A Histologic Evaluation on Tissue Reaction to Three Implanted Materials (MTA, Root MTA and Portland Cement Type I) in the Mandible of Cats

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Statement of Problem: Nowadays Mineral Trioxide aggregate (MTA) is widely used for root end fillings, pulp capping, perforation repair and other endodontic treatments. Investigations have shown similar physical and chemical properties for Portland cement and Root MTA with those described for MTA.

Purpose: The aim of this in vitro study was to evaluate the tissue reaction to implanted MTA, Portland cement and Root MTA in the mandible of cats.

Materials and Methods: Under asepsis condition and general anesthesia, a mucoperiosteal flap, following the application of local anesthesia, was elevated to expose mandibular symphysis. Two small holes in both sides of mandible were drilled. MTA, Portland cement and Root MTA were mixed according to the manufacturers, recommendation and placed in bony cavities. In positive control group, the test material was Zinc oxide powder plus tricresoformalin. In negative control group, the bony cavities were left untreated. After 3, 6 and 12 weeks, the animals were sacrificed and the mandibular sections were prepared for histologic examination under light microscope. The presence and thickness of inflammation, presence of fibrosis capsule, the severity of fibrosis and bone formation were investigated. The data were submitted to Exact Fisher test, chi square test and Kruskal-Wallis test for statistical analysis.

Results: No statistically significant differences were found in the degree of inflammation, presence of fibrotic capsule, severity of fibrosis and inflammation thickness between Root MTA, Portland cement and MTA (P>0.05). There was no statistical difference in bone formation between MTA and Portland cement (P>0.05). However, bone formation was not found in any of the Root MTA specimens and the observed tissue was exclusively of fibrosis type.

Conclusion: The physical and histological results observed with MTA are similar to those of Root MTA and Portland cement. Additionally, all of these three materials are biocompatible. However, in order to replace Root MTA and Portland cement type I as less expensive and suitable substitutes for MTA, more longer-term studies with larger number of samples are suggested.

Key words: MTA; Root MTA; Portland cement type I; Tissue reaction.

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Inflammation thickness refers to the thickness of the inflammatory multicellular region (mm) extending from the implanted material to the intact bony tissue. As a result, the inflammation thickness, based on its distance from the root end filling material was recorded as: mm 0.1>, 0.5>, 0.2>, <1

Dental pulp is fundamentally related to the periodontium through apical foramen, accessory and lateral canals. Periodontium consists of cementum, periodontal ligament and alveolar bone. Dental pulp and periodontium are physically separated from oral flora by enamel, dentin and gingival attachments. The exposure of pulp and periradicular tissues to microorganisms results in pulp and periradicular diseases. Generally, these microorganisms act as the main stimulating factor for pulp and periodontium. In order to protect dental pulp and prevent pathologic changes in periradicular tissues, mechanical exposures should be completely sealed and the inflammation resulted from caries in the pulp of tooth with open apices and without the signs of irreversible pulpitis, should be eliminated. Moreover, in root end filling surgeries, materials with considerable marginal seal and biocompatibility should be applied to regenerate the affected tissues to their normal condition.

In order to achieve these goals, a lot of materials have been already presented including: amalgam, cements with ZOE base such as Super-EBA, IRM and cavit, composite resins and glass ionomer cements. Microleakage, various degrees of toxicity and sensitivity to moisture are of major defects attributed to these materials. In recent decade, a new material known as MTA (Mineral Trioxide Aggregate) has been introduced by Torabinejad being capable to create a thorough seal between root canals and dental external surfaces. In vitro and in vivo studies have attributed considerable sealing ability and marginal adaptation for MTA. More acceptable tissue reaction to this material, as compared with others has been also observed, although its application due to its high price has been limited. On the other hand it has been reported that Portland cement is similar to MTA in antimicrobial and physical properties. Another material called Root MTA that is claimed to be similar to MTA has been produced in Tabriz. Considering these claims, the subject of this paper is to study the biocompatibility of these two materials (Root MTA, Portland cement type I) and to compare them with MTA. In case of similar comparative results and based on further studies on cellular culture and microbial leakage etc these materials may be introduced as suitable substitutes for MTA that because of its high price can not be commonly used.

