

Immunohistochemical Analysis of Biodentine Versus MTA in Repair of Experimental Furcation Perforation

Mahmoud Badr¹, Ashraf M. Abu-Seida^{2*}, Ahmed A. Hashem³, Salma H. El Ashry³

¹Department of Endodontic, Faculty of Dentistry, Future University, Cairo, Egypt

^{2*}Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary
Medicine, Cairo University, Giza, Egypt

³Department of Endodontic, Faculty of Dentistry, Ain Shams University, Cairo, Egypt

Short running title

Biodentine Versus MTA as Furcation Perforation Repair

***Corresponding author**

Prof. Ashraf M. Abu-Seida

Department of Surgery, Anesthesiology & Radiology

Faculty of Veterinary Medicine, Cairo University

Giza Square. PO: 12211- Egypt

E-mails:

ashrafseida@cu.edu.eg

ashrafseida@yahoo.com

ORCID:

<http://orcid.org/0000-0001-5466-2016>

Immunohistochemical Analysis of Biodentine Versus MTA in Repair of Experimental Furcation Perforation

Abstract

This study compared the Biodentine (BD) and MTA in repair of furcation perforation (FP) in a dog model in terms of inflammatory cell count and new hard tissue formation through histological and immunohistochemical evaluations. Ninety six teeth in six adult mongrel dogs were divided according to the time of repair into two groups (48 teeth/3 dogs each); immediate and delayed repair of the induced FP. Each group was divided into three subgroups (16 teeth each) according to the evaluation periods; 1, 2, and 3 months. Each subgroup was further subdivided into four subdivisions according to the material used; MTA (6 teeth), Biodentine (6 teeth), negative control (2 teeth) and positive control (2 teeth). In experimental and positive control samples, the teeth were instrumented and obturated then, a FP was performed. 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. In all groups and subgroups, there were no significant differences between MTA and BD in the inflammatory cell count and new hard tissue formation ($P>0.05$). Biodentine can alternate the MTA as a FP repair material.

Keywords: Biodentine; furcation perforation; MTA; new bone formation; Osteonectin

1. Introduction

Furcation perforation (FP) may occur throughout the procedure of endodontic access opening as a result of badly directed bur, or during 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 be able to seal the defect completely and enhance the new hard tissue formation. Numerous filling materials have been used for FP repair such as; MTA, Bioaggregate, Biodentine, platelet rich plasma, platelet rich fibrin and others [3-6].

The 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 disadvantages like long setting time (3h), less applicability and expensiveness [3-6].

Recently, BD has been developed for endodontic repairs such as; perforations, apexifications, resorptive lesions and retrograde filling material [8]. The BD has the same characteristics of the MTA, but it has faster setting time and better applicability [5,8]. A modified powder composition, adding of setting accelerators and softeners, as well as a new predosed capsule formulation improve the physical properties of BD [8,9]. There are scarce studies on the BD as a FP repair material, mostly *in vitro* studies [6,10]. The hypothesis of this study was that Biodentine will alternate the MTA as a FP repair material. Therefore, this study compared BD and MTA in repair of furcation perforation in dogs in terms of inflammatory cell count and new hard tissue formation through histological and immunohistochemical evaluations.

2. Material and Methods

2.1. Animal model

The research proposal was approved by the ethics committee at Faculty of Dentistry, Ain Shams University, Egypt (Protocol number 15-12-13-Endo). A total of 96 teeth in six adult mongrel dogs were selected. These dogs were 2-3 years old, 15-20 kg, and clinically normal. All teeth were intact, free from caries and had complete root development. The animals were divided according to the time of repair into two main groups (48 teeth/3 dogs each); immediate and delayed repair. Each group was divided into three subgroups (16 teeth each) according to the evaluation periods; 1, 2, and 3 months. Each subgroup was further subdivided into four subdivisions according to the material used; MTA (6 teeth), BD (6 teeth), negative control (2 teeth) and positive control (2 teeth).

2.2. Perforation creation

The dogs were pre-medicated with subcutaneous Atropine sulphate at a dose of 0.05 mg/kg and intramuscular Xylazine HCl at a dose of 1mg/kg. The anesthesia was induced by intravenous Ketamine HCl at a dose of 5mg/kg. The anesthesia was then maintained by intravenous Thiopental sodium 2.5% solution at a dose of 25 mg/kg (dose to effect).

