ORIGINAL ARTICLE

Assessment of the biocompatibility of mineral trioxide aggregate, bioaggregate, and biodentine in the subcutaneous tissue of rats

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Abstract

Objective: The objective of this study was to evaluate the tissue inflammation caused by three endodontic repair materials.

Materials and Methods: The materials included micro mega-mineral trioxide aggregate (MM-MTA), bioaggregate (BA), and biodentine (BD), which were implanted into the subcutaneous tissue of rats. The tissue samples for histological examination were prepared. The infiltration of lymphocytes and macrophages into the tissue was examined to assess the inflammatory response.

Results: Lymphocyte infiltration: A significant increase was detected in the MM-MTA and BA groups on the 7th and 14th days as compared with the control (7th day $P = 0.0001$, 14th day $P = 0.0176$). There was no difference between the groups on the 45th day ($P = 0.1730$). Lymphocyte infiltration had decreased over time in all groups. Macrophage infiltration: There was a significant increase by the 7th day in the test groups as compared to the control group ($P = 0.007$). However, there was no difference between the experimental groups on the 14th ($P = 0.2708$) and 45th ($P = 0.1291$) days.

Conclusion: While MM-MTA and BA showed a similar biocompatibility, BD was more biocompatible than MM-MTA and BA in the 1st week of the experiment. However, there was no difference between the materials at the end of the 45th day. MM-MTA, BA, and BD can be considered suitable endodontic repair materials.

Key words: Bioaggregate, biocompatible materials, biodentine, endodontics

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Introduction

Root repair materials are expected to have an acceptable biocompatibility, radiopacity, good sealing ability, physical and chemical stability and also set in a wet environment to achieve successful treatment.[1] Biocompatibility can be described as a biomaterial's ability to function as a medical device within the human tissue and to carry out a specific task in the presence of an appropriate host response. These materials must not cause an unacceptable degree of harm to the body and must not carry any risks.[2,3] In endodontics, they can be used to preserve pulp vitality, for disinfection of root canal space during endodontic treatment, and as a root canal filling.[2] The biocompatibility of root canal filling materials is important because they are in contact with vital periapical tissues and may penetrate through dentine.[4] The biocompatibility of dental materials can be evaluated via *in vitro* and *in vivo* tests. Some of the testing methods are as follows: Testing the general toxicity profile of dental materials in a cell culture; implantation tests; and usage tests in experimental animals.[5] While using animal testing to study the biocompatibility, the material can be implanted to the subcutaneous tissue of rats[6-8] inside polyethylene tubes.

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Mineral trioxide aggregate (MTA) is a bioceramic aggregate that was first introduced by Lee *et al*.[9] as a root end filling material; however, nowadays it has several clinical applications and is more commonly used in endodontic therapy.[10,11] Because of MTAs' superiority to other root end filling materials in terms of its biocompatibility and osteoconduction ability, it is recognized as the gold standard for various clinical applications.[11] It is preferred for vital pulp therapy, apical MTA plug, root repair for perforations, root end filling material, repair of vertical fractures, and coronal plug after root canal obturation.[12] *In vitro* and *in vivo* studies have shown that MTA is a bioactive and biocompatible material.[10,13] Micro mega MTA (MM-MTA; MM, Besançon, France) is manufactured in special capsules, which provide automatic mixing with a vibrating mixer contain powder and liquid. It has the similar content with MTA is a biocompatible, radiopaque and material with excellent adhesion to the dentine according to the manufacturer. Addition of calcium carbonate ($CaCO_3$) to the mixture, reduces the setting time considerably; thereby allowing for filling within the same treatment session.[14] Bioaggregate (BA; Innovative BioCeramix, Vancouver, BC, Canada) is a new bioceramic root end filling material, which is composed of tricalcium silicate, dicalcium silicate, calcium phosphate monobasic, amorphous silicon dioxide, and tantalum pentoxide. The differences between BA and MTA are that BA does not contain aluminum but contains calcium phosphate monobasic and tantalum pentoxide.[10] Another new calcium silicate-based material is Biodentine (BD; Septodont, Saint Maur-des Fossés, France), which contains tricalcium silicate, $CaCO_3$, zirconium oxide, a water-reducing agent and a water-based liquid-containing calcium chloride as the setting accelerator.[15] According to the manufacturer, BD has the advantages of similar bioceramic cements without their disadvantages; displaying a faster setting time and better mechanical properties.[15,16] Histological studies comparing the biocompatibility of these three bioceramic materials are lacking. Thus, the aim of this study was to evaluate the biocompatibility of the MM-MTA, BA, and BD through histological analyzes of the subcutaneous tissue of rats, in which the materials were implanted in polyethylene tubes.

