

Influence of white mineral trioxide aggregate on inflammatory cells before and after expiry date

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Abstract– Introduction: The aim of this study was to compare the effect of subcutaneously implanted white mineral trioxide aggregate (WMTA) on inflammatory reactions before and after expiry date. **Methods:** Fifty Wistar rats were used in this study. Polyethylene tubes were filled with WMTA with expiry dates of 2008, 2009, and 2011, and empty ones serving as the controls were implanted into the subcutaneous tissue. The rats were sacrificed at 7-, 14-, 28-, and 60-day intervals. 5- μ m sections were stained with hematoxylin and eosin and observed under a light microscope. Inflammatory reactions were categorized as 0, none (without inflammatory cells); 1, mild (inflammatory cells < 25); 2, moderate (25–125 inflammatory cells); and 3, severe (more than 125 inflammatory cells). Statistical analysis was performed with Kruskal–Wallis test. **Results:** All the experimental materials provoked moderate to severe inflammatory reactions after 7 days, which significantly differed from the control group ($P < 0.05$). At 14-day interval, WMTA with expiry date of 2008 and the control group elicited mild to moderate infiltration of inflammatory cells. However, WMTA with expiry dates of 2009 and 2011 provoked moderate to severe inflammatory reactions, which were significantly different from WMTA with expiry date of 2008 and the control group ($P < 0.05$). At 28- and 60-day intervals, the overall inflammation subsided in all the groups to mild to moderate infiltration of inflammatory cells without any significant differences ($P < 0.05$). **Conclusion:** It seems that the expiry date has less negative effects on the response of inflammatory cells. WMTA keeps its biocompatibility even after expiry date.

Mineral trioxide aggregate (MTA) was developed as a root-end filling material at Loma Linda University in 1993 (1). The principal components of this material are tricalcium silicate, tricalcium aluminate, tricalcium oxide, and silicate oxide (2). MTA is currently marketed in two forms: gray (GMTA) and white (WMTA). Investigations have shown that contrary to WMTA, the gray one contains lower amounts of iron, aluminum, and magnesium, leading to discoloration potential of GMTA (3). MTA has been used for pulp capping, pulpotomy, repair of root perforations, apical barrier formation in non-vital teeth with open apices, and root canal filling (4).

Although MTA has many indications in endodontics, its usage is not as widespread as that of materials such as composite resins, gutta-percha or root canal sealers, luting agents, etc. in a general practitioner dental office. It has been reported that the shelf life of almost half of dental cements and base/liner materials may be past the manufacturer's expiry date at the time of clinical use (5). The period that dental materials are stored before use and the conditions under which they are stored and shipped to the user may have detrimental effects on their

physical, mechanical, and chemical properties, and ultimately on their clinical efficacy (6).

Some of the effects of aging on different dental materials, such as gutta-percha (7), composite resins (8), dental luting agents (9), and root canal sealers (10), have been investigated. The influence of environmental conditions on mechanical and physical properties of WMTA has been investigated in some research studies, including the evaluation of sealing ability at different pH values (11), setting time (12, 13), surface hardness (14, 15), and the morphology of WMTA stored under various conditions (16). In addition, the effect of different storage temperatures on microhardness, surface topography, and phase structure of WMTA has been previously evaluated (17). However, there is no available data on the physical and chemical properties of expired MTA.

Several *in vitro* and *in vivo* tests are used to evaluate the biocompatibility of dental materials, such as testing the cytotoxicity of materials in a cell culture, implantation tests, and animal studies (4). The results of a meta analysis showed that MTA is more biocompatible than IRM, Super EBA, and silver amalgam (18).

A number of studies have investigated subcutaneous responses to various types of MTA (19–23). There have been some conflicting results regarding subcutaneous reactions to white MTA (WMTA) and gray MTA (GMTA; 19, 20). It has been reported that WMTA is more biocompatible than GMTA after 3 days, but GMTA is more biocompatible than WMTA after 7 days, with no significant differences in their biocompatibility after 21 days (19). A study found no differences between the inflammatory responses to GMTA and WMTA (20). In a study, 2.5 wt% Na₂HPO₄ was added to WMTA and a significantly lower inflammatory response was reported compared with WMTA when the materials were implanted subcutaneously (22). To date, there are no available data regarding the biocompatibility of expired MTA. Therefore, the aim of this study was to evaluate the effect of subcutaneously implanted WMTA on inflammatory reactions before and after expiry date.