Access cavity and exposure of the pulp chamber were done in all experimental and positive control teeth by #4 round bur (Dentsply maillefer, Ballaigues, Switzerland) with conventional speed hand piece mounted on electric micromotor. The pulp tissue was removed using large spoon excavator and the root canals were instrumented using hand files and crown down technique. 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, Switzerland). The mechanical preparation was performed using step back technique until master apical file ranging 40-55 according to the size of the initial file. The

canals were irrigated with normal saline after each file. Master cones were placed. After drying with paper points, the canals were obturated using cold lateral condensation technique with Gutta percha core and Endo-fill cement as a sealer (Dentsply Maillefer, Balaignes, Switzerland).

A #4 round bur at low speed hand piece was used to create a 1.4 mm-diameter perforation in the center of the pulp chamber floor to the furcation area of the 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 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. The perforation sites were immediately sealed in group I (immediate repair).

2.3. Perforation repair

2.3.1. Subdivision IA

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

2.3.2. Subdivision IB

Biodentine (Septodont, USA) was mixed according to the manufacturer's instructions. The capsule was gently tapped on a hard surface then, opened and placed on the white

capsule holder, and 5 drops of the liquid were poured into the capsule. The capsule was closed and placed on the amalgamator at a speed of 4000 rotations/min for 30 seconds. A thicker consistency was preferred, and then the material was collected and carried out 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.

2.3.3. Subdivisions IIA and IIB

After the infection period, the animal was re-anesthetized, and radiographs were taken to confirm the formation of furcal lesion. The perforation sites were curetted by using a small spoon excavator to remove the debris and inflamed tissues, cleaned with normal saline and dried with paper points. Then the perforations were treated with angelus MTA and BD as previously described subdivisions IA and IB.

2.3.4. Positive control subdivision

The perforations were left open without filling

2.3.5. Negative control subdivision

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

2.4. Histological evaluation

After each observation period, the dogs were sacrificed by anesthetic overdose (20 mL of 5% Thiopental sodium solution). Bone segments including the experimental and control teeth were cut and prepared for histological evaluation. The blocks were fixed in 10% buffered formalin solution with a ratio 1:50 for 2 weeks, then the blocks were decalcified using 17% EDTA solution for 120 days. After decalcification, the samples were prepared as usual and the blocks were sectioned in buccolingual sections of 6µm thickness. These sections were stained using hematoxylin and eosin dye for histopathological evaluation of the inflammatory cell count as follows:

For each slide, 3 representative fields were analyzed at x200 magnification power. Fields were characterized by well-preserved architecture, no artifacts and intense inflammatory cells infiltration. Total inflammatory cell number was counted using image analysis software.

2.5. Immunohistochemical evaluation

Immunohistochemical staining was performed using Osteonectin antibody. Immunohistochemical analysis was applied by using anti-Osteonectin antibody to identify the new hard tissue formation. The sections were deparaffinized in xylene. Antigen retrieval was performed 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 visualized using diaminobenzidine (Universal LSAB TM Kit, DAKO, Carpinteria, CA, USA). Counterstaining was performed using Mayer's hematoxylin, and the specimens were mounted in Permout. The new bone formation was measured according to Alhadainy et al. [11]. Briefly, Scores 0, 1, 2 and 3 represented no, slight, moderate and heavy bone formation, respectively.

2.6. 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 used to compare between the inflammatory cell counts in the groups,

subgroups and subdivisions. Tukey's post-hoc test was used for pair-wise comparisons between the groups when ANOVA test was significant.

For non-parametric data, Kruskal-Wallis test was used to compare between the groups subgroups and subdivisions. Mann-Whitney U test was used 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 performed with SPSS statistics version 20 for windows (IBM Corporation, NY, USA).

3. Results

3.1. Histological findings

The data are shown in table (1) and figure (1). There was a significant difference in the inflammatory cell count between the group I and group II in all subgroups ($P < 0.05$).

There was no significant difference in the inflammatory cell count between subdivision A (MTA) and subdivision B (BD) in both groups ($P > 0.05$). There was a significant difference in the inflammatory cell count between subgroup 1, subgroup 2 and subgroup 3 in both groups ($P < 0.05$).