Materials and Methods

In this study, 45 white female Wistar rats, 3-4 months old and weighing 150-200 g, were used with the approval of the Animal Ethics Research Committee of Inonu University (2013/A-16). Sterile polyethylene (nontoxic Scalp Vein 19G) tubes (1.3 mm internal diameter, 10 mm long, with one of the end sealed) filled with the test materials MM-MTA, BA and BD were used according to the manufacturer's instructions while empty tubes were used as control groups. Three separate groups were created, each consisting of 15 experimental animals.

The experimental animals were anesthetized with xylazine (7 mg/kg; Rompun, Bayer) and ketamine HCl (50 mg/kg; Ketalar, Parke-Davis). The surgical sites on the dorsal skin were shaved. Then, four equal sections at equidistance to each other were identified, and 2 mm incisions were performed with surgical scissors (Aesculap, Tuttlingen, Germany). Deep blunt dissection from opened incisions was made without opening the peritoneum, and two blank control tubes were placed in the right side of the back of each animal. Two tubes filled with repair material were placed in the left side of the back of the each experimental animal with the aid of presel (Aesculap, Tuttlingen, Germany). The incisions were sutured using silk 3/0, and the entire shaved area was disinfected with 5% iodine solution.

After periods of 7, 14, and 45 days, 5 animals from each experimental group were sacrificed by cervical dislocation after anesthesia and the tubes with surrounding tissues were removed.

Histological evaluation

The tissue samples containing the tubes were removed, fixed in 10% formalin for 24 h, and the specimens embedded in paraffin. Sections were cut at 5 μm, mounted on slides, and stained with hematoxylin and eosin for general tissue structure. Tissue injury was scored according to infiltration of lymphocyte and macrophage into the tissue. Lymphocyte and macrophage infiltration were scored on a scale of 0–3: 0 for normal tissue, 1 for 25% injury involvement, 2 for 26-75% injury involvement and 3 for >75% injury involvements. The total histology score is the sum score of all parameters. Tissues were examined (by E.T) using a Leica DFC280 light microscope and a Leica Q Win Image Analysis system (Leica Micros Imaging Solutions Ltd., Cambridge, UK). The intra-class coronation confidence test was performed.

Statistical analysis

Statistical analysis was performed using SPSS for Windows (version 15.0, SPSS Inc., Chicago, IL, USA) and given as mean ± standard deviation. Within the groups, normality of variables was measured using the Shapiro–Wilk test. Data were analyzed with the Kruskal–Wallis and Connover tests. The differences were considered significant when $P < 0.05$.

Results

Histopathological findings

The intra-class coronation confidence test was performed ($r = 0.89$). The data for each period of time were compared and are presented in Table 1.

