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ORIGINAL ARTICLE

Immunohistochemical analysis of Biodentine versus MTA in repair of furcation perforation: an animal study

ABSTRACT

Aim: *This study compared Biodentine (BD) and mineral trioxide aggregate (MTA Angelus) in repair of furcation perforation (FP) in terms of inflammation and new hard tissue formation.*
Methodology: *Ninety-six teeth in six adult mongrel dogs were divided into two equal groups (48 teeth/3 dogs each) according to the time of repair; immediate and delayed repair of the induced FP. These groups were divided into three subgroups (16 teeth each) according to the evaluation period; 1, 2, and 3 months. Each subgroup was further subdivided into four subdivisions according to the FP repair material used; MTA Angelus (6 teeth), BD (6 teeth), negative control (2 teeth) and positive control (2 teeth). Instrumentation, obturation and FP were performed in the experimental and positive control teeth. The perforations were sealed according to the groups and subdivisions. Histopathology and immunohistochemical analysis using Osteonectin antibodies were performed for assessment of the inflammatory cell count and new hard tissue formation. All data were statistically analyzed.*
Results: *In all groups and subgroups, there were no significant differences between MTA and BD in the inflammatory cell count and new hard tissue formation (P>0.05).*
Conclusion: *Compared to MTA Angelus as a FP repair material, Biodentine induces similar degree of inflammation and new hard tissue formation.*

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Introduction

Furcation perforation (FP) may happen during opening an endodontic access as a result of a bad direction of the bur, or at post space preparation and localization of the calcified canals (1).
The prognosis of FP depends mainly upon its size, site, the duration between the defect creation and repair, and the degree of periodontal damage. Therefore, immediate therapy using suitable filling material is essential to avoid the complications, and to enhance favorable prognosis. (2).
The ideal filling material used for treatment of the FP should have an efficient sealing ability and enhance the new hard tissue formation. Numerous filling materials have been used for FP repair. These materials include both manufactured cements such as; MTA, Bioaggregate and Biodentine and natural biological materials such as; platelet rich plasma and platelet rich fibrin (3-6).
MTA is commonly applied FP repair material because it has good biocompatibility and sealing ability as well as low bacterial leakage and cytotoxicity (7). However, MTA has few shortcomings such as delayed setting (3h), less applicability, discoloration and expensiveness (3-6).
Recently, BD has been developed for endodontic repairs like perforations, apexifications, resorptive tissues and retrograde filling cement (8). BD has the same characteristics of MTA, but it has faster setting time and better applicability (5-8).
To improve the physical properties of BD, a modified chemical constitution, addition of setting accelerators and softeners, and a predosed capsule were carried out (8,9). There are scarce studies on BD as a treatment for the FP, mostly *in vitro* studies (6,10). The hypothesis of this study was that Biodentine can alternate MTA for treatment of the FP. Therefore, the present study compared BD and MTA in repair of furcation perforation in dogs in terms of inflammation and new hard tissue formation through histological as well as immunohistochemical evaluations.

Material and Methods

Animal model
This study was accepted by the Ethics Committee at Faculty of Dentistry, Ain Shams University, Egypt (Protocol number 15-12-13-Endo). All international and institutional guidelines for animal use and care were followed up in the current study. Ninety-six premolar and molar teeth in six adult mongrel dogs were selected. These dogs were 2-3 years old, 15-20 kg, and clinically normal. All selected teeth were healthy with complete root development. These dogs were randomly divided according to the time of repair into two groups (48 teeth/three animals each); immediate (group I) and delayed repair (group II). Based on the evaluation periods, the main groups were divided into three subgroups (16 teeth each); one month (subgroup 1), two months (subgroup 2), and three months (subgroup 3). Each subgroup was further subdivided into four subdivisions according to the material used; MTA Angelus (subdivision A, n=six teeth), BD (subdivision B, n=six teeth), negative control (subdivision C, n=two teeth) and positive control (subdivision D, n=two teeth).

