An Investigation on the Histologic Responses of Periapical Tissues Following Retrofilling with Root MTA and Portland Cement Type I Versus Pro Root MTA in the Canine Teeth of Cats

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Statement of the Problem: Endodontic surgery if required is considered as an additive therapy after classic endodontic treatments. The most prevalent type of endodontic surgeries are apicoectomy and corrective surgeries. Type of material applied in apicoectomies is of high importance. Root end filling material should not only be biocompatible but also provide appropriate seal. Considering these findings, the subject of this paper was to compare the histologic responses of the periradicular tissues after using Root MTA and Portland cement, as Root end fillings, with Pro Root MTA.

Purpose: The aim of the present study was to introduce a suitable material for root end filling.

Materials and Methods: The canals of sixty maxillary canines of cats, after cutting the crowns and exposing the pulps, were instrumented 1 mm beyond the apex until 25 # file to develop periapical lesion after 28 days. They were then randomly divided into five groups. After apicoectomy, Pro Root MTA, Root MTA and Portland cement were placed, as root end filling materials in three experimental groups whereas the two remaining groups were considered as positive and negative controls.

After 3, 6 and 12 weeks the animals were sacrificed and the specimens were stained for histologic evaluation under light microscope. The data were submitted to statistical analyses (Fisher exact test, k_2 test and one- way variance analysis).

Results: No statistically significant difference was found between these three materials in cementum apposition on roots and implanted materials, fibrous capsule formation and the severity of inflammation, however, bone formation in Root MTA group was significantly less than pro Root MTA and Portland cement groups. The extent of inflammation thickness in Portland cement group was significantly less than Pro Root MTA group.

Conclusion: According to these findings if long term investigations with larger sample size confirm the obtained results of the present study, Root MTA and Portland cement which are cost- effective and readily available, can replace Pro Root MTA in endodontic surgeries.

Key Words: Histology; Root end filling; Pro Root MTA; Root MTA; Portland Cement type I.

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Nowadays, endodontic surgeries have extensively developed ranging from a simple incision for drainage to apicoectomy, retrofilling, hemisection, bicuspidzation and corrective surgeries such as perforation sealing, external resorption and the treatment of growth-developmental disorder as well as endo and endoosseous implants.

Along with the scientific and technical improvements combined with the use of newly invented instruments for endodontic surgeries, a lot of efforts have been taken to present a material for root end filling, being capable of healing and the correction of defects as perforations and resorptions. Generally, an ideal root end filling material should provide a thorough apical seal, should be tolerated by periradicular tissues and be biocompatible. Moreover, dimensional stability, simple manipulation, radio opacity, rapid healing and being non- absorbable are considered as other characteristics for an ideal material.(1)

Several materials have been introduced for root end fillings including amalgam, Super EBA, IRM, Composite and Gutta-percha, however, none of them possess all characteristics mentioned for an ideal material.(2-3)

A lot of studies have been reported on the cytotoxicity of these materials through cellular culturing and on their biocompatibility by subcutaneous soft tissue response as well as material implantation inside bone,(4-6) but there is no study to compare Pro Root MTA a material recently introduced for root end filling,(7) with Root MTA a material presented by Mehrdad lotfi in Tabriz dental faculty 2 years ago(8) almost physically identical to pro Root MTA and Portland cement type I with similar chemical composition to Pro Root MTA.(9)

Considering these findings, the aim of this study was to evaluate the periradicular responses to Root MTA and Portland cement and to compare them with Pro Root MTA in maxillary canines of cats and finally to introduce a material with similar biocompatibility to Pro Root MTA, as well as being available on Iran market

Materials and Methods

The maxillary canines of 30 healthy male mature cats of Iranian race were used in this experimental research. All the procedures were performed under general anesthesia, with ketammin hydrochloride $30^{mg}/_{kg}$ and 20% asperomazine maleate $0.05^{mg}/_{kg}$ (IM) combined with intraoral infiltration injection of 2% Lidocaine with Epinephrine $\frac{1}{100.000}$ (1.8ml).