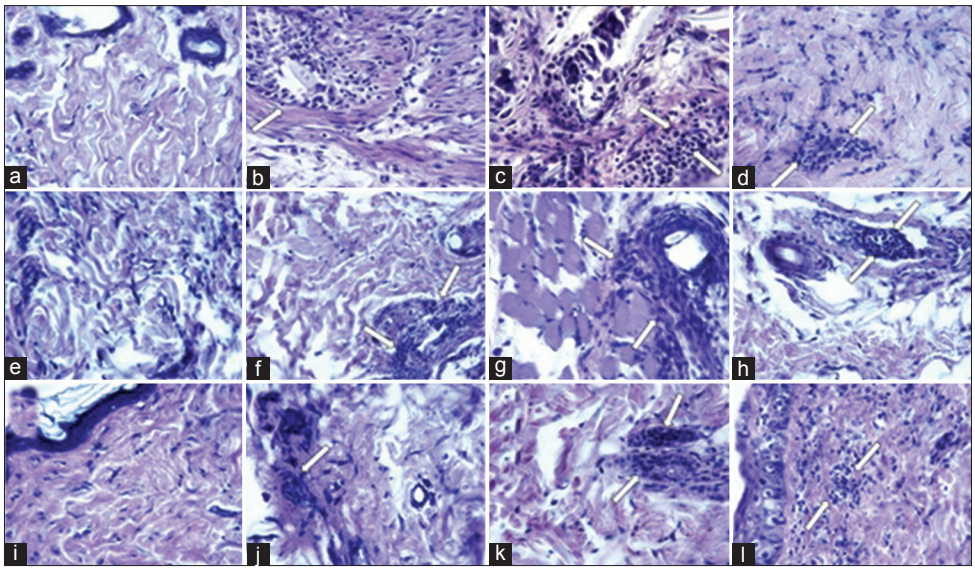

Figure 1: The view of lymphocyte cells (arrows) in test groups at all the experimental periods. Slight inflammatory reaction was observed in all test groups. Control (empty tube) groups: (a, e, i) 7, 14 and 45 days of test period, respectively. 7 days: (b-d) Lymphocyte infiltration of micro mega-mineral trioxide aggregate, bioaggregate and biodentine experimental groups, respectively. 14 days: (f-h) Lymphocyte infiltration of groups as described above. 45 days: (j-l) Lymphocyte infiltration of groups as described above (H and E, ×40)

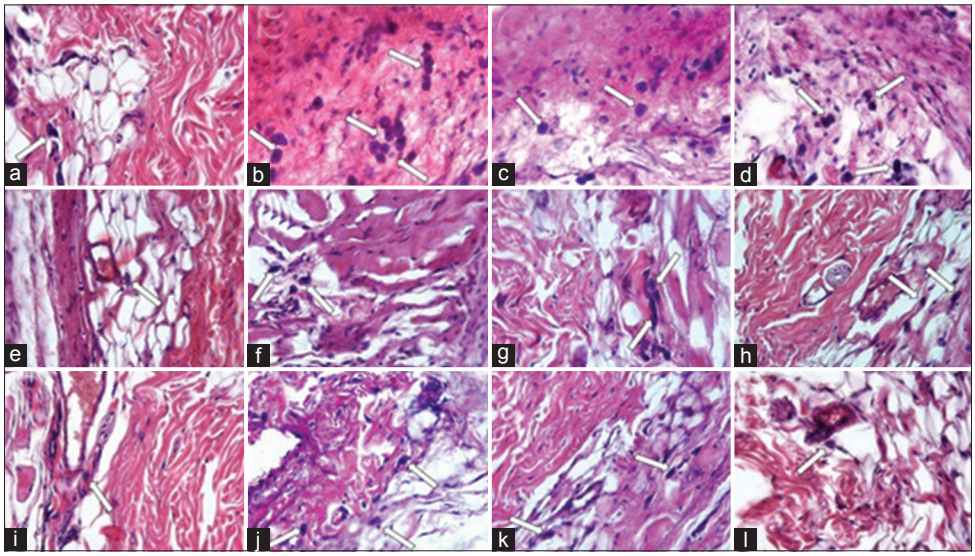

Figure 2: The view of macrophage cells (arrows) in test groups at all the experimental periods. Slight inflammatory reaction was observed in all test groups. Control groups: (a, e, i) 7, 14 and 45 days of test period, respectively. 7 days: (b-d) Macrophage infiltration of micro mega-mineral trioxide aggregate, bioaggregate and biodentine experimental groups, respectively. 14 days: (f-h) Macrophage infiltration of groups as described above. 45 days: (j-l) Macrophage infiltration of groups as described above (H and E, ×40)

Lymphocyte infiltration

In the MM-MTA and BA groups, there was a statistically significant increase in lymphocyte infiltration on the 7th day (P = 0.0001) as compared with the control group. The BD group showed an increase of lymphocyte infiltration on the 7th day, but this change was not statistically significant. On the 14th day, the lymphocyte infiltration had decreased in all groups, but there was still a statistically significant difference in the MM-MTA and BA groups (P = 0.0176). By the 45th day of the experiment, lymphocyte infiltration was the same in all groups (P = 0.1730) [Figure 1].