Creation of furcation perforations
The dogs were pre-medicated with subcutaneous Atropine sulphate (Atropine sulphate 1%®, ADWIA, Egypt) at a dose of 0.05 mg/kg and intramuscular Xylazine HCl (Xylaject 2%®, ADWIA, Egypt) at a dose of 1mg/kg. The anesthesia was induced by intravenous Ketamine HCl (Keiran®, EIMC Pharmaceuticals Co., Egypt) at a dose of 5mg/kg. The anesthesia was then maintained by intravenous Thiopental sodium 2.5% solution (Thiopental sodium®, EPICO, Egypt) at a dose of 25 mg/kg (dose to effect). Access cavity and exposure of the pulp chamber were carried out in both experimental and positive control teeth by using a #4 round bur (Dentsply maillefer, Ballaigues, Switzerlad) with conventional speed hand piece mounted on an electric micromotor. Removal of the pulp tissue by a large spoon excavator and instrumentation of the root canals by hand files and crown down technique were performed. A K-file

#15 (Root ZXII – J Morita – Japan) was placed until the limit of cemento-dentinal junction with watch winding motion and working length was confirmed by apex locator (Dentsply Maillefer, Balaigues, Switzerlad). The mechanical preparation was done by step back technique until master apical file ranging 40-55 according to the size of the initial file. Irrigation of the root canal with normal saline solution was carried out after each file. Master cones were placed. Obturation of the root canal was performed by the cold lateral condensation technique with Gutta percha core and Endo-fill cement as a sealer (Dentsply Maillefer, Balaigues, Switzerlad).
A 1.4 mm-diameter furcation perforation was done by a #4 round bur at low speed hand piece in the center of the pulp chamber floor in both experimental and positive control subgroups until the hemorrhage was noted. The perforation depth was limited to 2 mm into the alveolar bone by a rubber stopper. Hemostasis was performed by irrigation with normal saline solution and drying by the paper points. For confirmation of the furcation perforation, radiographs were taken.
In the group II (delayed repair), the perforation sites were left open for a month to induce bacterial infection and inflammatory lesion in the furcation area (4). The perforation sites were immediately sealed in group I (immediate repair).

Perforation repair

- Subdivision IA

Angelus MTA (Angelus, Londrina, PR, Brazil) was mixed according to the manufacturer's instructions. One part of water was mixed gradually to 3 parts of the powder using MTA mixing plastic stick. The paste was carried out into the perforation sites by a small amalgam carrier and compacted with a suitable size plugger. The access cavity was closed by chemical cured Glass ionomer (Ivoclar vivadent pvt. Ltd., India). Radiographs were taken to confirm the perforation repair.

- Subdivision IB

Biodentine (Septodont, USA) was mixed according to the manufacturer's instructions. The capsule was tapped on a hard surface then, opened and placed on the white capsule holder. Five drops of the liquid were poured into the capsule then; the capsule was closed and placed on the amalgamator at a speed of 4000 rotations/min for 30 seconds to obtain a thick consistency. The material was transferred to the perforation sites by a small amalgam carrier and compacted with a suitable size plugger. The same steps for filling of the access cavity and radiography were carried out as in subdivision IA.

- Subdivisions IIA and IIB

At the end of the infection time, the dogs were re-anesthetized, and radiographs were taken for confirmation of the induced furcal lesion. The perforation site was cleaned by using a small spoon excavator for removal of necrotic and inflamed tissues. Cleaning of the perforation site was carried out with normal saline. After drying the sites with paper points, the perforations were treated with angelus MTA and BD as previously described subdivisions IA and IB.

- Positive control subdivision

The perforations were kept open with no filling.

- Negative control subdivision

Intact teeth were left without perforation to show the normal histology.

Histological evaluation

At the end of each observation time, each dog was sacrificed by anesthetic overdose (20 mL of 5% Thiopental sodium solution). Each experimental and control tooth was sectioned with its surrounding bone for preparation for histological evaluation. Fixation of the blocks was carried out in 10% buffered formalin solution for 2 weeks. After decalcification of the samples in17% EDTA solution for 120 days, the samples were prepared as usual for histopathology. The blocks were cut in buccolingual sections of 6um thickness. These specimens were stained by hematoxylin and eosin dye for assessment of the inflammatory cell count as follows: three representative fields in each slide were evaluated at x200 magnification power. These fields had well-preserved architecture, no artifacts and intense inflammatory cells.