Materials and Methods
In this experimental study thirty healthy mature male cats of Iranian race were initially selected. It should be mentioned that International Standard Organization (ISO), for material implantation inside bones recommends tibia, Femur and mandible of laboratory animals such as rabbits Rats Guinea pigs and cats. Under general anesthesia, with Ketamine and Asepromazine, the lower lip region was completely disinfected by Beta dine. A horizontal mucoperiosteal incision was then made by blade no. 15 between the lower lip and alveolar mucosa extending from the distal of one side canine to the other side. Following this the lip and gingival regions were completely elevated by a periosteal elevator until the mandibular symphysis was exposed. A horizontal mucoperiosteal incision was then made by blade no. 15 between the lower lip and alveolar mucosa extending from the distal of one side canine to the other side. Following this the lip and gingival regions were completely elevated by a periosteal elevator until the mandibular symphysis was exposed. The selection of the symphysis region was due to its appropriate bone thickness and accessibility, based on mandibular radiographs and anatomic investigations. Tow small holes, with a depth equal to the cutting length of the angle round bur no. 3 were then drilled at both sides of mental symphysis. Such a depth not only provides enough material thickness, but also prevent from lingual cortex perforation. Based
on a random classification, eight cats were placed in each of the MTA group (Dentsply factory), Root MTA group (Tabriz- Iran), and Portland cement group (Tehran cement factory), totally 24 cats. In each of the positive and negative control groups three cats were also placed.

Each cement was mixed with distilled water (3.1). The paste was then formed by filling a cylinder (3mm in depth) by an amalgam carrier and placed into mandibular holes so as 1mm depth inside bone was left empty. In positive control group, for a maximum tissue reaction, zinc oxide plus formalin solution was applied, whereas in negative control group bony cavities were left untreated to induce minimum tissue reaction. The tissues were then replaced and sutured (Fig. 1).

After one week, the sutures were removed. During this week, all sutures were rinsed twice with Beta dine solution to decrease the possibility of secondary infection. After 3, 6 and 12 weeks, the animals were sacrificed based on vital perfusion technique.

In this technique, following general anesthesia a by Ketamine- Asepromazine mixture, 2cc of heparin was injected I.V to prevent blood coagulation. Head and neck vessels were then thoroughly washed with 40cc of sterile saline solution followed by the injection of 15% formalin solution (40cc) into cervicular right and left carotid arteries for a complete fixation of head and neck tissues. The animals were then sacrificed and the mandibular sections, from canine to canine, were fixed in 10% formalin solution and prepared for histologic examination.

After decalcification, the paraffin embedded serial sections (6-µm thick) were prepared vertically to the long axis of the implanted materials and stained with hematoxylin eosin and examined under a light microscope. For each section, 3 cross- sections in the middle, buccal and lingual of the implanted material were made. For better results, the pieces were studied without soft tissues.

During the histological analysis of the results of this study, details were considered regarding the type of tissue (bone or connective), the inflammation thickness (mm), the presence of inflammation and fibrotic capsule adjacent to the implanted materials. For data analysis, SPSS statistical software was used. In order to determine the relationship between qualitative variables, the data were submitted to statistical analyses (Fisher exact test and \( \chi^2 \) test).

Due to the small sample size, Kruskal- Wallis non- parametric test was used to determine the relationship between the inflammation thickness and implanted materials.

Result

In this study, from among the total number of 30 cats, one cat in a 3- week study from MTA group, one cat in a 6- week study from Root MTA group, tow cats in a 12-week study from MTA group and one cat from Portland cement group died and were excluded from the present study. Finally, 26 cats were investigated statistically. The total number of investigated bony cavities was 52, considering that two cavities in the mandible of each cat were prepared. The results of histological analyses on the tissues surrounding the implanted materials inside bony cavities after 3,6 and 12 weeks are shown in tables I, II and III.
Chi-Square and Exact fisher tests showed no statistically significant differences in the degree of inflammation among MTA, Root MTA and Portland cement (P> 0.05).

Table I- Histological findings around the bone implanted materials in relation to MTA/ Root MTA and Portland cement after 3 weeks

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>MTA</th>
<th>Root MTA</th>
<th>Portland cement</th>
<th>Control (+)</th>
<th>Control (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The number of bony cavities</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Degree of inflammation</td>
<td>Moderate**</td>
<td>Moderate**</td>
<td>Moderate;***</td>
<td>Severe:***</td>
<td>Moderate;**</td>
</tr>
<tr>
<td>Inflammation thickness (mm)</td>
<td>0.08&lt;0.1</td>
<td>0.13&lt;0.2</td>
<td>0.105&lt;0.2</td>
<td>0.38&lt;0.5</td>
<td>0.13&lt;0.2</td>
</tr>
<tr>
<td>Fibrosis capsule (Yes/No)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bone formation (Yes/No)</td>
<td>S.F.2</td>
<td>M.F.3</td>
<td>M.F.4</td>
<td>M.F.2</td>
<td>S.F.1</td>
</tr>
<tr>
<td>S.F or MF</td>
<td>M.F.2</td>
<td>M.F.3</td>
<td>M.F.4</td>
<td>M.F.2</td>
<td>S.F.1</td>
</tr>
</tbody>
</table>