Macrophage infiltration

All groups showed a statistically significant increase on the 7th day when compared to the control groups (P = 0.007). However, there was no statistically significant change on the 14th and 45th days (P = 0.2708, P = 0.1291), and the macrophage infiltration had decreased in all groups [Figure 2].

Table 1: Means and SD of the histopathological score of lymphocyte and macrophage infiltration

Groups	Mean±SD					
	7th days		14th days		45th days	
	Lymphocytes infiltration	Macrophages infiltration	Lymphocytes infiltration	Macrophages infiltration	Lymphocytes infiltration	Macrophages infiltration
MM-MTA control	0.07±0.26[a]	0.05±0.22[a]	0.07±0.26[acd]	0.07±0.26	0.07±0.26	0.05±0.22
MM-MTA	0.35±0.48[b]	0.27±0.45[b]	0.27±0.45[b]	0.20±0.40	0.17±0.38	0.17±0.38
BA control	0.05±0.22[a]	0.05±0.22[a]	0.05±0.22[c]	0.07±0.26	0.05±0.22	0.07±0.26
BA	0.32±0.47[b]	0.25±0.43[b]	0.20±0.40[bd]	0.17±0.38	0.15±0.36	0.15±0.36
BD control	0.05±0.22[a]	0.10±0.30[a]	0.10±0.30[ac]	0.07±0.26	0.10±0.30	0.05±0.22
BD	0.25±0.43[a]	0.22±0.42[b]	0.25±0.43[b]	0.17±0.38	0.22±0.42	0.20±0.40
P	0.0001	0.007	0.0176	0.2708	0.1730	0.1291

Different superscript letters in the same column mean statistical significant difference within the group. MM-MTA=Micro mega-mineral trioxide aggregate; BA=Bioaggregate; BD=Biodentine; SD=Standard deviation

Discussion

In endodontic treatment, it is expected and desired that dental materials used, will be biocompatible and possibly stimulate healing in the tissues with which they have close or direct contact. In the literature different methods[10,15,16] are available to evaluate the biocompatibility and the cytotoxicity of the dental materials used in endodontics. One common method is the placement of dental materials into polyethylene tubes before placing them in the connective tissues of rats.[13] Using this method, the histological response of biological tissue to the dental materials over time can be examined and compared.[17] At sites of acute inflammation where the irritant is cleared, and the process is resolved, macrophages eventually die or wander off into lymphatics. In contrast, the accumulation of macrophages in chronic inflammation is permanent, and macrophages can proliferate. Sustained secretion, of the factors through which lymphocytes originate in an inflamed region, is an important mechanism by which macrophages are recruited or immobilized.[18]

The biocompatibility of MTA has been investigated in various studies and compared with different materials.[19,20] In one study, which examined the response of the periradicular tissues in dogs to MTA after 1-5 weeks, it was reported that MTA (used as root end filling material) is biocompatible and stimulates repair in periradicular tissues.[21] Holland *et al*. implanted MTA and calcium hydroxide materials in the connective tissue of rats. They took connective tissue samples from the experimental animals on the 7th and 30th days and evaluated them histologically and morphologically. The study concluded that the mechanism of action of MTA encourages hard tissue deposition and that calcium hydroxide showed a similar effect.[20]

In a cell study in which BA was used as a root end filling material, BA was reported as nontoxic to mouse osteoblast cells. Furthermore, when they compared BA group and MTA group, BA group caused significantly increase (induced genes) in some proteins such as collagen type 1, osteocalcin and osteopontin in 2nd and 3rd days of the cell culture.[11] In another cell culture study, it was shown that BA is nontoxic to the periodontal ligament fibroblast cells.[21,22] Similarly, another study indicated that BA and MTA are bioactive material.[10] However, when their cytotoxic effects were compared and evaluated in an *in situ* study, there was no significant difference between them,[23] but they had a similar effect on *Enterococcus faecalis* bacterium.[24] Another study that compared BD and MTA; both calcium silicate-based materials described them as bioactive materials and suitable for clinical applications like direct pulp capping.[16] The similarities in the effects of BD and MTA has led to the use of the former as an alternative pulp capping material in vital pulp therapy.[19]

