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Comparative study of subcutaneous tissue responses to a novel root-end filling material and white and grey mineral trioxide aggregate

M. Parirokh[1], B. Mirsoltani[2], M. Raoof[1], H. Tabrizchi[3] & A.A. Haghdoost[4,5]

[1]Oral and Dental Diseases Research Center, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran; [2]Endodontic Department, School of Dentistry, Qazvin University of Medical Sciences, Qazvin, Iran; [3]Pathology Department, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran; [4]Physiology Research Center, Kerman University of Medical Sciences, Kerman, Iran; and [5]London School of Hygiene and Tropical Medicine, London, UK

Abstract

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Aim To compare the subcutaneous tissue response to grey mineral trioxide aggregate (GMTA), white mineral trioxide aggregate (WMTA) and a new experimental cement (calcium enriched cement, CEM).

Methodology Thirty-six Wistar male albino rats each received three implants, containing one of the tested materials, and an empty tube as a control. Seven, 30 and 60 days after implantation, the animals were sacrificed. After histological preparation and H&E staining, the specimens were evaluated for capsule thickness, necrosis, and for the type, the severity, and the extent of inflammation. Kruskal Wallis and Chi-square tests were used for data analysis.

Results After 1 week, CEM produced no necrosis compared to both types of WMTA and GMTA ($P = 0.007$). After 30 days, GMTA specimens had significantly less inflammation compared with WMTA and CEM ($P = 0.011$). After 60 days, less inflammation was associated with CEM specimens ($P = 0.0001$) compared to the other materials. Dystrophic calcifications in the connective tissue adjacent to all experimental material were detected.

Conclusion Histological observation illustrated that all materials were well tolerated by the subcutaneous tissues.

Keywords: biocompatibility, grey, mineral trioxide aggregate, novel cement, white.

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Introduction

The aim of periapical surgery is to remove diseased tissue and to carry out root-end resection and root-end filling to seal the communication between the periapical tissues and the root canal system (Johnson & Witherspoon 2006). The purpose of a root-end filling is to produce a hermetic seal after root-end resection (Torabinejad & Pitt Ford 1996, Kim & Kratchman 2006). Root-end filling materials are in direct contact with the periapical tissues and for this reason, an ideal material should be biocompatible, impervious to dissolution or breakdown by the tissue fluids, non-resorbable, adapting as closely as possible to the dentinal walls of the root-end preparation and possess good handling characteristics (Torabinejad & Pitt Ford 1996). MTA is a relatively recent root-end filling

Correspondence: Dr Maryam Raoof, Endodontic Department, School of Dentistry, Shafa Street, Jomhori Eslami Boulevard, Kerman, Iran (Tel.: +98 341 2112093; fax: +98 341 2118073; e-mail: maryam.raoof@gmail.com).

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material and has shown greater resistance to leakage in root-end cavities when compared with other materials (Parirokh & Torabinejad 2010a, Torabinejad & Parirokh 2010). In addition, its biocompatibility as well as favourable clinical results have resulted in it being the material of choice for clinical applications such as pulp capping and root-end filling (Parirokh & Torabinejad 2010b, Torabinejad & Parirokh 2010).

Efforts to develop new root-end filling and pulp capping materials continue (Chng *et al.* 2005, Gandolfi *et al.* 2007, 2008a,b, Tay *et al.* 2007, Ozok *et al.* 2008, Gomes-Filho *et al.* 2009a,b, Martínez Lalis *et al.* 2009, Saliba *et al.* 2009, de Vasconcelos *et al.* 2009, Camilleri 2010, Camilleri & Gandolfi 2010). Most of these new materials have been compared with MTA for their physical and chemical properties, biocompatibility and clinical applications. Recently, a new dental material has been introduced with appropriate setting time, handling characteristics, chemical properties, colour and sealing ability (Asgary *et al.* 2008a,b,c). Results of recent laboratory experiments have shown that in a synthetic tissue fluid, such as phosphate-buffered saline, hydroxyapatite-like crystals precipitated over both calcium enriched mixture (CEM) and MTA surfaces after 1 week (Asgary *et al.* 2009b). The authors attributed the biocompatibility, sealing ability and favourable clinical applications of both materials to their bioactivity when in contact with tissue fluid.