At the first session, after taking periapical radiographs, the teeth crowns were cut horizontally at 3 mm above C.E.J to expose the pulp, then the upper portion of the canals were widened with gates glidden burs no. #2, 3 & 4 and the pulps were removed by a barbed broach. Using Periapical radiographs, the working length was determined by # 15 file and each canal was then instrumented 1mm beyond the apex up to # 25 file. All the teeth were left exposed for 28 days to develop periapical lesion. (10)

At the second session, 28 days later, radiographs were again taken to determine the canal length and to ensure periapical lesion development.

All canals were then prepared using step back technique and washing with 5.25% sodium hypochlorite. Calcium hydroxide was then put into canals for one week. Cavit past was used to seal the access cavity.

At the third session, after redebridement the canals were washed with 5.25% sodium hypochlorite and the root lengths were redetermined. Then the canals were filled with gutta-percha applying lateral condensation technique. Then final radiographs were taken and the crowns were filled 3 mm in depth with varnish and amalgam.

During the fourth session, a mucogingival flap was elevated and the cortical buccal bone of each canine tooth was removed using turbine and round carbide bur no.6. Then, the root end

was cut at 45° angles to the long axis of the tooth with a conic diamond bur. A class I cavity 3 mm in depth was then prepared at the root end with a cavitron retro tip without periapical curettage and filled with one of these three materials according to the manufactures, instructions:

Pro Root MTA (Dentsply, U.S.A made by Dr. Torabinejad), Root MTA (made in Tabriz Dental faculty by Dr. Lotfi) and Portland cement (Tehran cement factory). All the flaps were sutured with silk thread 30 and reverse cutting needle.

Three cats were considered as negative control group and no operation was performed on them whereas in three other cats, as positive control group, the root ends were filled with zinc oxide powder plus tricresoformalin as the test material. During 3-6 weeks after the operation, two cats from each of the experimental groups and one cat from each of the positive and negative control groups were sacrificed based on vital perfusion technique.

During 12 weeks, four other cats from experimental groups and one cat from each of the positive and negative control groups were also sacrificed based on the mentioned technique. Complete fixation of head, neck and mandible areas was achieved through 10% formalin solution injection to the brain vascular system of each animal.

Each canine tooth combined with the surrounding bone was block sectioned then, three sections were prepared for each tooth and the best one consisting of tooth, apical portion and bony tissue was selected for the intended lamella.

After processing, at least 3 sections (5-6 µm in diameter) were prepared by a microtome and stained with hematoxylin and eosin.

Severity and thickness of the inflammation and the type of predominant inflammatory cells existing in the periradicular tissues adjacent to the root endings were recorded.

Severity of inflammation was classified as follows:

Free of Inflammation: without any inflammatory cell

Mild: with a few inflammatory cells

Moderate: Inflammatory cells did not surround the whole normal tissue.

Severe: Inflammatory calls replaced the normal tissue

Inflammation thickness based on its distance from the root end filling material was recorded as: <0.1mm, <0.2mm, <0.5mm and <1mm. The presence of fibrous capsule, cementum apposition on root ending and the implanted material and bone formation were determined by a pathologist based on histological staining. Moreover, the existence of bacteria in periradicular tissues were investigated by H&E staining, gram staining and if required Bown and Brenn technique. The data were submitted to SPSS (version 10) statistical software and Fisher's exact, Pearson Chi-square and one-way ANOVA tests.

Results

Out of thirty cats at the beginning, four of them died by the end of the investigation period including one cat from pro Root MTA group in a 3-week study, one cat from Root MTA group in a 6-week study, one cat from Pro Root MTA group in a 12-week study and one cat from Portland cement type I group.

Totally, twelve teeth of Pro Root MTA, fourteen teeth of Root MTA and fourteen teeth of Portland cement groups were investigated histologically, from them 14 cases in 3 weeks, 14 other cases in 6 weeks and 24 cases in 12 weeks were studied.

The histological results are summarized in tables I through III.

The following conclusions can be drawn from the statistical evaluation of table I-III.