Image analysis software was applied to count the total inflammatory cells.

Immunohistochemical evaluation
Immunohistochemical staining was performed using Osteonectin antibody. Immunohistochemical analysis was applied by using anti-Osteonectin antibody to identify the new hard tissue. The sections were deparaffinized in xylene. Antigen retrieval was carried out using citrate (pH 6.0) in a microwave oven followed by blocking of endogenous peroxidase using a solution of 50% Methyl alcohol and Hydrogen peroxide (1:1). The samples were incubated in bovine serum albumin (BSA) for 1h inside a moist chamber to block nonspecific antigens. Samples were incubated with the primary antibodies (1:400, overnight at the room temperature) followed by incubation with a secondary antibody (Universal LSAB TM Kit, DAKO, Carpinteria, CA, USA) for 30 min. A final incubation was performed using the tertiary complex Streptavidin peroxidase (Universal LSAB TM Kit, DAKO, Carpinteria, CA, USA) for an additional 30 min. The reaction was seen using diaminobenzidine (Universal LSAB TM Kit, DAKO, Carpinteria, CA, USA). Counterstaining was performed using Mayer's hematoxylin, and the specimens were mounted in Permount. The new bone formation was evaluated according to Alhadainy *et al.* (11). Briefly, Scores 0, 1, 2 and 3 represented no, slight, moderate and heavy bone formation, respectively.

Statistical analysis
Numerical data were presented as mean and standard deviation (SD) values. Inflammatory cell count data showed normal (parametric) distribution. One-way ANOVA was applied for comparison between the inflammatory cell counts in the groups, subgroups and subdivisions. Tukey's post-hoc test was applied to pair-wise compare between the groups when ANOVA test was significant.
For non-parametric data, Kruskal-Wallis test was applied for comparison between the groups, subgroups and subdivisions. Mann-Whitney U test was applied for pair-wise comparisons between the groups when Kruskal-Wallis test was significant. Prevalence of new hard tissue formation was presented as frequencies and percentages. The significance level was set at $P<0.05$. The statistical analysis was carried out with SPSS statistics version 20 for windows (IBM Corporation, NY, USA).

Results

Histological findings
The data are presented in table (1) and figure (1). A significant difference was seen in the inflammatory cell count between the group I (immediate repair) and group II (delayed repair) in all subgroups ($P<0.05$).
No significant difference was noticed in the inflammatory cell count between subdivision A (MTA Angelus) and subdivision B (BD) in both groups ($P>0.05$). There was a significant difference in the inflammatory cell count between subgroup 1 (one month), subgroup 2 (two months) and subgroup 3 (three months) in groups I and II ($P<0.05$).
In positive control, there was a significant difference in the inflammatory cell count between subgroup 1 and each of subgroup 2 and subgroup 3 ($P<0.05$). However, no significant difference was found between subgroup 2 and subgroup 3 ($P>0.05$).
In negative control, no significant difference was seen in the inflammatory cell count between subgroup 1, subgroup 2 and subgroup 3 ($P>0.05$).
A significant difference was noticed in the inflammatory cell count between subgroup 1, subgroup 2 and subgroup 3 in both MTA Angelus and BD subdivisions ($P<0.05$).