The new experimental material (CEM cement, Yektazdandan; Bionique Dent, Tehran, Iran) contains CaO, SiO2, MgO, SO3, P2O5, Na2O and Cl (Asgary *et al.* 2008c, 2009a). Antibacterial activity, cytotoxicity and clinical applications of CEM have been evaluated in several studies (Asgary & Kamrani 2008, Asgary & Ehsani 2009, Hasan Zarrabi *et al.* 2009, Samiee *et al.* 2009, Asgary & Eghbal 2010b, Asgary *et al.* 2010, Tabarsi *et al.* 2010).

The biocompatibility of all experimental dental materials that might come in contact with tissues should be examined. The biocompatibility of dental materials is an important requirement because the toxic components present in these materials could produce irritation or even degeneration of the surrounding tissues (Ingle *et al.* 2002). Subcutaneous tissue reaction is one of the *in vivo* biocompatibility tests that has been used for examining several root-end filling materials (Torabinejad & Pitt Ford 1996).

Thus, the purpose of this study was to compare the biocompatibility of grey and white MTA and CEM.

Material and methods

The research protocol was approved by the Research Ethics Committee of Kerman University of Medical Sciences (protocol no. KA/85/75). The experiment was carried out in accordance with the European Economic Community's directive of 24 November 1986 (86/609/EEC) and Institutional Animal Care and Use Committee (IACUC) recommendations regarding the care and use of laboratory animals.

Thirty-six male Wistar albino rats weighing 200–250 mg were used. The animals were anaesthetized by intraperitoneal administration of 47.5 mg kg^{-1} ketamine HCL (Alfasan, Woerden, the Netherlands) and 0.1 mg kg^{-1} Rompun 2% (Alfasan). In each animal, two anterior sites and two posterior sites right and left of the dorsal skin were shaved (total of four sites). For pain relief, 0.1 mL of 2% lidocaine with 1/80 000 epinephrine (Darupakhsh, Tehran, Iran) was used as a local anaesthetic at the sites of implantation. The areas were first disinfected with povidine iodine 10% and then a 12-mm incision was made in each area using a No. 15 blade (Carl Martin, Solingen, Germany). Subsequently, a blunt dissecting instrument was used to create a 20-mm-deep pocket in the subcutaneous tissues to receive the implants. Each rat received three implants, containing grey ProRoot MTA (Dentsply Tulsa Dental, Tulsa, OK, USA), white ProRoot MTA (Tooth Colored Formula; Dentsply Tulsa Dental) or CEM (Yektazdandan; Bionique Dent).

All the materials were prepared according to the manufacturers' instructions in a 3 : 1 powder to liquid ratio. The ethylene dioxide sterilized polyethylene tubes consisted of a single lumen 7 mm long and an internal diameter of 1.7 mm. Each tube was filled with one of the materials. Because the materials have a putty consistency after mixing with distilled water, they did not flow out of the tube or into the adjacent tissue. The animals were randomly divided into three groups consisting of 12 animals each.

Seven, 30 and 60 days after implantation, the animals were sacrificed by administering an overdose of ketamine HCL (Alfasan). The implantation areas were shaved, and the skin and underlying connective tissue containing the implant were excised as a block section and kept in 10% formalin for a minimum of 48 h. After fixation, a section parallel to the long axis of the tube was made. The tissues were prepared for haematoxylin & eosin (H&E) staining.

A pathologist who was unaware of the materials and time intervals evaluated the specimens. For evaluating

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tissue reaction, a modification of the criteria described by Yaltirik *et al.* (2004) was used. The tissue reactions at both ends of the tubes were assessed and measured with the histological criteria outlined below.

I. The thickness of connective tissue capsule:

Defined by the extent of connective tissue formation around the tube in ×10 field of vision and measured by a micrometre

0: without capsule

1: capsule thickness is less than 150 μm

2: capsule is thicker than 150 μm

II. Severity of inflammation:

Defined by the concentration of inflammatory cells in/around the connective tissue capsule in ×40 field of vision:

0: without inflammation

+1: <25 cell counts

+2: 25 < cell count < 50

+3: 50 < cell count < 75

+4: Over 75 cell counts

III. Extent of inflammation

Defined by the extension of inflammation in ×40 field of vision:

+1: Inflammatory cells could be observed just at the superficial layer of the capsule

+2: Inflammatory cells are limited to the fibrous capsule

+3: Inflammatory cells could be observed beyond the capsule

IV. Necrosis:

Defined as present or not in ×40 field of vision.

0: the absence of necrosis

1: The presence of necrosis

V. The type of inflammatory cells:

The type of inflammatory cells seen under ×40 field of vision was also recorded.

Because most of the variables in this study had an ordinal scale, non-parametric statistical tests were used. The difference between groups was assessed using Kruskal Wallis and Chi-square tests. The level of significance was set at 0.02 to minimize the effect of multiple tests.