The amount of bone formation in Pro Root MTA group was statistically more than the positive control (P=0.005) and Root MTA (P=0.001) groups.

Table I- Histologic findings in the periradicular tissues of roots retrofilled with Pro Root MTA, Root MTA and Portland cement type (I) in 3 weeks

Number of roots	Negative control 2	Positive control 2	Portland cement 4	Root MTA 4	Pro Root MTA 2
Severity of inflammation	Severe: 2	Severe: 2	Moderate : 3 Severe: 1	Severe: 4	Moderate: 1 Severe: 1
Inflammation thickness (mm)	0.14 <0.2	0.37 <0.5	0.08 <0.1	0.13 <0.2	0.09 <0.1
Fibrosis Capsule (Yes/ total)	$\frac{2}{2}$	$\frac{0}{2}$	$\frac{4}{4}$	$\frac{3}{4}$	$\frac{1}{2}$
Cementum apposition on root (Yes/total)	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{1}{4}$	$\frac{0}{4}$	$\frac{0}{2}$
Cementum apposition on the material (Yes/ total)	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{0}{4}$	$\frac{0}{4}$	$\frac{0}{2}$
New bone formation (Yes/ total)	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{0}{4}$	$\frac{0}{4}$	$\frac{0}{2}$
The presence of bacteria (Yes/total)	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{0}{4}$	$\frac{0}{4}$	$\frac{0}{2}$

Table II- Histologic findings in the periradicular tissues of roots retrofilled with Pro Root MTA, Root MTA and Portland cement type (I) in 6 weeks

Number of roots	Negative control 2	Positive control 2	Portland cement 4	Root MTA 4	Pro Root MTA 2
Severity of inflammation	No : 2	Severe: 2	Mild : 4	Moderate: 2	Moderate: 3 Mild : 1
Inflammation thickness (mm)	No.	0.33 <0.5	0.04 <0.1	0.05 <0.1	0.06 <0.1
Fibrosis Capsule (Yes/ total)	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{4}{4}$	$\frac{2}{2}$	$\frac{4}{4}$
Cementum apposition on root (Yes/total)	$\frac{2}{2}$	$\frac{0}{2}$	$\frac{0}{4}$	$\frac{1}{2}$	$\frac{0}{4}$
Cementum apposition on the material (Yes/total)	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{0}{4}$	$\frac{0}{2}$	$\frac{3}{4}$
New bone formation (Yes/total)	$\frac{2}{2}$	$\frac{0}{2}$	$\frac{4}{4}$	$\frac{0}{2}$	$\frac{3}{4}$
The presence of bacteria (Yes/total)	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{0}{4}$	$\frac{0}{2}$	$\frac{0}{4}$

Table III- Histologic Findings in the periradicular tissues of roots retrofilled with Pro Root MTA, Root MTA and Portland cement type (I) in 12 weeks

Number of roots	Negative control 2	Positive control 2	Portland cement 6	Root MTA 8	Pro Root MTA 6
Severity of inflammation	No inflammation	Severe: 2	Mild: 1 Moderate: 5	Mild: 5 Moderate:2 Severe: 1	Mild : 4 Severe: 2
Inflammation thickness (mm)	No	0.30 <0.5	0.04 <0.1	0.07 <0.1	0.16 <0.2
Fibrosis Capsule (Yes/total)	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{6}{6}$	$\frac{8}{8}$	$\frac{6}{6}$
Cementum apposition on root (Yes/total)	$\frac{2}{2}$	$\frac{0}{2}$	$\frac{3}{6}$	$\frac{8}{8}$	$\frac{3}{6}$
Cementum apposition on the material (Yes/total)	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{3}{6}$	$\frac{4}{8}$	$\frac{4}{6}$
New bone formation (Yes/total)	$\frac{2}{2}$	$\frac{0}{2}$	$\frac{6}{6}$	$\frac{3}{8}$	$\frac{6}{6}$
The presence of bacteria (Yes/ total)	$\frac{0}{2}$	$\frac{0}{2}$	$\frac{0}{6}$	$\frac{0}{8}$	$\frac{0}{6}$

No statistically significant difference was found in bone formation between Pro Root MTA and Portland cement groups.