Table II- Histological findings around the bone implanted materials in relation to MTA/ Root MTA and Portland cement after 6 weeks

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>MTA</th>
<th>Root MTA</th>
<th>Portland cement</th>
<th>Control (+)</th>
<th>Control (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The number of bony cavities</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Degree of inflammation</td>
<td>Mild*</td>
<td>Moderate**</td>
<td>Mild *.4</td>
<td>Severe:***</td>
<td>Inflammation 2</td>
</tr>
<tr>
<td>Inflammation thickness (mm)</td>
<td>0.05&lt;0.1</td>
<td>0.12&lt;0.2</td>
<td>0.04&lt;0.1</td>
<td>0.33&lt;0.5</td>
<td>0.33&lt;0.5</td>
</tr>
<tr>
<td>Fibrosis capsule (Yes/No)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bone formation (Yes/No)</td>
<td>S.F.4</td>
<td>S.F.2</td>
<td>S.F.4</td>
<td>M.F.2</td>
<td>S.F.2</td>
</tr>
<tr>
<td>S.F or MF</td>
<td>S.F.2</td>
<td>S.F.4</td>
<td>S.F.6</td>
<td>S.F.8</td>
<td>S.F.6</td>
</tr>
</tbody>
</table>

Table III- Histological findings around the bone implanted materials in relation to MTA/ Root MTA and Portland cement after 12 weeks

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>MTA</th>
<th>Root MTA</th>
<th>Portland cement</th>
<th>Control (+)</th>
<th>Control (-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The number of bony cavities</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Degree of inflammation</td>
<td>Mild. *4</td>
<td>Moderate;**</td>
<td>Mild *.4</td>
<td>Moderate **2</td>
<td>Inflammation 2</td>
</tr>
<tr>
<td>Inflammation thickness (mm)</td>
<td>0.14&lt;0.2</td>
<td>0.102&lt;0.2</td>
<td>0.06&lt;0.1</td>
<td>0.31&lt;0.5</td>
<td>0.31&lt;0.5</td>
</tr>
<tr>
<td>Fibrosis capsule (Yes/ No)</td>
<td>6</td>
<td>8</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bone formation (Yes/ No)</td>
<td>S.F.6</td>
<td>S.F.8</td>
<td>S.F.6</td>
<td>M.F.2</td>
<td>S.F.2</td>
</tr>
<tr>
<td>S.F or MF</td>
<td>S.F.6</td>
<td>S.F.8</td>
<td>S.F.6</td>
<td>M.F.2</td>
<td>S.F.2</td>
</tr>
</tbody>
</table>

M.F: (Incomplete) Mild Fibrosis
S.F: (Complete) Server Fibrosis
* Mild inflammation: The number of inflammatory cells (mm) under light microscope was small
** Moderate inflammation: Inflammatory cells did not surround the whole normal tissue
*** Severe inflammation: Inflammatory cells substituted the normal tissue
\(^{\Delta}\) Fibrotic capsule refers to some degrees of fibrosis around the implanted material
Considering the presence of fibrotic capsule the difference between three kinds of implanted materials was not statistically meaningful (P>0.05).

There was no statistical difference in bone formation between MTA, PC and the negative control group (P>0.05).

Analysis of the numerical data showed no significant differences between three groups in the presence of mild and severe fibrosis (P>0.05).

Non-parametrical Kruskal- Wallis test exhibited no meaningful difference in inflammation thickness between MTA, Root MTA and PC groups.

Some of the histological investigations are shown in figures 2, 3, 4, 5 and 6.

**Discussion**

Materials used for root treatments, particularly those for root end filling, are frequently in direct contact with soft & hard tissues of periodontium.

**Fig. 2** - The formation of fibrotic capsule adjacent to Root MTA after 12 weeks (×200)

**Fig. 3** - Newly formed bone adjacent to MTA after 6 weeks (×200)

**Fig. 4** - Newly formed bone adjacent to Portland cement after 6 weeks

**Fig. 5** - Severe inflammation in positive control group (×400)

**Fig. 6** - Complete fibrosis and bone formation combined with osteoblasts in negative control group
Therefore, a root end filling material seems necessary to be highly compatible and not toxic. In order to evaluate the material biocompatibility, three stages of cytotoxicity, local toxicity and usage tests on small laboratory animals should be investigated.\(^{(9)}\) Such evaluations lead to the elimination of improper materials, reduction in the number of studied animals and finally a decrease in the application of incompatible materials for human beings.