Results

Histological evaluation illustrated chronic inflammation around both ends of the tubes in all the specimens tested as well as controls. Calcific precipitations were also found in 33% of grey mineral trioxide aggregate (GMTA) and CEM as well as 22% of white mineral trioxide aggregate (WMTA) specimens (Fig. 1a,b).

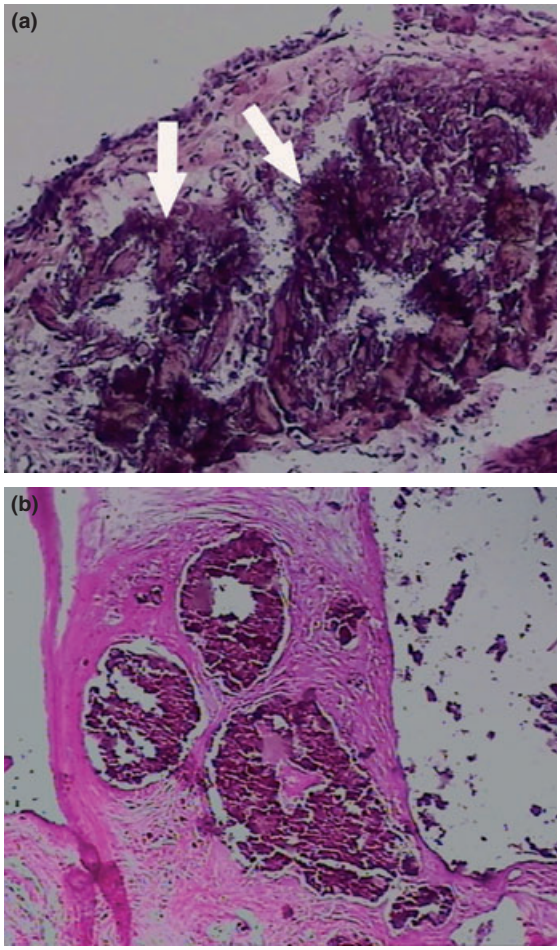

Figure 1 (a) Dystrophic calcification around grey mineral trioxide aggregate material (white arrows) (×10); (b) dystrophic calcification around calcium enriched cement material (×10).

Comparisons between experimental materials at different time intervals were as follows:

Seven days interval

The capsule thickness surrounding the tubes was significantly thinner in the control specimens when compared with the experimental materials ($P = 0.008$). There was no significant difference amongst the tested materials in the extension and severity of inflammation ($P > 0.05$). CEM specimens were not associated with necrosis; this was significantly different to the other specimens in the two MTA groups ($P = 0.007$) (Fig. 2a,b). Control specimens were not significantly different in terms of the

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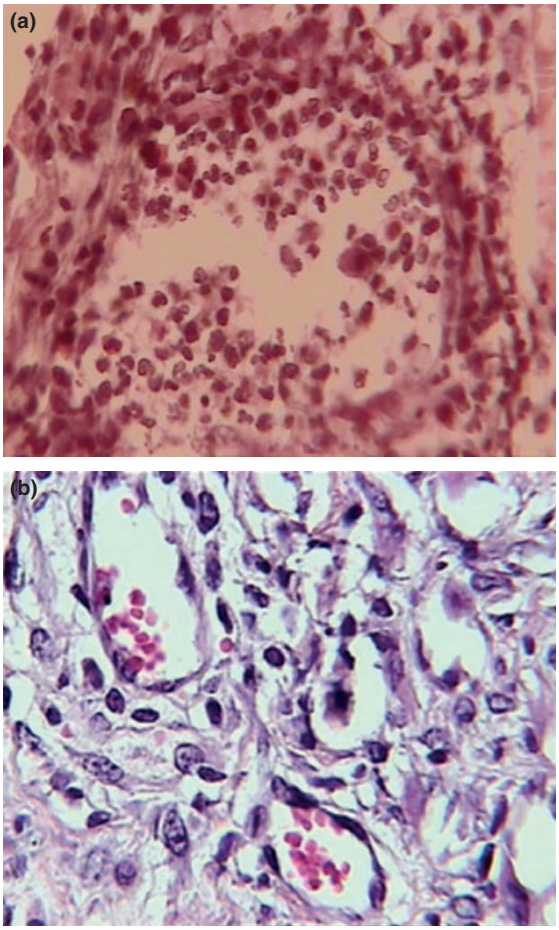

Figure 2 (a) Connective tissue necrosis 7 days following grey mineral trioxide aggregate implantation (×40); (b) chronic inflammation 7 days after subcutaneous implantation of calcium enriched cement (×40).

extent of inflammation compared to the tested materials ($P > 0.05$).