Regarding bone formation, the results observed with Portland cement group was significantly more than Root MTA (P=0.001) and positive control (P=0.024) groups. The reported results on bone formation in negative control group were similar to those reported for Pro Root MTA, Root MTA and Portland cement groups.

It was found that root cementum apposition in Root MTA cases was significantly more than Portland cement (P=0.027) and positive control (P=0.012) groups.

Similar results were obtained among the four groups of Pro Root MTA, Portland cement, positive and negative controls in root cementum apposition.

No statistically significant difference was found in fibrosis capsule formation between Pro Root MTA, Root MTA and Portland cement groups.

The results observed with Pro Root MTA (P=0.000), Root MTA (P=0.000) and Portland cement (P=0.000) groups, on the amount of fibrosis capsule formation was significantly more than that of positive control group.

The amount of fibrosis capsule formation in Pro Root MTA (P=0.022), Root MTA (P=0.014) and Portland cement (P=0.003) group were found to be significantly more than negative control group.

Similar results were observed on inflammation thickness between Pro Root MTA group and Root MTA groups, however, the results observed with Pro Root MTA were significantly more than those reported for Portland cement group (P=0.009) and negative control group (P=0.019) but less than positive control group (P=0.001). It should be mentioned that inflammatory cells in all samples were of mononuclear type.

Analysis of the numerical data showed no significant difference on inflammation thickness between Root MTA, Portland cement and negative control groups, but the results observed with Root MTA group was significantly less than that of positive controls (P=0.001).

The Portland group had results on inflammation thickness very similar to the negative control group although comparing to the positive control, significantly less inflammation thickness was observed (P=0.001).

Histological views of some of the investigated lamellas are shown in figures 1-5.

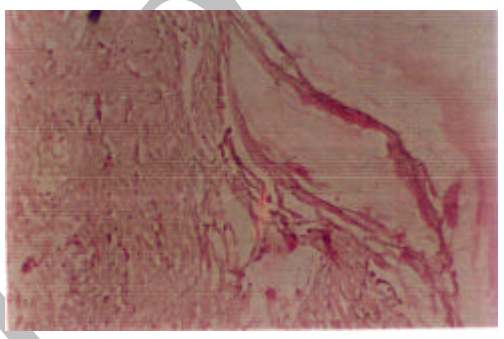

Fig 1- Fibrous capsule formation on root end adjacent to Root MTA (×200)

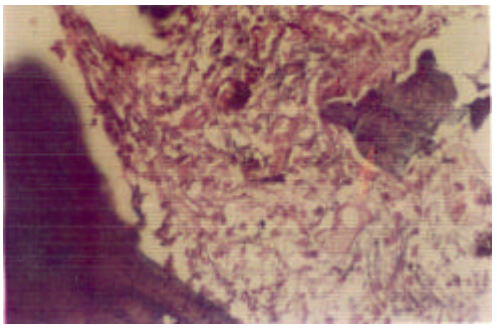

Fig 2- Bone formation on root end adjacent to Portland cement (×200)

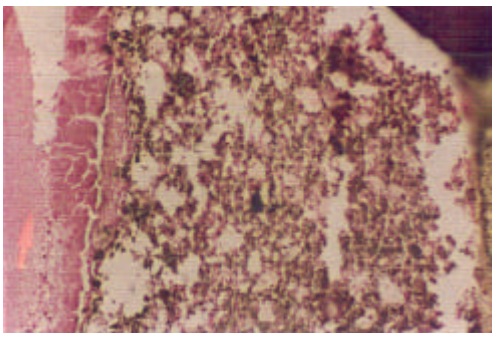

Fig 3- New cementum formation on Pro Root MTA (×200)

Fig 4- Natural connective tissue, bone marrow, bone formation and the absence of inflammation in negative control group (×200)