To the best of our knowledge, no histological studies have examined a combination of the three repair materials (MM-MTA, BA, and BD) studied to determine their degrees of bioactivity or cytotoxicity. Our study investigated the macrophages, which play a major role in chronic inflammation; the lymphocytes correlated with the macrophages; and the severity of chronic inflammation.

In this study, a comparison of the MM-MTA and BA groups with the control groups, showed a statistically significant increase in lymphocyte and macrophage infiltration by the 7th day, but the inflammation was not severe. However, in terms of lymphocyte infiltration on the 14th day BD group showed more decline in the inflammation but it was not statistically significant when compared with the control group. Calcium compounds in both the powder and the liquid of BD may have accelerated the healing.[25] This is important because $CaCO_3$ is used for both its biocompatibility and its calcium content. The inflammation had decreased negligibly in all experimental groups by the 45th day.

The evaluation of both the materials and the control groups revealed a slight or mild inflammatory reaction in the connective tissue. Batur *et al*.[26] state that BA is more biocompatible than MTA in a study in which BA and MTA in polyethylene tubes were implanted in rat subcutaneous

connective tissues immediately after their preparation. However, according to this study, neither of these two materials is superior. In their study, Gomes-Filho *et al*.[27] evaluated the response of tissue to Endo-CPM-Sealer (CPM Sealer; EGEO S.R.L., Buenos Aires, Argentina), Sealapex (SybronEndo, Glendora, CA), and MTA materials (Angelus, Londrina, Brazil), which were prepared, placed in polyethylene tubes, and implanted in rats' subcutaneous tissues. They reported that MTA showed lymphocyte and macrophage cell infiltration of moderate severity in fibrous capsules after 7 days. However, the severity of inflammation decreased after 15, 30, 60, and 90 days and also almost no inflammatory cells was observed, and only a thin capsule formed around the polyethylene tubes over time. In our study, we observed that MM-MTA created a mild inflammatory response after 7 days.

Conclusions

This study found that MM-MTA and BA repair materials are similar to each other in biocompatibility, but inflammation declines more quickly with the use of BD. After 45 days, histological evaluations showed that none of the materials is statistically superior to the other in terms of their bioactive properties. Further research about these materials is recommended for a deeper insight into their properties.