Thirty days interval

Calcium enriched cement specimens contained a thinner capsule compared with both types of GMTA, WMTA and the control specimens ($P = 0.012$), whereas GMTA had the least extent of inflammation (Fig. 3) in comparison with the other tested materials ($P = 0.011$). Control specimens had significantly less severity of inflammation in comparison with the experimental materials ($P = 0.018$).

All tested materials and control specimens had significantly less severity of inflammation compared to

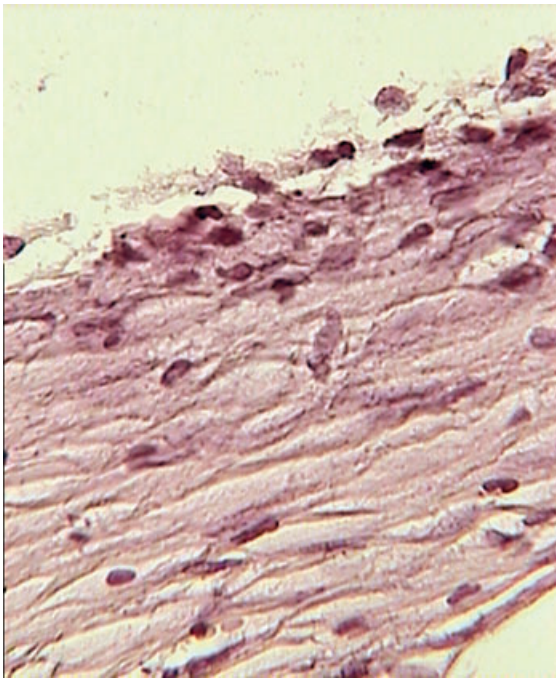

Figure 3 Mild inflammation grey mineral trioxide aggregate samples 30 days after implantation (×40).

7 days specimens ($P < 0.0001$). None of the specimens revealed necrosis.

Sixty days interval

No significant difference amongst the tested materials and control specimens was observed in terms of capsule thickness and severity of inflammation ($P > 0.01$) (Fig. 4a,b). The extent of inflammation was lower in the CEM group in comparison with the other tested materials ($P < 0.0001$). None of the specimens in the three groups were associated with necrosis.

Discussion

Recently, CEM was introduced as a root-end filling material, perforation repair material and pulp capping agent (Asgary *et al.* 2008a,b, Asgary & Ehsani 2009, Samiee *et al.* 2009, Asgary & Eghbal 2010a,b, Tabarsi *et al.* 2010). The sealing ability, physical and chemical properties of the material have been investigated (Asgary *et al.* 2008a,c, 2009a). The present study demonstrated promising results for the material in terms of subcutaneous implantation.

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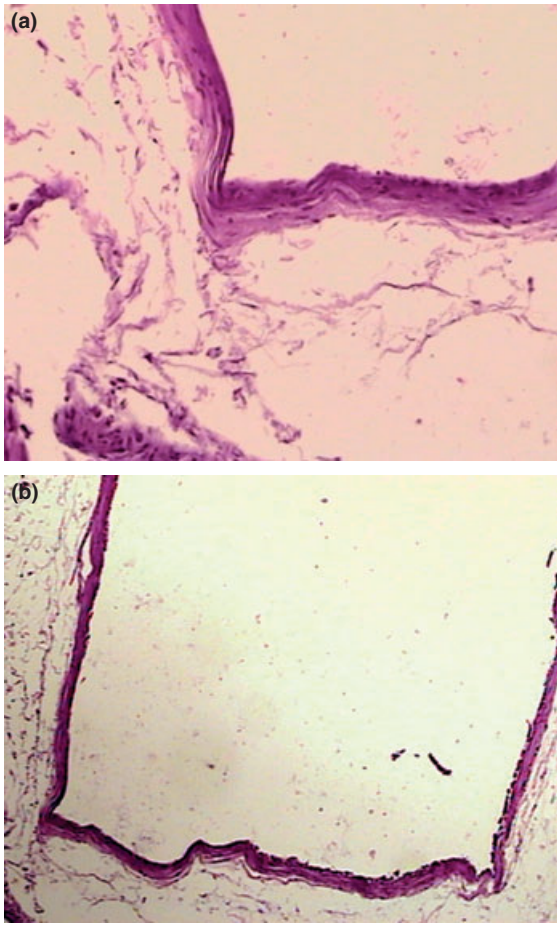

Figure 4 (a) Thin capsule around control specimens 60 days after implantation (×10); (b) thin capsule around white mineral trioxide aggregate 60 days after implantation (×10).

