Received: 24 December 2021 | Revised: 7 March 2022 | Accepted: 8 March 2022

DOI: 10.1111/iep.12439

ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF Experimental Pathology WILEY

Inflammatory response and immunohistochemical characterization of experimental calcium silicate-based perforation repair material

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Funding information
This research did not receive any funding from any agency.

Abstract

This study compares the immunohistochemical reaction of a new experimental tricalcium silicate perforation repair material to mineral trioxide aggregate (MTA) and Biodentine. A total of 162 mature premolar teeth from 12 dogs were divided into three experimental groups ($n = 54$ teeth each) according to the evaluation period: 1, 2 and 3 months. Each group was further divided into two equal subgroups ($n = 27$ teeth each) according to the time of repair: immediate repair and delayed repair. Each subgroup was subdivided according to the material used into three experimental subdivisions ($n = 8$ teeth each): MTA, Biodentine (Septodont) and experimental material, and two control subdivisions: positive control ($n = 2$ teeth) and negative control (one tooth). Under general anaesthesia, access cavity was done. Cleaning and shaping were performed using ProTaper universal rotary instruments. The canals were obturated using cold lateral compaction technique with Gutta percha and Adseal sealer. Furcation perforations were created then randomly sealed using the three materials either immediately or after one month (delayed repair). Inflammatory cell count and immunohistochemical analysis of osteopontin-positive area fraction were digitally analysed using the IMAGEJ software. Delayed furcal perforation repair showed significantly higher inflammatory cell count than immediate repair. No significant difference in inflammatory cell count and immunohistochemical analysis was detected between the three tested materials. The immunohistochemical analysis revealed the highest immunopositive area fraction in the 3-month evaluation period. The experimental tricalcium silicate cement performed similarly to Biodentine and MTA regarding the osteopontin expression during perforation repair, suggesting it is a suitable alternative with favourable handling characters.

KEYWORDS

Biodentine, furcal repair, inflammation, mineral trioxide aggregate, osteopontin

1 | INTRODUCTION

Root perforation is an adverse incident that may occur during root canal therapy leading to iatrogenic communication between the pulp space and the periodontal tissues.[1] Such mistakes can induce a negative effect on the prognosis of the diseased tooth.[2]

Prognosis of such cases depends on several factors like the aetiology, location and size of the perforation, as well as the time elapsed from the development of the defect.[3] A delay in repairing a perforation increases bacterial contamination.[4]

Calcium silicate cements have increasingly become the material of choice for the treatment of all types of dentinal defects, creating communication pathways between the root canal system and the periodontal tissues.[5–7] Their primary role in bone tissue repair is due to their confirmed biocompatibility and ability to enhance calcium–phosphate precipitation at the periodontal tissue interface.[5–7]

Several materials have been used to repair perforations including MTA, bioaggregate, Biodentine, calcium-enriched matrix cement (CEM) and others.[6,8–10]

Mineral trioxide aggregate was introduced in 1993 by Torabinejad[11] and became the material of choice for repair of root perforations because of its excellent biocompatibility, good sealing ability, insolubility on tissue fluids and capability of promoting periradicular tissue regeneration.[12]

Biodentine cement is a part of a new approach seeking to simplify the clinical techniques. A modified powder composition, the addition of setting accelerators and softeners and a new pre-dosed capsule formulation for use in a mixing device mainly improve the physical characteristics of this cement, making it much more user-friendly.[13–15]

As far as we are aware there have been conflicting results when the sealing ability of current repair materials are compared. There is always a continuous need to develop newer materials for furcal perforation repair with better clinical outcome, lower cost and easier handling. Thus, this study compares the immunohistochemical reaction of an experimental perforation repair material (tricalcium silicate cement) to MTA and Biodentine.

2 | MATERIALS AND METHODS

2.1 | Preparation of tricalcium silicate paste

Tricalcium silicate powder was prepared by the sol-gel method, as described by Zhao and Chang.[16] Mixing of 0.5 mol Si (O $_2C_2H_5)_4$ (tetra-ethyl orthosilicate, TEOS), nitric acid and 200 ml water was performed under continuous stirring; then, 1.5 mol Ca (NO_3) d4H2O was added to the prepared solution. The mixture was stored at 60°C until gelation occurred. The gel was allowed to dry at 120°C to form a powder.

For the preparation of the paste, an equal mass of Ca_3SiO_5 powder was mixed with deionized water with a liquid/powder ratio of 0.8 ml/g. The mixture was placed in a stainless steel mould of 10 mm diameter and stored in a 37°C, 100% humidity water bath for various times.