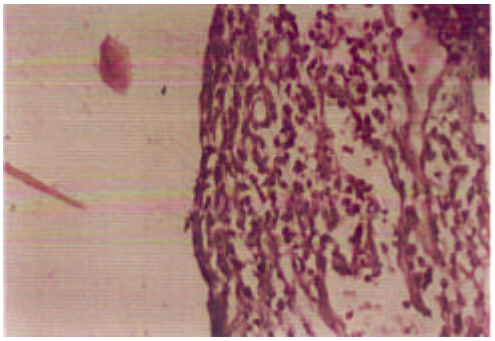

Fig 5- Severe tissue inflammation in positive control group (×200)

Discussion

Various methods have been suggested to evaluate materials applied in dentistry including cytotoxicity assessment through cellular culturing and biocompatibility evaluation using material implantation inside bone or soft tissue. These sequential tests lead to the elimination of improper materials and reduction of animal studies and finally the probability of applying incompatible materials, in human beings, would decrease.

Invitro and in-vivo studies have proved pro Root MTA as a suitable material for root end filling[1,11-14], however, a new material Known as Root MTA has been recently introduced by Lotfi, being claimed of having microleakage similar to that of Pro Root MTA.[8] Moreover, Estrela showed identical chemical compositions for pro Root MTA and Portland cement.[9]

Saidon study on the implantation of Pro Root MTA and Portland cement in guineapigs mandible attributed similar bone healing process and inflammatory reaction to both studied materials.[15] Considering these similarities and the availability of Root MTA and Portland cement as compared with Pro Root MTA, the subject of this paper is to compare these three mentioned materials.

Sharifian and Ghobadi investigated the cytotoxicity of these three materials. Razmi and Ramezankhani studied the tissue response to bone implantation of these materials. But due to non-similarity between in vivo studies and implantation tests with periradicular tissues, the results of these studies should be considered mainly as an index.

The findings of the present study revealed that Pro Root MTA and Portland cement application as root end filling materials cause bone formation, however, all three studied materials could induce cementoblasts for cementum deposition on the implanted materials.

The main difference in periradicular reaction between Pro Root MTA, Root MTA and Portland cement groups was attributed to the amount of bone formation, being significantly less in Root MTA group, comparing to Pro Root MTA and Portland cement groups, however, no statistically significant difference was found between three groups in cementum deposition on the implanted materials and roots, formation of fibrosis capsule and severity of inflammation.

Torabinejad[17], in his study, reported bone formation and the presence of fibrosis capsules for almost all Pro Root MTA cases, similar to those obtained with the present study and the minor difference between two studies can be justified due to the existence of root end lesion in the present study, contrary to one conducted by Torabinejad.

Considering cementum apposition, the obtained results were almost identical and the differences may be justified as the above.

The main observed difference between this study and that by Torabinejad was the severe

inflammatory reaction among Pro Root MTA cases of the present study, which can be attributed to the periradicular lesion.

In another investigation by Torabinejad[7] similar results even on the severity of inflammation were reported.

The only observed difference was the presence of fibrosis capsule in the amalgam group opposing to Pro Root MTA cases.

In a short-term study by Economides et al on periradicular tissue respone to pro Root MTA as the root end filling material, almost identical results were obtained in inflammatory reaction and hard tissue formation. Saidon [15] compared tissue reaction between pro Root MTA and portland cement and reported similar results in bone healing process and inflammatory response for both studied materials approving the results of the present study.

Moreover, the findings of the present study showed considerable healing even at the presence of periapical lesion in the negative control group with just one acceptable root treatment indicating general evaluation as a necessity before initiating the operation phase.

Conclusions

Considering Pro Root MTA as the most appropriate material comparing to other root end filling materials and its high price on the other hand, the subject of this paper was to study the histological responses of periradicular tissues to this material and compare it with those of Root MTA and Portland cement type I.

Analysis of the numerical date showed no significant differences between these three materials. Therefore, longer-term investigations with larger population are suggested.

If the results of the present study are confirmed, Root MTA and particularly Portland cement type I which are more cost-effective and available can possibly replace Pro Root MTA in apicoectomies.

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