Immunohistochemical findings
The data are presented in Table (2) and figures (2-4). Both positive and negative control exhibited zero score in the new hard tissue formation. A significant difference was recorded in the new hard tissue formation between group I and group II in both MTA Angelus and BD subdivisions ($P<0.05$).
In group I and group II, no significant differences were reported in the new hard tissue formation between MTA Angelus and BD subdivisions ($P>0.05$).
A significant difference was seen in the

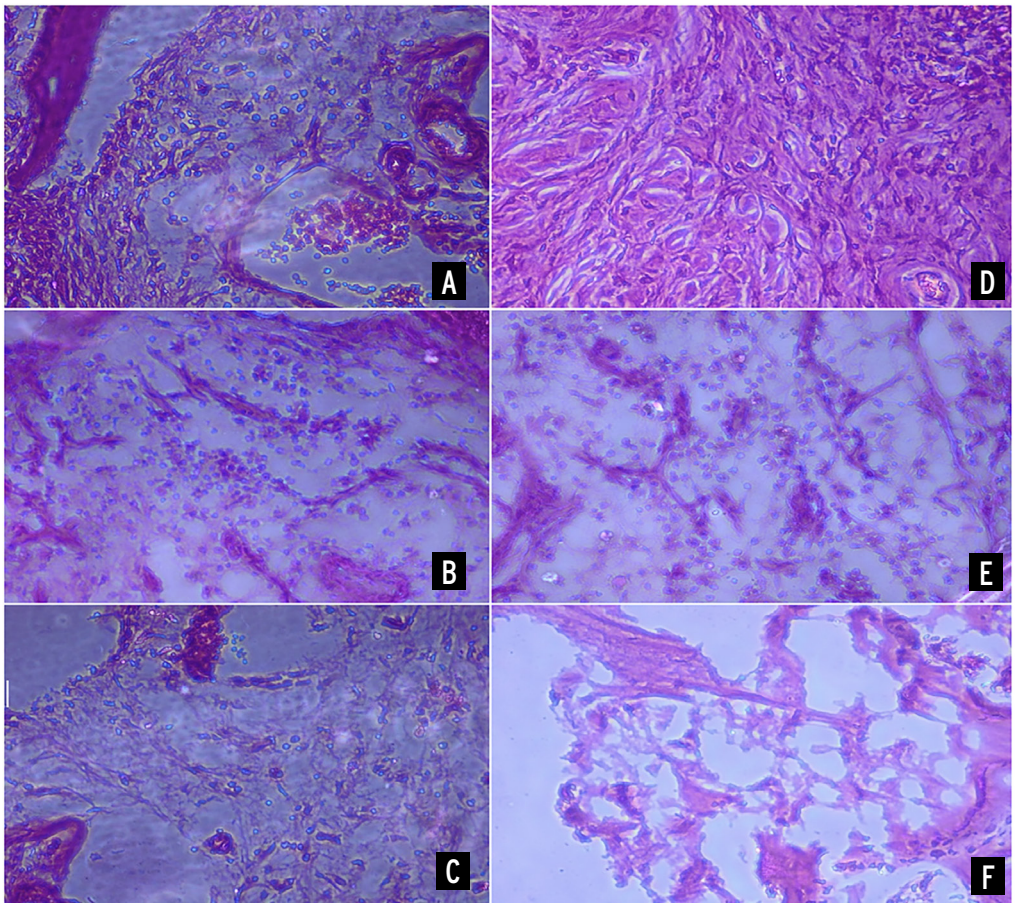

Figure 1
Representative photomicrographs of group II showing mild **(A)**, moderate **(B)** and severe **(C)** inflammatory reaction in the MTA subdivision after 1, 2 and 3 months, respectively. Representative photomicrographs of group II showing mild **(D)**, moderate **(E)** and severe **(F)** inflammatory reaction in the Biodentine subdivision after 1, 2 and 3 months, respectively (H&E, X 200).

Table 1

The mean and standard deviation (SD) values of the inflammatory cell count in different groups, subgroups and subdivisions