2.2 | Animal model

The research proposal was approved by the Ethics Committee at the Faculty of Dentistry, Ain Shams University, Egypt (FDASU-REC-15-02-2015). Also, this experiment is reported using the Animal Research: Reporting of in Vivo Experiments (ARRIVE) guidelines.[17]

Twelve healthy male mongrel adult dogs were enrolled in this study. The dogs weighed 15–20 kg and were aged 2–3 years. Before the experiment, the dogs were examined clinically and radiographically to assure the health of the periodontium. They were kept in the animal house at Faculty of Veterinary Medicine, Cairo University under proper conditions of nutrition, ventilation, clean environment and 12 h light/dark cycle. They were kept on separate kennels (1.5 × 2.5 × 3 m) and acclimatized to housing and diet for 2 weeks before the experiment. The dogs were given two meals per day (soft food and milk) and freshwater ad libitum.

2.3 | Classification of samples

A total number of 162 premolars and first molars in 12 dogs were used in this study. The dogs were divided into three main experimental groups (54 teeth/4 dogs each) according to the evaluation period: 1, 2 and 3 months. Each group was subdivided into two subgroups (27 teeth/2 dogs each), according to the time of repair: subgroup A, immediate repair, and subgroup B, delayed repair (after one month of infection). Each subgroup was further subdivided into five subdivisions according to the material used for perforation repair: subdivision 1: MTA (8 teeth), subdivision 2: Biodentine (8 teeth), subdivision 3: experimental material (8 teeth), subdivision 4: positive control, no repair of perforation (2 teeth), and subdivision 5: negative control, intact teeth with no perforation (one tooth).

2.4 | Experimental procedures

The anaesthetic regimen for each dog included injection of atropine sulphate (Atropine Sulphate®: ADWIA

Co.) at a dose of 0.05 mg/kg body weight given subcutaneously and xylazine HCl (Xylaject®: ADWIA Co.) at a dose of 1.1 mg/kg body weight given intramuscularly. The anaesthesia was induced by ketamine HCl (Keiran®: EIMC pharmaceuticals Co.) at a dose of 5 mg/kg bodyweight via a 20 gauge IV cannula using the cephalic vein. The anaesthesia was maintained during the operation by 25 mg/kg incremental doses of 2.5% solution of thiopental sodium (Thiopental sodium®: EIPICO) given intravenously.

Under complete aseptic techniques, teeth were accessed using a #4 round bur with conventional speed handpiece mounted on electric micromotor. Moisture was controlled by using cotton rolls isolation and atropine sulphate to reduce salivary flow. The working length was confirmed by the apex locator. Instrumentation was performed by ProTaper Next rotary instruments (Dentsply Maillefer) until reaching X3, and then, mechanical preparation was completed with K-files, #35 and #40 (Dentsply Maillefer). The canals were continuously irrigated with 20 ml NaOCl 2.6% solution. The canals were dried by paper points and obturated using cold lateral compaction technique with Gutta Percha and ADSEAL cement (Meta Biomed Co.).

A sterile size #4 round bur in a low-speed handpiece was used to create a 1.4-mm-diameter perforation in the centre of the floor of the pulp chamber into the periodontal tissue until haemorrhage was observed. The perforation depth was limited to 2 mm into the alveolar bone guided by the use of rubber stopper as a marker on the shank of the bur. The perforation was irrigated with normal saline solution.

In subgroup A, the perforation sites were sealed immediately for all experimental subdivisions, using MTA angelus (Angelus, Londrina, Paraná, Brazil) for subdivision 1, Biodentine (Septodont, Saint Maur des Faussés, France) for subdivision 2 and the experimental material for subdivision 3. After perforation repair, access openings were sealed with chemical-cured glass ionomer cement (Ivoclar Vivadent, Schaan, Liechtenstein).

In subgroup B, the perforations were left open for one month to allow salivary contamination. After this period, the animals were re-anaesthetized. Radiographs were taken before repair to confirm the presence of furcal lesion. The perforation sites were curetted to remove the inflamed tissues and debris and cleaned using normal saline irrigation. Then, the perforation sites were sealed as previously described for the three subdivisions.

In subdivision 4, the perforation sites were left open without sealing.

In subdivision 5, the teeth were left intact with no perforations to show normal histology.

2.5 | Histological evaluation

According to the evaluation periods, the dogs were sacrificed using 20 ml of 5% thiopental sodium rapidly injected through the cephalic vein. Block sections, including the experimental teeth, were obtained, including each tooth with its surrounding bone. The blocks were prepared for histological examination. The chronic inflammatory cell number was counted using IMAGE analysis software (IMAGEJ, 1.41a; NIH). The histopathological assessor was blinded to the treatment groups. Data were collected and statistically analysed.