Although the biocompatibility tests currently available are not ideal, they do provide data from which reasonable deductions about a dental material may be compiled. It has been suggested that implantation in subcutaneous tissues of small experimental animals is one of the most suitable methods to determine the local effects of materials (Olsson *et al.* 1981, Safavi *et al.* 1983). Therefore, the results of the present study could be used as a preliminary source of information on the biocompatibility of CEM.

According to Olsson *et al.* (1981), the placement of an experimental material in a polyethylene tube before implantation prevents the diffusion of the material into the connective tissue, which simulates the situation in the root canal. Another advantage of this method is that the material comes into direct contact with the connective tissues. Makkes *et al.* (1977) have shown that polyethylene tubes are a suitable container for root canal sealers as they do not produce tissue reactions. Many other research studies have also used polyethylene for the same purpose (Kim *et al.* 2004, Yaltirik *et al.* 2004, Moreira *et al.* 2005, Shahi *et al.* 2006, Vosoughhosseini *et al.* 2008). The inert nature of polyethylene tubes and their ability to expose a test material to living tissue in a controlled and effective manner is why they are often employed in biocompatibility studies.

There are many methods for evaluating the severity of subcutaneous reaction to dental materials; however, they may produce conflicting results (Shahi *et al.* 2006, Vosoughhosseini *et al.* 2008). Yaltirik *et al.* (2004) evaluated the extension of inflammation, number of inflammatory cells, presence of fibrous capsule and necrosis as did the present study with slight modification. After 30 days, capsule thickness around the CEM was significantly less than GMTA and WMTA and even the control groups ($P = 0.012$). Makkes *et al.* (1977) concluded that decreasing capsule thickness is a sign of biocompatibility.

The deferred harmful effects of a material are considered to be more important than its initial effects in biocompatibility tests (Stanford 1980). Control and the tested material specimens in the present study had significantly less severity of inflammation at longer time intervals (30 and 60 days) compared to 7 days specimens. The results of subcutaneous implantation of both types of MTA are consistent with the results of previous investigations (Holland *et al.* 1999, 2002, Moretton *et al.* 2000, Yaltirik *et al.* 2004, Modaresi *et al.* 2005, Shahi *et al.* 2006, Sumer *et al.* 2006).

The responses to MTA have ranged from necrosis to dystrophic calcification in various reports (Holland *et al.* 1999, 2002, Yaltirik *et al.* 2004, Modaresi *et al.* 2005, Shahi *et al.* 2006, Sumer *et al.* 2006). Koh *et al.* (1998) suggested that necrosis occurs as an early reaction to MTA because of the high pH of the freshly mixed material, its high temperature during setting and the production of cytokines such as IL1 and IL6. A recent study on the physical properties of CEM and WMTA reported a similar pH value for both materials (Asgary *et al.* 2008b). Therefore, the absence of necrosis around CEM at the early time interval in the present study may be attributed to the difference in cytokine induction or the lower temperatures produced during setting. However, this cannot be confirmed.

Calcific precipitation was also observed around implantation sites (33% of GMTA, CEM and 22% of WMTA specimens). Several studies have reported this

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phenomenon when MTA was implanted subcutaneously (Holland *et al.* 1999, 2002, Yaltirik *et al.* 2004); conversely, others have not found such calcified structures (Modaresi *et al.* 2005, Shahi *et al.* 2006, Vosoughhosseini *et al.* 2008). The production of calcific structures in subcutaneous investigations is a sign of osteoinductivity of the experimental material (Moretton *et al.* 2000).

Two laboratory investigations reported that CEM can release calcium and phosphorus ions (Amini Ghazvini *et al.* 2009) and form hydroxyapatite over its surface in synthetic tissue fluid as well as normal saline solution (Asgary *et al.* 2009b). Formation of hydroxyapatite over MTA has been demonstrated previously (Parirokh & Torabinejad 2010b). The biocompatibility and osteoinductivity and conductivity of CEM may be attributed to the release of calcium and phosphorous as well as the formation of hydroxyapatite crystals over the material.

Conclusion

Both MTA and CEM were tolerated well by the subcutaneous tissues. Presence of calcifications in response to the materials revealed their osteoinductive ability when they come in contact with connective tissues.

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