Materials and methods

The research protocol was approved by the Research Ethics Committee of Kamal Asgar Research Center (protocol no. KARC/10B2010-45-11). This study was similar to those carried out previously (20, 22). Fifty 2–3-month-old male Wistar albino rats weighting 250 ± 30 g were used in this study. All the ethical and human criteria contained in Helsinki declaration and all the recommended points by Institutional Animal Care and Use Committee in the care and use of laboratory animals were observed in the different stages of the project. The following materials were examined: ProRoot white MTA sachets (Tooth-colored Formula; Dentsply Tulsa Dental, Johnson City, TN, USA) were selected and divided into three groups of 10 each as shown in Table 1. Each sachet was mixed with its ampoule according to manufacturer's instructions under aseptic conditions.

The animals were anesthetized with diethyl ether using the chamber induction method. Three separate 2-cm incisions were made on the back of the rats at least 2 cm away from each other. Freshly mixed materials were prepared according to the recommendations of the manufacturer. In expired ampoules, sterile distilled water for injection was used to avoid the use of un-sterile distilled water and compensate for lack of water to meet powder/liquid ratio of 3:1 by weight. All the samples were placed in sterile polyethylene tubes with a 1.1-mm inner diameter and 8-mm length and were immediately implanted subcutaneously in two separate incisions. An empty polyethylene tube was implanted in the third incision in each animal as a control at 7-, 15-, 30-, and 60-day intervals. The rats were euthanized by administering a high dose of diethyl ether in an induction chamber.

Table 1. Groups of MTA used in this study

Group	Lot number	Expiry date
I	083006	01/2011
II	06002895	07/2009
III	05004913	12/2008

The tubes and surrounding tissues were removed in block and fixed in 10% buffered formalin solution for 2 weeks. 5- μ m tissue sections were prepared longitudinally through the midline of the tubes and stained with hematoxylin and eosin. Quantitative evaluations of inflammatory cells (lymphocytes, plasmocytes, polymorphonuclear leukocytes, macrophages, and giant cells) were made in microscopic fields adjacent to the test materials at the end of the tubes under a light microscope (Carl Zeiss, Oberkochen, Germany) at $\times 500$ magnification. An average value for each specimen was obtained from the sum of cells counted in four separate areas (24–26). The observer did not have any knowledge of the materials used in specimens. The overall mean value for each material was determined in subjects at each period.

The inflammatory reactions were categorized as:

- 0: none (without inflammatory cells);
 - 1: mild (<25 inflammatory cells);
 - 2: moderate (25–125 inflammatory cells);
 - 3: severe (more than 125 inflammatory cells).
- Kruskal–Wallis and Mann–Whitney tests were used for statistical analyses. Statistical significance was defined at $P < 0.05$.

Results

At 7-day interval, the mean inflammation grades were 2.6 ± 0.51 , 2.4 ± 0.51 , and 2.8 ± 0.42 for WMTA that expired in 2008, 2009, and 2011, respectively, consisting of moderate to severe infiltration of inflammatory cells (Fig. 1). There were no significant differences among the groups ($P = 0.2$). At 14-day interval, the mean inflammation grades were 1.8 ± 0.42 , 2.3 ± 0.48 , and 2.5 ± 0.52 for WMTA that expired in 2008, 2009, and 2011, respectively. MTA expiring in 2008 elicited mild to moderate infiltration of inflammatory cells (Fig. 1). MTA groups with expiry dates of 2009 and 2011 provoked moderate to severe infiltration of inflammatory cells.