2.6 | Immunohistochemical analysis

Ab8448-staining osteopontin was used by immunohistochemistry. Tissue samples were fixed with paraformaldehyde followed by permeabilization with 0.1% Triton and blocking with 20% serum for an hour at RT. The samples were incubated with primary antibody (1/100–1/500) in 20% FBS/PBS for 16 h at 4°C. An Alexa fluor 448-conjugated polyclonal to rabbit IgG was used as a secondary antibody at 1/100–1/500 dilutions. For each positive section, three microscopic fields showing the highest immunopositivity were selected, and photomicrographs were taken at an original magnification of 40×. The immunohistochemical assessment was carried out using the IMAGE analysis software (IMAGEJ, 1.41a; NIH). Images were autocorrected for brightness and contrast then converted into an 8-bit grayscale. After colour sampling, colour thresholding was accomplished and then adjusted. In photomicrographs, where positive immunoreaction was present, the area fraction of immunopositivity was measured automatically. The area fraction represented the percentage of immunopositive area to the total area of the microscopic field. The mean area fraction for each case was calculated. The collected data were tabulated and statistically analysed.

2.7 | Statistical analysis

Two-way ANOVA test was used to compare between more than two groups in related samples followed by Tukey post hoc test for pairwise comparison. The significance level was set at $p \leq .05$. Statistical analysis was performed with SPSS® (IBM) Statistics Version 20 for Windows.

3 | RESULTS

Histological evaluation of inflammatory cell counts showed a significant difference between the three evaluation periods, whether immediate or delayed repair, with the lowest inflammatory cell counts recorded in the 3-month period ($p < .05$).

In general, delayed repair showed a significantly higher inflammatory cell count than immediate repair ($p < .05$). After immediate repair (subgroup A), the experimental

TABLE 1 Mean values of inflammatory cell count of different materials at different follow-up periods

	Subgroup A (Immediate repair)			Subgroup B (Delayed repair)			Control subdivisions		
Groups	**MTA**	**BD**	**Exp Mat**	**MTA**	**BD**	**Exp Mat**	**Positive control**	**Negative control**	***p*-Value**
Group 1 (1 month)	$84.08^{aA} \pm 6.53$	$83.91^{aA} \pm 5.11$	$80.69^{aA} \pm 6.70$	$165.71^{aB} \pm 11.53$	$161.92^{aB} \pm 7.44$	$160.75^{aB} \pm 6.08$	$176.34^{cB} \pm 2.99$	$3.75^{aC} \pm 0.50$	$p < .0001$
Group 2 (2 months)	$70.83^{bA} \pm 9.63$	$67.09^{bA} \pm 9.73$	$65.42^{abA} \pm 8.01$	$102.57^{bB} \pm 4.55$	$101.97^{bB} \pm 9.30$	$99.19^{bB} \pm 5.70$	$247.50^{bC} \pm 28.27$	$3.75^{aD} \pm 0.50$	$p < .0001$
Group 3 (3 months)	$56.66^{bA} \pm 4.53$	$55.41^{bA} \pm 6.51$	$54.24^{bA} \pm 4.02$	$85.79^{bB} \pm 11.66$	$80.50^{cB} \pm 6.83$	$74.98^{cB} \pm 4.37$	$279.75^{abC} \pm 6.40$	$3.75^{aD} \pm 0.50$	$p < .0001$
p-Value	.024*	.025*	.017*	.002*	$p < .001$*	$p < .001$*	.006*	1 Ns	

Note: Means with different superscript small letters indicate a significant difference in the same column. Different superscript capital letters indicate a significant difference in the same row: *Significant at $p < .05$.

Abbreviations: MTA, mineral trioxide aggregate; BD, Biodintine; Exp Mat, Experimental material.