In-vitro and in-vivo studies have proved considerable biocompatibility, sealing ability and marginal adaptation for MTA.\(^{(10,11)}\) On the other hand, the results observed with PC are similar to those reported about MTA on physical and antimicrobial properties.\(^{(6)}\) Moreover, a material known as Root MTA has been produced in Tabriz, that is claimed to possess properties much similar to those described for MT.\(^{(7)}\)

Considering these observations, the subject of this paper is to study the local biocompatibility of PC type I and Root MTA in the mandible of cats, as compared with MTA.

Generally, biocompatibility tests on bone biomaterials such as those applied in endodontics should be performed in the special bones identical to their clinical applications since bone healing process and its quality depend on the location of the implanted material.\(^{(12)}\)

It should be also mentioned that International Standard Organization (ISO) recommends bones as Tibia, femur and the mandible of laboratory animals for material implantation investigation and among small animals rabbits, rats, guinea pigs, and cats are more popular.\(^{(8)}\) Herve Tassery et al (1999) reported mandible as a more suitable location for biocompatibility studies, comparing to femur.\(^{(13)}\) Based on these findings it is concluded that the special characteristics of the mandible of cats, particularly due to the bony thickness of mental symphysis, are in agreement with ISO recommendation. The results of this study showed no statistically significant differences in inflammatory reaction between the studied materials (MTA, Root MTA and Portland cement) (P>0.05).

No statistically significant difference was also found between three materials on the presence of fibrotic capsule (P>0.05). The same results were also observed regarding the presence of a mild or chronic fibrosis (P>0.05).

There was no statistically significant difference in bone formation between MTA, Portland cement and the negative control group, too (P>0.05).

However none of the Root MTA specimens exhibited bone formation, similar to those reported by Saidon and Safavi (2003) on guinea pigs mandible, they also found no significant difference in bone healing and minimum inflammation between MTA and Portland cement.\(^{(14)}\) Holland et al (2001) studied the rat subcutaneous connective tissue response to implanted polyethylene tubes filled with MTA, Portland cement and calcium hydroxide Ca (OH)\(_2\) and found very similar mechanisms of action and healing process.\(^{(15)}\) Considering that both studied materials showed similar tissue reaction to that of MTA, one can assume that Root MTA and Portland cement are as biocompatible as MTA which can be attributed to the presence of Portland cement as the chemical base of these three compositions. It should be also noted that their cytotoxicity safety has been also confirmed.\(^{(16)}\)

Regarding the absence of newly formed bony tissues around Root MTA, it may be concluded that this material does not induce any effects on local cells for bone synthesis, Root MTA specimens displayed a granulation tissue implying a proper and acceptable response to this implanted material. The creation of a framework for bone formation and an environment with lower bacteria (due to high alkaline pH) can be considered as favorable
MTA and Portland cement properties for an appropriate healing process.

Generally, the presence of inflammatory reaction, after 3 weeks around the implanted materials was an indicative of their initial stimulating effects due to caustic properties (alkaline) and foreign body reaction (giant cell in some cases), which subsequently developed into repair. Finally it should be mentioned that the white color of the applied Portland cement reject the possibility of its tissue tattooing in endodontics.

As previously stated, the results of this study revealed that the histological response to Root MTA and Portland cement is identical to that of MTA. Zarabin and Moosavi also reported similar histological findings in an animal study on the biocompatibility of these so-called retrograde materials. Moreover, the cytotoxicity studies by Sharifian and Ghobadi showed similar results for all three studied materials. According to Safavi and Saidon investigation along with that conducted by Holland and Souza, the tissue reaction of the Portland cement was similar to that reported for MTA. Based on a study by Esterla et al regarding the similarity in physical and antimicrobial properties between MTA and Portland cement in addition to the identical physical and micro leakage properties between MTA and Root MTA expressed by Lotfi, Parirokh and Bolhari, it can be concluded that MTA, Root MTA and Portland cement seem almost identical physically and histologically. They were also reported to be biocompatible. However, further long-term investigations are required to replace Root MTA and Portland cement type I, as more cost effective and appropriate substitutes for MTA.

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