Subdivisions	Group I (Immediate sealing)			Group II (Delayed sealing)			P-value
	Subgroup 1 (One month)	Subgroup 2 (2 months)	Subgroup 3 (3 months)	Subgroup 1 (One month)	Subgroup 2 (2 months)	Subgroup 3 (3 months)	
MTA	714.17±7.88^{Ab}	511.67±14.3^{Bb}	296.67±6.41^{Cb}	755.67±10.23^{De}	546.00±10.64^{Ee}	311.67±9.33^{Fe}	≤0.001*
BD	702.50±16.33^{Ab}	504.50±7.89^{Bb}	292.83±8.95^{Cb}	735.17±10.80^{De}	536.17±9.64^{Ee}	317.67±5.20^{Fe}	≤0.001*
Positive	842.50±3.54^{Aa}	922.00±14.14^{Ba}	962.50±3.54^{Ba}	860.50±4.54^{Dd}	945.00±15.14^{Ed}	992.51±3.54^{Ed}	≤0.001*
Negative	61.02±1.42^{Ac}	69.00±1.41^{Ac}	64.03±1.44^{Ac}	65.02±1.62^{Df}	71.00±1.61^{Df}	74.03±1.64^{Df}	≤0.001*
P-value	≤0.001*	≤0.001*	≤0.001*	≤0.001*	≤0.001*	≤0.001*	

Means with different capital letters in the same row indicate significant difference.
Means with different small letters in the same column indicate significant difference.
**Significant at P<0.05.*
NS: non significant.