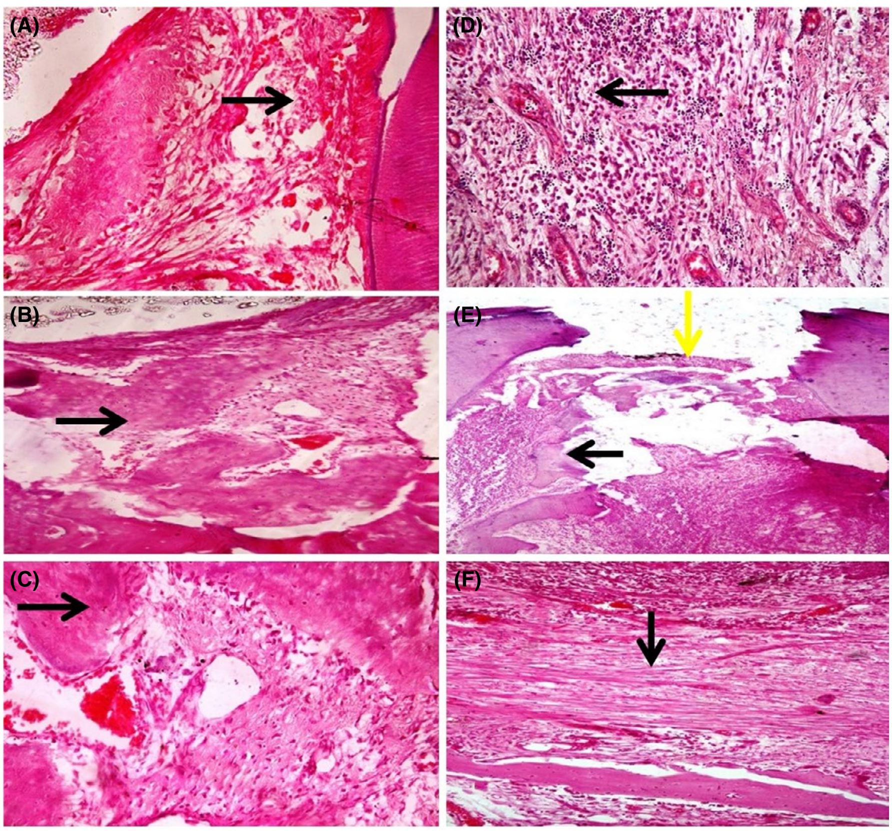

FIGURE 1 Photomicrographs of subdivision 3 (experimental material) after immediate (A–C) and delayed repair (D–F) of the furcation defect showing the following: (A) chronic inflammatory cells (black arrow) with calcified tissue after 1 month of immediate repair (H&E, ×40). (B) Hard tissue/osseous-like structure (black arrow) beneath the experimental material after 2 months of immediate repair (H&E, ×10). (C) Highly cellular hard tissue like structure (black arrow) beneath the experimental material after 3 months of immediate repair (H&E, ×20). (D) Chronic inflammatory cell infiltrate (black arrow) with hyalinized connective tissue beneath the experimental material after 1 month of delayed repair (H&E, ×40). (E) Hard tissue like structure bridging the defect (black arrows) after 2 months of delayed repair (H&E, ×4). (F) Linear calcification (black arrow) and highly cellular hard tissue like structure in connective tissue underlying the experimental material after 3 months of delayed repair (H&E, ×20)

TABLE 2 Mean values of immunopositive area fraction for different materials at different follow-up periods

Groups	Subgroup A (Immediate repair) MTA	BD	Exp Mat	Subgroup B (Delayed repair) MTA	BD	Exp Mat	Control subdivisions Positive control	Negative control	*p*-Value
Group 1 (1 month)	$9.81^{aA} \pm 1.34$	$10.42^{aA} \pm 0.56$	$11.03^{aA} \pm 0.82$	$4.43^{cB} \pm 1.21$	$4.86^{aB} \pm 0.88$	$4.94^{aB} \pm 0.36$	$0^{aC} \pm 0$	$0^{aC} \pm 0$	$p < .001$
Group 2 (2 months)	$11.22^{abA} \pm 0.89$	$11.96^{abA} \pm 0.36$	$12.68^{abA} \pm 0.80$	$7.42^{bB} \pm 0.65$	$8.35^{bB} \pm 1.21$	$9.51b^{bB} \pm 1.26$	$0^{aC} \pm 0$	$0^{aC} \pm 0$	$p < .001$
Group 3 (3 months)	$13.21^{bA} \pm 0.90$	$14.24^{bA} \pm 0.59$	$14.59^{bA} \pm 0.62$	$9.47^{bB} \pm 0.91$	$10.24^{bB} \pm 0.63$	$11.06^{bB} \pm 0.75$	$0^{a}C \pm 0$	$0^{aC} \pm 0$	$p < .001$
p-Value	.013*	$p < .001$*	.007*	.003*	.003*	.002*	$p < .001$*	$p < .001$*	

Note: Means with different superscript small letters indicate a significant difference in the same column. Different superscript capital letters indicate a significant difference in the same row: *Significant at $p < .05$.

Abbreviations: MTA, mineral trioxide aggregate; BD, Biodentine; Exp Mat, Experimental Material.

