), similar to that induced by Angelus MTA® and faster than that induced by Sealapex®. During all observational periods, Von Kossa-positive areas for calcium and birefringent structures were observed similarly to those reported in reaction to Angelus MTA® (18). These calcifications are thought to originate from CaO present in MTA FILLAPEX® and in MTA (14, 19).

When in contact with water, CaO can be converted into $Ca(OH)_2$ and dissociated into $Ca2^{2+}$ and OH^-. The diffusion of hydroxyl ions from the root canal increases the pH at the surface of the root adjacent to the periodontal tissues, possibly interfering with osteoclastic activity and promoting alkalinization in the adjacent tissues, which favors healing (19, 20). Calcium ions participate in the activation of calcium-dependent adenosine triphosphatase (19, 21) and react with carbonic gas to form calcium carbonate crystals (birefringent to polarized light), which serve as a nucleus for calcification and favor mineralization (19, 21). A rich extracellular network of fibronectin in close contact with these crystals strongly supports the role of calcite crystals and fibronectin as an initiating step in the formation of a hard tissue (19, 21). Calcium is also needed for cell migration and differentiation (19, 22). Because MTA FILLAPEX® and MTA have similar chemical composition and produce similar tissue reactions, it is expected that MTA FILLAPEX® will act similarly to MTA when used in clinical situations, but be easier to handle because of its paste–paste combination.

Sealapex® was developed as a sealer and was used in the present study as a reference. The results observed with Sealapex® were similar to those of the control group. The moderate chronic inflammatory reaction observed initially was diminished over time concomitantly with the presence of positive Von Kossa areas and birefringent structures, showing that this material stimulated the formation of mineralized tissue. These calcifications can originate from the CaO present in Sealapex®, which reacts with tissue fluids to form $Ca(OH)_2$ (14). The birefringent granulations observed next to Sealapex® were probably calcite crystals that originated from the reaction of calcium ions with the carbon dioxide in the tissues (14, 23). Interestingly, more calcification areas were observed in the Sealapex® group than in the other groups. This was probably due to differences in the composition of the materials, resulting in differences in the degree of ionic dissolution.

Mineral trioxide aggregate has been used mainly in perforation repairs or as a root-end filling materials with good biocompatibility (24). It has the ability to induce hard tissue formation to pulpal tissues when used as a direct pulp cap or as a pulpotomy material (25–29). In animal studies, MTA consistently induces the formation of structurally superior dentin at a greater rate when compared with $Ca(OH)_2$ (25, 26, 29).

In conclusion, in the rat model MTA FILLAPEX® produced similar tissue reactions to Angelus MTA® and Sealapex®, including stimulation of mineralization. Thus, MTA FILLAPEX® is a biocompatible material. Further study is necessary to better characterize the behavior of this material and to confirm the findings of the present study.

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