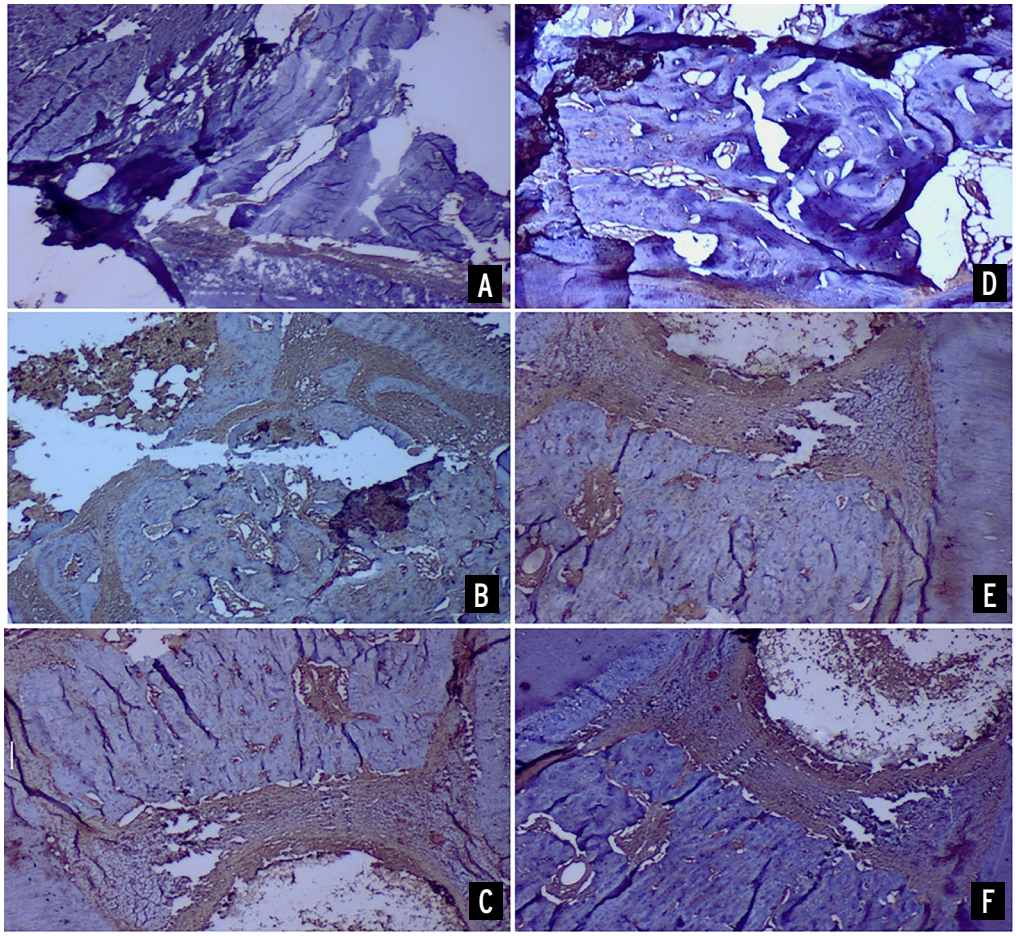

Figure 2
Representative photomicrographs of Osteonectin section of group I showing mild **(A)**, moderate **(B)** and dense **(C)** new bone formation at the FP site in the MTA subdivision after 1, 2 and 3 months, respectively. Representative photomicrographs of Osteonectin section of group I showing mild (**D)**, moderate **(E)** and dense **(F)** new bone formation at the FP site in the Biodentine subdivision after 1, 2 and 3 months, respectively.

Table 2

The mean and standard deviation (SD) values of the new hard tissue formation scores in different groups, subgroups and subdivisions

Subdivisions	Group I (Immediate sealing)			Group II (Delayed sealing)			P-value
	Subgroup 1 (One month)	Subgroup 2 (2 months)	Subgroup 3 (3 months)	Subgroup 1 (One month)	Subgroup 2 (2 months)	Subgroup 3 (3 months)	
MTA	1.67±0.52^{Aa}	2.50±0.55^{Ba}	3.00±0.00^{Ca}	1.00±0.00^{Dd}	1.67±052^{Ed}	2.17±0.41^{Ed}	≤0.001*
BD	1.67±0.52^{Aa}	2.83±0.41^{Ba}	3.00±0.00^{Ba}	1.00±0.00^{Dd}	2.00±0.63^{Ed}	2.33±0.52^{Ed}	≤0.001*
P-value	1 Ns	0.241 Ns	1 Ns	1 Ns	0.336 Ns	0.523 Ns	

Means with different capital letters in the same row indicate significant difference.
Means with different small letters in the same column indicate significant difference.
**Significant at P<0.05).*
Ns: non-significant at P>0.05.