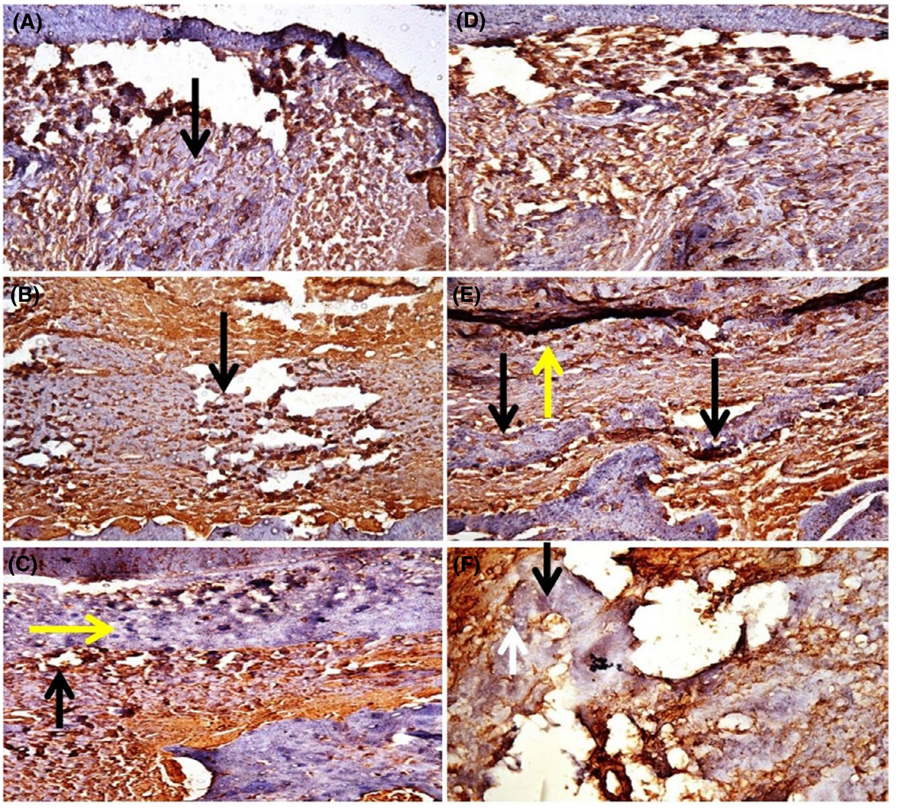

FIGURE 2 Photomicrographs of immunohistochemical analysis of mineral trioxide aggregate (MTA) subdivision after immediate repair of the furcation defect (A–C) showing expression of osteopontin +ve cells (arrows) with newly formed bone in Group 1 (A), Group 2 (B) and Group 3 (C) as well as after delayed repair (D–F) showing osteopontin +ve cell expression (arrows) with newly formed bone at the furcation defect in Group 1 (D), Group 2 (E) and Group 3 (F)

material recorded the lowest inflammatory cell count in comparison with MTA and Biodentine.

No significant difference was found among the materials after immediate or delayed repair ($p > .05$), as shown in Table 1 and Figure 1.

The immunohistochemical analysis revealed a significant difference ($p < .05$) between the three evaluation periods (groups) when any of the three materials was used to repair perforations, with the highest immunopositive area fraction in the 3-month evaluation period. Nevertheless, there was no significant difference among the three study materials when used to repair furcal perforations ($p > .05$), with the highest immunopositive area fraction in the experimental material (subdivision 3) as shown in Table 2 and Figures 2–4.

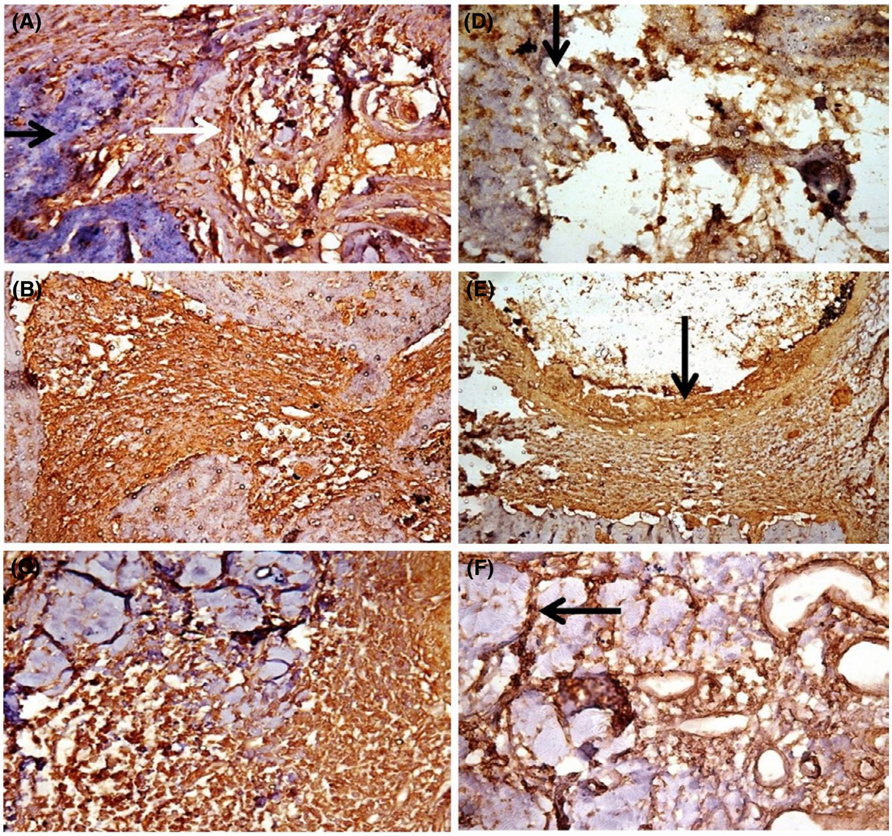

FIGURE 3 Photomicrographs of immunohistochemical analysis of Biodentine subdivision after immediate (A–C) and delayed repair (D–F) of the furcation defect showing the same findings in Figure 2