There were no significant differences between WMTA with expiry dates of 2009 and 2011 ($P = 0.374$), but WMTA with expiry date of 2009 and 2011 showed significant differences from WMTA with expiry date of 2008 and the control group ($P < 0.05$) (Fig. 2). At 28- and 60-day intervals, the overall inflammation subsided in all the groups to mild to moderate infiltration of inflammatory cells with no significant differences ($P > 0.05$) (Fig. 2).

Discussion

In this study, the 2008 group was selected to simulate long-expired situation, 2009 was selected for short aging, and the 2011 (before expiry) was selected as gold standard. The executive part of this study was carried out in spring 2010.

In this study, subcutaneous implantation was used to evaluate the biocompatibility of materials. This method was introduced in 1966 (24) and approved in 1981 (27).

In the current study, at the 7-day interval, all the experimental groups evoked moderate to severe inflammatory reactions, which is partially consistent with the findings of a research study (20), reporting that the subcutaneous reaction to both types of MTA (WMTA

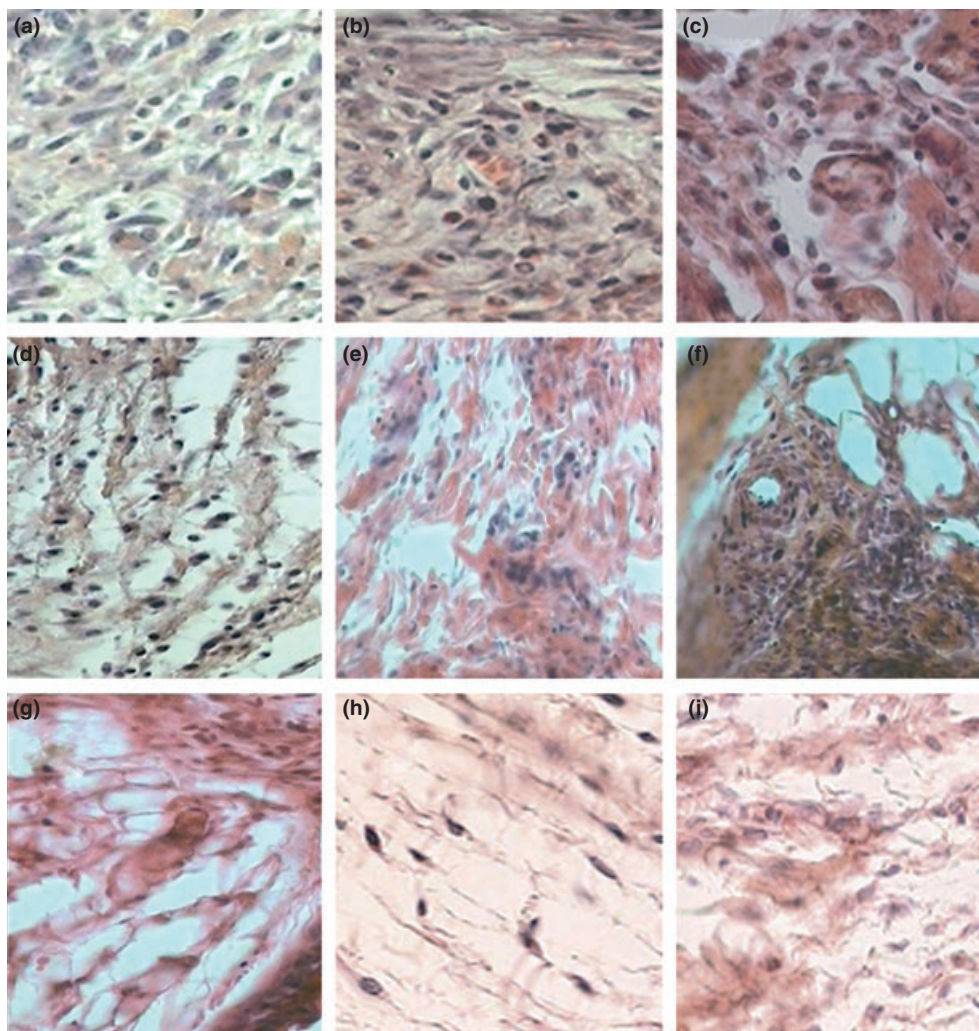


Fig. 1. Histologic images of inflammatory cell infiltration at the end of implanted tubes in the experimental groups (hematoxylin-eosin staining; original magnification, $\times 400$). (a, b, c) Seven-day WMTA with expiry dates of 2008, 2009, and 2011, respectively; moderate infiltration (25–125) of inflammatory cells (lymphocytes, plasmacytes, polymorphonuclear leukocytes, and macrophages), grade 2. (d, e, f) Fourteen-day WMTA with expiry dates of 2008, 2009, and 2011, respectively; mild to moderate infiltration of lymphocytes, plasmacytes, and polymorphonuclear leukocytes is seen in WMTA with expiry dates of 2008. However, moderate to severe infiltration of lymphocytes, plasmacytes, and polymorphonuclear leukocytes is seen in WMTA with expiry dates of 2009 and 2011. (g, h, i) Twenty-eight-day WMTA with expiry dates of 2008, 2009, and 2011, respectively; mild to moderate infiltration of inflammatory cells (lymphocytes, plasmacytes, polymorphonuclear leukocytes, and macrophages) is seen in WMTA with expiry dates of 2008, 2009, and 2011.