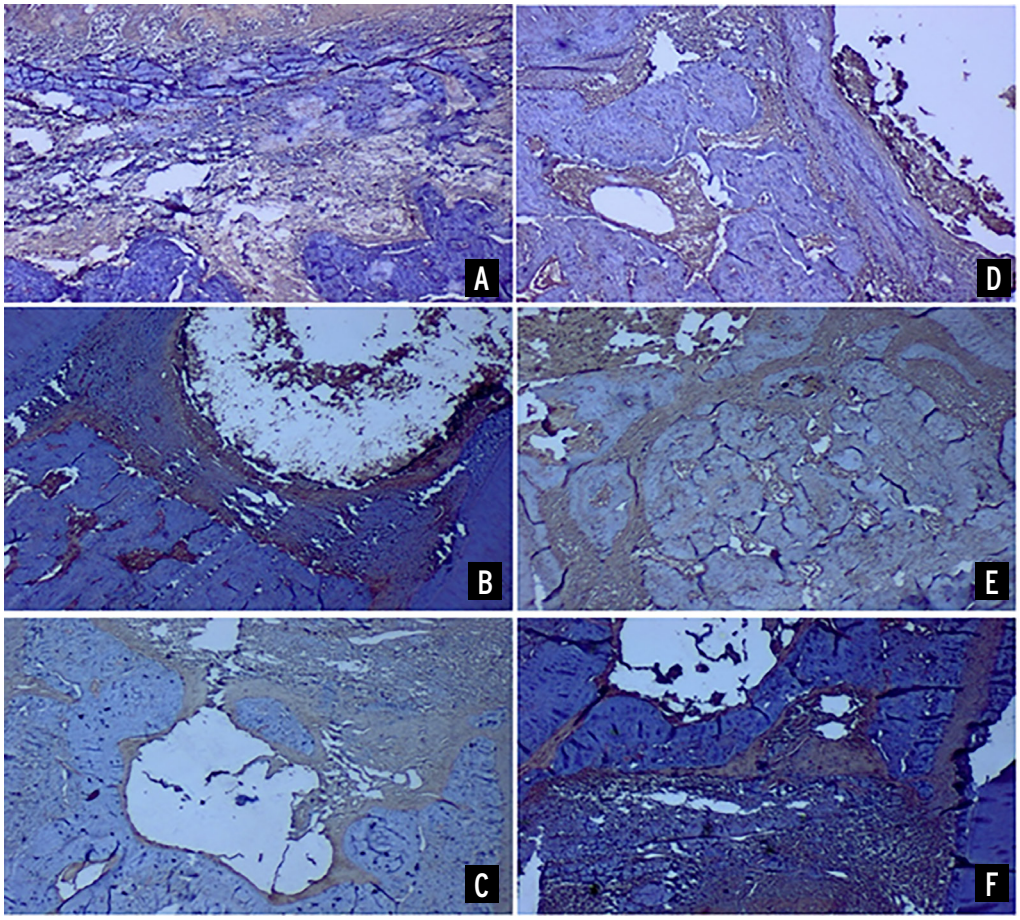

Figure 3
Representative photomicrographs of Osteonectin section of group II showing mild **(A)**, moderate **(B)** and dense **(C)** new bone formation at the FP site in the MTA subdivision after 1, 2 and 3 months, respectively. Representative photomicrographs of Osteonectin section of group II showing mild **(D)**, moderate **(E)** and dense **(F)** new bone formation at the FP site in the Biodentine subdivision after 1, 2 and 3 months, respectively.

new hard tissue formation between subgroup 1, subgroup 2 and subgroup 3 in MTA Angelus subdivision of group I ($P<0.05$). Significant differences were reported between subgroup 1 and subgroup 2 and subgroup 3 in BD subdivision of group I ($P<0.05$). However, no significant difference was noticed between subgroup 2 and subgroup 3 in BD subdivision of group I ($P>0.05$). In group II, significant differences were recorded in the new hard tissue formation between subgroup 1 and subgroup 2 as well as subgroup 3 in both MTA Angelus and BD subdivisions ($P<0.05$). There was no significant difference between subgroup 2 and subgroup 3 in both MTA Angelus and BD subdivisions ($P>0.05$).

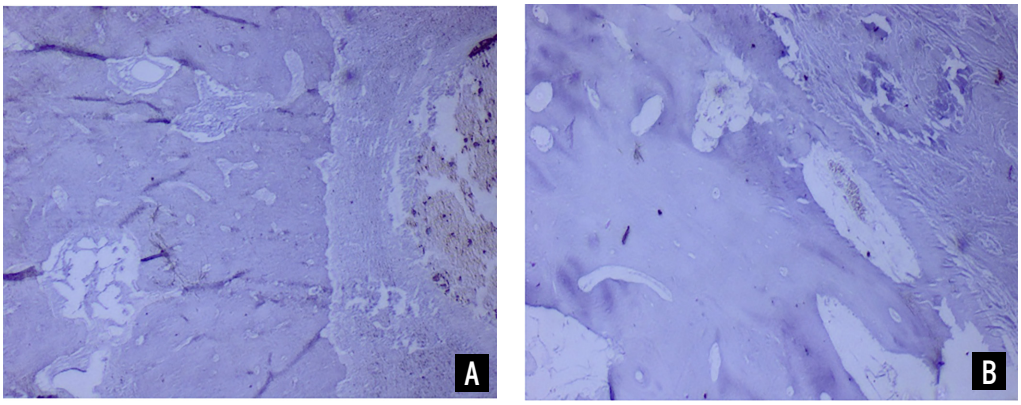

Figure 4
Representative photomicrographs of Osteonectin section showing no bone formation at the FP site in the control positive subdivision in group I **(A)** and group II **(B)**.

Discussion

During treatment of FP, using of an ideal repair material is an important factor for good prognosis. The FP repair material should have the abilities of good sealing and induction of new hard tissue (3, 4). This study compared BD against MTA as a FP repair material. According to the present results, the hypothesis of this study is accepted and BD can alternate MTA in the repair of FP.

In last few years, BD has been simplified the clinical techniques due to its superior mechanical properties and faster setting time compared with other calcium silicate-based materials (12).