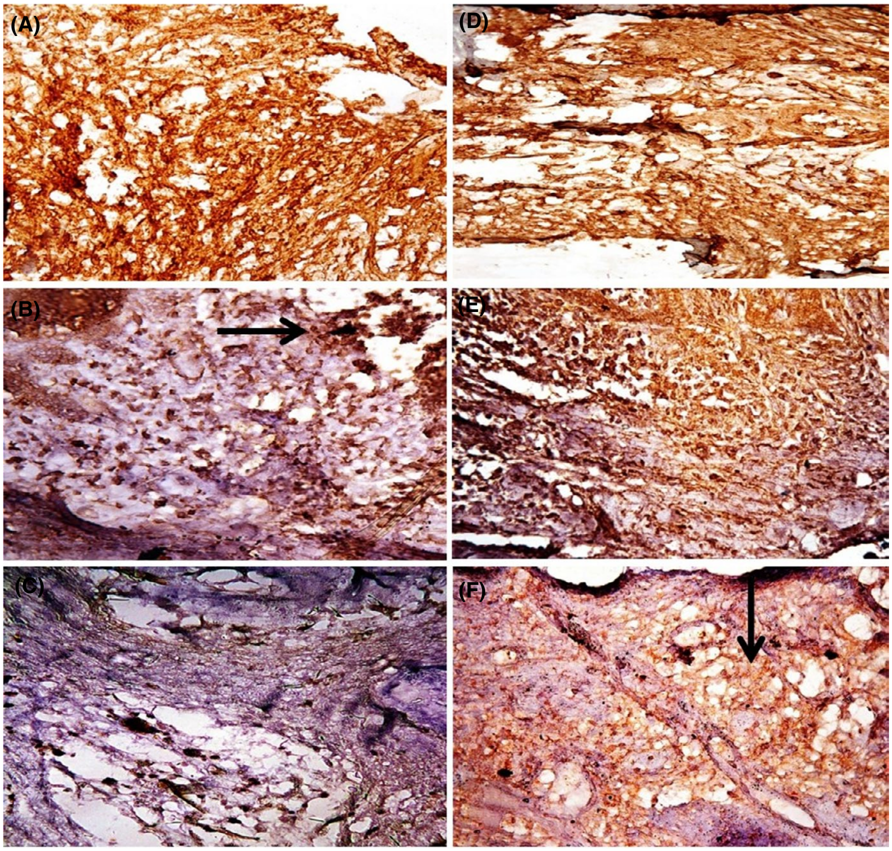

FIGURE 4 Photomicrographs of immunohistochemical analysis of experimental material subdivision after immediate (A–C) and delayed repair (D–F) of the furcation defect showing the same findings in Figure 2

4 | DISCUSSION

Furcal perforation is one of the most common endodontic problems that can affect the case prognosis.[18] The usage of biocompatible materials to repair perforations might be encouraged to reduce the incidence of inflammatory reactions in the surrounding tissues.[19]

Among the different materials used for perforation repair, MTA showed outstanding biocompatibility, good seal even in the presence of moisture/blood and a high pH, which stimulates the growth of cementum and regeneration of periodontal ligament.[20,21]

Biodentine with Active Biosilicate Technology was introduced as a fast-setting calcium silicate dentine substitute that can be used for perforation repair, resorption repair, apexification and root-end fillings.[22]

In recent years, more attention is focusing on developing novel bioactive ceramics with improved properties. Tricalcium silicate is analogous with calcium silicate and dicalcium silicate in component. In the current study, we used an experimental tricalcium silicate bone cement manufactured through sol-gel synthesis, aiming to be an alternative to MTA and Biodentine in repairing perforations.[16,23]

The perforation site was either repaired immediately or left opens for 1 month to allow for salivary contamination in the delayed subgroup, ensuring inter-radicular lesions to simulate clinical cases with delayed repair.[24]

Unlike in human practice, rubber dam could not be used for moisture control in this study due to some anatomical considerations of the canine teeth that hinder the fixation of the dam. The moisture was controlled by using cotton rolls isolation and injection of atropine sulphate to reduce salivary flow. Similar findings were reported by earlier workers.[8,9]