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References

1. Jiang Y, Zheng Q, Zhou X, Gao Y, Huang D. A comparative study on root canal repair materials: A cytocompatibility assessment in L929 and MG63 cells. Sci World J 2014;2014:463826.
2. Hauman CH, Love RM. Biocompatibility of dental materials used in contemporary endodontic therapy: A review. Part 1. Intracanal drugs and substances. Int Endod J 2003;36:75-85.
3. Williams DF. On the mechanisms of biocompatibility. Biomaterials 2008;29:2941-53.
4. Hauman CH, Love RM. Biocompatibility of dental materials used in contemporary endodontic therapy: a review. Part 2. Root-canal-filling materials. Int Endod J 2003;36:147-60.
5. Torabinejad M, Parirokh M. Mineral trioxide aggregate: a comprehensive literature review – part II: Leakage and biocompatibility investigations. J Endod 2010;36:190-202.
6. Grecca FS, Kopper PM, Santos RB, Fossati AC, Carrard VC, Acasigua GA, *et al.* Biocompatibility of RealSeal, its primer and AH Plus implanted in subcutaneous connective tissue of rats. J Appl Oral Sci 2011;19:52-6.
7. Holland R, Souza Vd, Nery MJ, Faraco Júnior IM, Bernabé PF, Otoboni Filho JA, *et al.* Reaction of rat connective tissue to implanted dentin tubes filled with a white mineral trioxide aggregate. Braz Dent J 2002;13:23-6.
8. Onay EO, Ungor M, Ozdemir BH. *In vivo* evaluation of the biocompatibility of a new resin-based obturation system. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:e60-6.
9. Lee SJ, Monsef M, Torabinejad M. Sealing ability of a mineral trioxide aggregate for repair of lateral root perforations. J Endod 1993;19:541-4.
10. Shokouhinejad N, Nekoofar MH, Razmi H, Sajadi S, Davies TE, Saghiri MA, *et al.* Bioactivity of EndoSequence root repair material and bioaggregate. Int Endod J 2012;45:1127-34.
11. Yuan Z, Peng B, Jiang H, Bian Z, Yan P. Effect of bioaggregate on mineral-associated gene expression in osteoblast cells. J Endod 2010;36:1145-8.
12. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. J Endod 1999;25:197-205.
13. Shahi S, Rahimi S, Lotfi M, Yavari H, Gaderian A. A comparative study of the biocompatibility of three root-end filling materials in rat connective tissue. J Endod 2006;32:776-80.
14. Celik D, Er K, Serper A, Tasdemir T, Ceyhanli KT. Push-out bond strength of three calcium silicate cements to root canal dentine after two different irrigation regimes. Clin Oral Investig 2014;18:1141-6.
15. Zhou HM, Shen Y, Wang ZJ, Li L, Zheng YF, Häkkinen L, *et al. In vitro* cytotoxicity evaluation of a novel root repair material. J Endod 2013;39:478-83.
16. Zanini M, Sautier JM, Berdal A, Simon S. Biodentine induces immortalized murine pulp cell differentiation into odontoblast-like cells and stimulates biomineralization. J Endod 2012;38:1220-6.
17. Simsek N, Akinci L, Gecor O, Alan H, Ahmetoglu F, Taslidere E. Biocompatibilty of a new epoxy resin-based root canal sealer in subcutaneous tissue of rat. Eur J Dent 2015;9:31-5.
18. Kumar V, Cotran RS, Robbins SL. Robbins Basic Pathology. 7th ed. Philadelphia, PA: Saunders; 2003.
19. Nowicka A, Lipski M, Parafiniuk M, Sporniak-Tutak K, Lichota D, Kosierkiewicz A, *et al.* Response of human dental pulp capped with biodentine and mineral trioxide aggregate. J Endod 2013;39:743-7.
20. Holland R, de Souza V, Nery MJ, Otoboni Filho JA, Bernabé PF, Dezan Júnior E. Reaction of rat connective tissue to implanted dentin tubes filled with mineral trioxide aggregate or calcium hydroxide. J Endod 1999;25:161-6.
21. Economides N, Pantelidou O, Kokkas A, Tziafas D. Short-term periradicular tissue response to mineral trioxide aggregate (MTA) as root-end filling material. Int Endod J 2003;36:44-8.
22. Yan P, Yuan Z, Jiang H, Peng B, Bian Z. Effect of bioaggregate on differentiation of human periodontal ligament fibroblasts. Int Endod J 2010;43:1116-21.
23. De-Deus G, Canabarro A, Alves G, Linhares A, Senne MI, Granjeiro JM. Optimal cytocompatibility of a bioceramic nanoparticulate cement in primary human mesenchymal cells. J Endod 2009;35:1387-90.
24. Zhang H, Pappen FG, Haapasalo M. Dentin enhances the antibacterial effect of mineral trioxide aggregate and bioaggregate. J Endod 2009;35:221-4.
25. Berbert FL, Leonardo MR, Silva LA, Tanomaru Filho M, Bramante CM. Influence of root canal dressings and sealers on repair of apical periodontitis after endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002;93:184-9.
26. Batur YB, Acar G, Yalcin Y, Dindar S, Sancakli H, Erdemir U. The cytotoxic evaluation of mineral trioxide aggregate and bioaggregate in the subcutaneous connective tissue of rats. Med Oral Patol Oral Cir Bucal 2013;18:e745-51.
27. Gomes-Filho JE, Watanabe S, Bernabé PF, de Moraes Costa MT. A mineral trioxide aggregate sealer stimulated mineralization. J Endod 2009;35:256-60.

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