and GMTA) was a moderate, not severe, infiltration of inflammatory cells at the 7-day interval. Another study demonstrated mild to moderated inflammatory reactions after subcutaneous implantation of WMTA after 7 days (22). In this study, the difference between experimental and control groups at 7-day interval was significant, which is consistent with the results of several studies (20, 22).

In this study, at 14-day interval, WMTA with expiry dates of 2011 (un-expired WMTA) and 2009 provoked moderate to severe inflammatory responses. Accordingly, the results of some studies (20, 22) have shown moderate to severe reactions to un-expired implanted WMTA. In our study, WMTA expired in 2008 significantly provoked fewer inflammatory reactions compared with WMTA with expiry dates of 2009 and 2011 at 14-day interval.

This study showed that at 28- and 60-day intervals, all the study groups (experimental and control) induced mild to moderate infiltration of inflammatory cells. Similarly, an investigation (20) reported mild to moderate inflammatory reactions to 30- and 60-day subcutaneously implanted WMTA and GMTA samples. When MTA powder is mixed with water, calcium phosphate and calcium oxide are released from MTA, which produce calcium hydroxide in contact with tissue fluids (28). Formation of calcium hydroxide is the cause of high alkalinity of MTA after hydration (29), which is considered an initial tissue irritant when MTA comes into contact with tissues (30). This would explain inflammatory reactions subsequent to subcutaneous implantation of MTA (31).

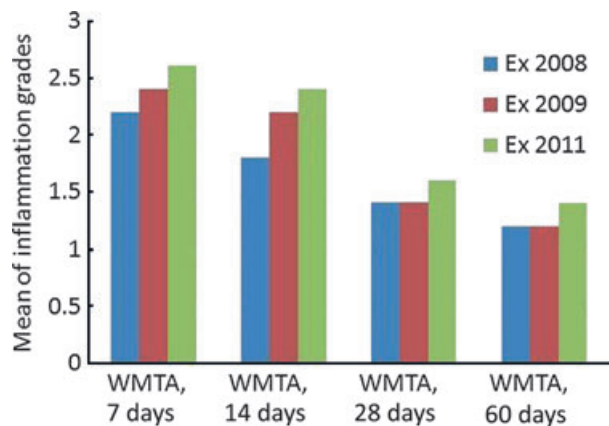


Fig. 2. The mean inflammation grades in white mineral trioxide aggregate with different expiry dates and test periods.

In conclusion, although the physical properties of WMTA may change over time, it seems that the expiry date has less negative effects on the inflammatory cells response. WMTA keeps its biocompatibility even after expiry date. Further *in vitro* and *in vivo* experiments are necessary to provide direct evidence for manufacturer-determined expiry date for WMTA.

Conflict of interest

The authors deny any conflicts of interest related to this study.

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