Immunohistochemical analysis has been used to reveal the expression of OPN during bone tissue repair. Osteopontin is an adhesive glycoprotein localized explicitly in the mineralized extracellular matrix (ECM) of bone and synthesized primarily by osteoblasts, endothelial cells and megakaryocytes.[25] Osteopontin is a major noncollagenous protein of bone, and it is responsible for the regulation of bone mineralization.[26]

The results of immunohistochemical analysis coincided with the histological results within the immediate repair. All tested materials demonstrated the highest immunopositive area fraction mean values in the immediate repair group. These results might be attributed to the acidic environment in the delayed repair subgroup, which might decrease the formation of calcium hydroxide and consequently affect the calcium ions release and hard tissue formation rate.[27]

There was no significant difference among the tested materials, with the highest immunopositive area fraction in the experimental material, and the least was for MTA. These findings may be attributed to the excellent sealing ability, alkalinity, surface bioactivity and biocompatibility of the experimental material, Biodentine[13,14] and MTA[11] and the ability of bone-forming cells to connect and spread on their surfaces and subsequent expression of OPN, which is highly specific for mineralized tissues including bone and cementum. Besides, the alkaline pH levels and calcium ions release in the fluid surrounding the three tested material might be conductive to hard tissue precipitation. Calcium ions released could enhance osteoblastic viability, proliferation and differentiation, besides the hydroxyl ions released from the three tested materials increase the alkalinity of the environment, which is unfavourable for bacterial growth.[10,11] Moreover, the tricalcium silicate bone cement manufactured by sol-gel synthesis has a porous structure with high surface energy for chemical reaction, which might have enhanced the hydration reaction and be less affected by environmental interferences such as acidic conditions, when used to repair perforations.[16,28] These results are consistent with many previous studies.[22,29–33]

5 | CONCLUSIONS

Under the conditions of this study, the experimental tricalcium silicate bone cement performed similarly to Biodentine and MTA regarding the osteopontin expression during perforation repair, suggesting it is a suitable alternative with favourable handling characters. Delayed furcal perforation repair presented a delayed healing than the immediately repaired furcal perforations regardless of the material used.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

Hend Okasha conceptualized, contributed to animal study, wrote original draft and analysed the data. Ashraf Abu-Seida contributed to animal study, analysed the data, wrote manuscript and supervised the work. Ahmed Hashem supervised the work, analysed the data and wrote manuscript. Salma El Ashry supervised the work, analysed the data and wrote manuscript. Mohamed Nagy contributed to animal study, analysed the data and wrote manuscript.