MTA is considered as a gold standard material for the FP repair because it has favorable biocompatibility and sealing ability (7). Therefore, we compared BD with MTA in the present study.

The iatrogenic FP is considered as one of the most serious complications of endodontic therapy that may lead to unsuccessful treatment of the root canal. Also, FP may occur pathologically by dental caries or resorption (13). Therefore, the present study assessed both immediate and delayed sealing of the FP to simulate both types of clinical cases.

Biocompatibility and bioactivity of the FP repair materials are major properties that should be taken in consideration because the material is in close contact with the vital tissues during the procedure and can affect the viability of the periradicular cells (10,14). Therefore, this study evaluated the inflammatory cell count and new hard tissue formation induced by both MTA and BD.

The selection of dogs in the present study is based on the facts that the dog has a comparative apical repair mechanism with human in shorter duration and the good accessibility and visibility of their dental roots and roots furcation (15,16). However, the roots furcation is present at 1-2 mm from the cemento-enamel junction (CEJ) in dogs (17). Therefore, any technique produces acceptable outcome in dogs may produce a more acceptable result in humans because the distance from the CEJ to the furcation is more prominent (17).

Similarly to previous studies, the perforation size in this study was standardized at 1.4 mm (3, 4, 18, 19). The alveolar bone was penetrated for 2 mm to induce the inflammatory response. Also, leaving the FP open for one month for saliva contamination was another cause for induction of inter-radicular lesion in the group II (4, 20).

Two evaluation methods (Histological and immunohistochemical methods) were applied in the current study to overcome the shortcomings of each method. These methods showed various degrees of osteoblastic and osteoclastic reactions that indicate a bony reaction to the various treatments. Immunohistochemical analysis was performed using Osteonectin antibody. Osteonectin is a non-collagenous protein of bone that shares in the osteoid maturation and mineralization (21). Therefore, immunohistochemistry with polycolonal Osteonectin antibodies shows a high specific marking of actively matrix-producing osteoblasts (21).

Biodentine has almost similar physicochemical properties of MTA (10, 22, 23). Therefore, it was not surprising that there were no significant differences in the inflammatory cell count and new hard tissue formation between the MTA and BD when used as FP repair materials in the present study. Similar findings were recorded in a recent study (24). MTA was able to repair the FP due to its antimicrobial action and its high pH (12.5) that enhances the cementogenesis and osteogenesis (25).

It was not surprising that no significant difference was recorded between the group I (immediate sealing) and group II (delayed sealing). The time of repair plays an important role in the repair of FP due to the crucial role of infection (3,4).

After one month, both MTA and BD samples exhibited high inflammatory cell count but statistically less significant than that in the positive control. This was explained by the inadequate time for repair of the defect. Similar findings were recorded before in several studies (26, 27).

After two and three months, the positive control samples exhibited significant highest mean of inflammatory cell count com-

pared to MTA and BD samples. This could be attributed to the presence of microorganisms and continued inflammatory reaction in the positive control samples due to direct communication with the oral cavity. On the other hand, MTA and BD samples exhibited a significant lower mean of inflammatory cell count than that of the positive control due to the sealability, biocompatibility, and alkaline pH of the repair materials. This is in agreement with several previous results (24, 28-32). Quantitative field emission gun-scanning electron microscope (FEG-SEM) observations illustrated that there is no significant difference between Biodentine™ and ProRoot MTA in the mean gap at the dentin–furcation repair material interface (29). Moreover, calcium silicate materials have excellent effects on human periodontal ligament stem cells regarding cell adhesion and morphology (33).

The main limitations of this study were the small number of used teeth and relatively short times of evaluation. Therefore, future studies are recommended to evaluate the efficacy of BD as a furcation perforation repair material on a large sample size and for longer duration.

Conclusion

Compared to MTA as a FP repair material, Biodentine induces similar degree of inflammation and new hard tissue formation.

Clinical Relevance

Biodentine is an alternative to MTA when used as a furcal repair material.

Conflict of Interest

There are no conflicts of interest.

Acknowledgments

None.

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