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REFERENCES

1. Wong R, Cho F. Microscopic management of procedural errors. *Dent Clin North Am*. 1997;41:455-479.
2. Estrela C, Decurcio DA, Rossi-Fedele G, Silva JA, Guedes OA, Borges AH. Root perforations: a review of diagnosis, prognosis and materials. *Braz Oral Res*. 2018;32:133-146.
3. Sinai IH. Endodontic perforations: their prognosis and treatment. *J Am Dent Assoc*. 1977;95:90-95.
4. Fuss Z, Trope M. Root perforations: classification and treatment choices based on prognostic factors. *Endod Dent Traumatol*. 1996;12:255-264.
5. Gomes-Cornélio AL, Rodrigues EM, Salles LP, et al. Bioactivity of MTA Plus, Biodentine and an experimental calcium silicate-based cement on human osteoblast-like cells. *Int Endod J*. 2017;50:9-47.
6. Torabinejad M, Parirokh M, Dummer PMH. Mineral trioxide aggregate and other bioactive endodontic cements: an updated overview – part II: other clinical applications and complications. *Int Endod J*. 2018;51:284-317.
7. Al-Sherbiny IM, Farid MH, Abu-Seida AM, Motawea IT, Bastawy HA. Chemico-physical and mechanical evaluation of three calcium silicate-based pulp capping materials. *Saudi Dent J*. 2021;33:207-214.
8. Abboud KM, Abu-Seida AM, Hassanien EE, Tawfik HM. Biocompatibility of NeoMTA Plus® versus MTA Angelus as delayed furcation perforation repair materials in a dog model. *BMC Oral Health*. 2021;21:192.
9. Alazrag MA, Abu-Seida AM, El-Batouty KM, El Ashry SH. Marginal adaptation, solubility and biocompatibility of TheraCal LC compared with MTA-angelus and biodentine as a furcation perforation repair material. *BMC Oral Health*. 2020;20:298.
10. Nabeel M, Tawfik HM, Abu-Seida AM, Elgendy AA. Sealing ability of Biodentine versus ProRoot mineral trioxide aggregate as root-end filling materials. *Saudi Dent J*. 2019;31:16-22.
11. Torabinejad M, Watson TF, Pitt Ford TR. Sealing ability of a mineral trioxide aggregate when used as a root end filling material. *J Endod*. 1993;19:591-595.
12. Main C, Mirzayan N, Shabahang S, Torabinejad M. Repair of root perforations using mineral trioxide aggregate: a long-term study. *J Endod*. 2004;30:80-83.
13. Rajasekharan S, Martens LC, Cauwels RGE, Verbeeck RMH. Biodentine™ material characteristics and clinical applications: a review of the literature. *Eur Arch Paediatr Dent*. 2014;15:147-158.
14. Camilleri J. Investigation of Biodentine as dentine replacement material. *J Dent*. 2013;41:600-610.
15. Koubi G, Colon P, Franquin JC, et al. Clinical evaluation of the performance and safety of a new dentine substitute, Biodentine, in the restoration of posterior teeth – a prospective study. *Clin Oral Investig*. 2013;17:243-249.
16. Zhao W, Chang J. Sol-gel synthesis and in vitro bioactivity of tricalcium silicate powders. *Mater Lett*. 2004;58:2350-2353.
17. Kilkenny C, Browne W, Cuthill IC, Emerson M, Altman DG. Animal research: reporting in vivo experiments: the ARRIVE guidelines. *Br J Pharmacol*. 2010;160:1577-1579.
18. ElDeeb ME, ElDeeb M, Tabibi A, Jensen JR. An evaluation of the use of amalgam, Cavit, and calcium hydroxide in the repair of furcation perforations. *J Endod*. 1982;8:459-466.
19. Balla R, LoMonaco CJ, Skribner J, Lin LM. Histological study of furcation perforations treated with Tricalcium phosphate, hydroxylapatite, amalgam, and Life. *J Endod*. 1991;17:234-238.
20. Roberts HW, Toth JM, Berzins DW, Charlton DG. Mineral trioxide aggregate material use in endodontic treatment: a review of the literature. *Dent Mater J*. 2008;24:149-164.
21. Nagy MM, Tawfik HE, Hashem AA, Abu-Seida AM. Regenerative potential of immature permanent teeth with necrotic pulps after different regenerative protocols. *J Endod*. 2014;40:192-198.
22. Jung S, Mielert J, Kleinheinz J, Dammaschke T. Human oral cells' response to different endodontic restorative materials: an in vitro study. *Head Face Med*. 2014;10:55.
23. Zhao W, Chang J. Preparation and characterization of novel tricalcium silicate bioceramics. *J Biomed Mater Res A*. 2005;73:86-89.
24. Jew RCK, Weine FS, Keene JJ, Smulson MH. A histologic evaluation of periodontal tissues adjacent to root perforations filled with Cavit. *Oral Surg Oral Med Oral Pathol*. 1982;54:124-135.
25. Lallier TE, Spencer A, Fowler MM. Transcript profiling of periodontal fibroblasts and osteoblasts. *J Periodontol*. 2005;76:1044-1055.
26. Fisher LW, Termine JD. Noncollagenous proteins influencing the local mechanisms of calcification. *Clin Orthop Relat Res*. 1985;200:362-385.
27. Pitt Ford TR, Torabinejad M, McKendry DJ, Hong CU, Kariyawasam SP. Use of mineral trioxide aggregate for repair of furcal perforations. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 1995;78:756-763.
28. Zhao W, Wang J, Zhai W, Wang Z, Chang J. The self-setting properties and in vitro bioactivity of tricalcium silicate. *Biomaterials*. 2005;26:6113-6121.
29. Koh ET, Torabinejad M, Pitt Ford TR, Brady K, McDonald F. Mineral trioxide aggregate stimulates a biological response in human osteoblasts. *J Biomed Mater Res*. 1997;37:432-439.
30. Lee BN, Lee KN, Koh JT, et al. Effects of 3 endodontic bioactive cements on osteogenic differentiation in mesenchymal stem cells. *J Endod*. 2014;40:1217-1222.
31. Xuereb M, Sorrentino F, Damidot D, Camilleri J. Development of novel tricalcium silicate-based endodontic cements with sintered radiopacifier phase. *Clin Oral Investig*. 2016;20:967-982.
32. Tawfik HE, Abu-Seida AM, Hashem AA, El-Khawlani MM. Treatment of experimental furcation perforations with mineral trioxide aggregate, platelet rich plasma or platelet rich fibrin in dogs' teeth. *Exp Toxicol Pathol*. 2016;68:321-327.
33. Hassanien EE, Abu-Seida AM, Hashem AA, Khanbash SS. Histologic evaluation of furcation perforation treated with mineral trioxide aggregate and bioaggregate. *Asian J Anim Sci*. 2015;9:148-156.

How to cite this article: Okasha H, Abu-Seida AM, Hashem AA, El Ashry SH, Nagy MM. Inflammatory response and immunohistochemical characterization of experimental calcium silicate-based perforation repair material. *Int J Exp Path*. 2022;00:1–8. doi:10.1111/iep.12439