In positive control, a significant difference was found 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, there was no significant difference in the inflammatory cell count between subgroup 1, subgroup 2 and subgroup 3 ($P > 0.05$).

There was a significant difference in the inflammatory cell count between subgroup 1, subgroup 2 and subgroup 3 in both MTA and BD subdivisions ($P < 0.05$).

3.2. Immunohistochemical findings

The data were collected in table (2) and figures (2-4). Both positive and negative control exhibited zero score in the new hard tissue formation (Figure 4). There was a significant difference in the new hard tissue formation between group I and group II in both MTA and BD subdivisions ($P < 0.05$).

In the group I (Figure 2) and group II (Figure 3), there were no significant differences in the new hard tissue formation between the MTA and BD subdivisions ($P > 0.05$).

There was a significant difference in the new hard tissue formation between subgroup 1, subgroup 2 and subgroup 3 in MTA subdivision of group I ($P < 0.05$). There were significant differences between subgroup 1 and subgroup 2 and subgroup 3 in the BD subdivision of group I ($P < 0.05$). However, there was no significant difference between subgroup 2 and subgroup 3 in BD subdivision of group I ($P > 0.05$).

In group II, there were significant differences in the new hard tissue formation between subgroup 1 and subgroup 2 as well as subgroup 3 in both MTA and BD subdivisions ($P < 0.05$). No significant difference was found between subgroup 2 and subgroup 3 in both MTA and BD subdivisions ($P > 0.05$).

4. 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 the BD against the MTA as a FP repair material. According to the results of the present study, the hypothesis of this study is accepted and the BD can alternate the 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].

The MTA is considered as a gold standard material for the FP repair because it has favorable biocompatibility and sealing ability [7]. Therefore, we compared the 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 [14]. Therefore, this study evaluated the inflammatory cell count and new hard tissue formation induced by both MTA and BD.

In this study, selection of dogs as an animal model is based on the facts that dogs have comparative apical repair mechanism with human in shorter duration and the good accessibility and visibility of their dental roots and roots furcation [15]. However, the roots furcate as close as 1-2 mm from the cemento-enamel junction (CEJ) in dogs [16]. Therefore, any technique produces favorable results in dogs may have a more favorable response in humans, where the distance from the CEJ to the furcation is more prominent [16].

Similarly to previous studies, the perforation size in this study was standardized at 1.4 mm [3,4,17,18]. The bur was allowed to penetrate 2 mm into the alveolar bone in order to enhance the inflammatory response. Also, leaving the FP open for one month for saliva contamination was another factor that enhances the formation of the inter-

radicular lesion in the group II [19].

Two evaluation methods (Histological and immunohistochemical methods) were used in this study to overcome the limitations of each method. These methods demonstrated variable degrees of osteoblastic and osteoclastic activities that reflect a bony reaction to different treatment modalities. Immunohistochemical analysis was performed using Osteonectin antibody. Osteonectin is a non-collagenous protein of bone that is involved in the process of osteoid maturation and mineralization [20]. Therefore, immunohistochemistry with polyclonal Osteonectin antibodies shows a high specific marking of actively matrix-producing osteoblasts [20].

Biodentine has almost similar physicochemical properties of the MTA [21,22]. 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 this study. The ability of MTA to repair the FP may be due to its antimicrobial action and its high pH (12.5) that promotes growth of the cementum and formation of bone [23].

It was not surprising that there was significant difference between the group I (immediate sealing) and group II (delayed sealing). The timing of repair is an important factor in the treatment 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 could be attributed to the short time that was not enough for repair of the defect. Similar findings were recorded before in several studies [24,25].

After two and three months, the positive control samples showed significant highest mean of inflammatory cell count compared to the MTA and BD samples. This was 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 showed a significant lower mean of inflammatory cell count than that of the positive control due to the sealing ability, biocompatibility, and alkaline pH of the repair materials. This in agreement with the results of previous studies [23,26]

5. Conclusion

Biodentine can alternate the MTA as a furcation perforation repair material

Author Contributions

Conceptualization, S.H.E.; data curation M.B.; investigation and methodology, M.B., A.M.A. and A.A.H.; supervision, S.H.E. and A.A.H.; writing—original draft, A.M.A. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Silva, L.A.B.; Pieroni, K.A.; Nelson-Filho, P.; Silva, R.A.B.; Hernández-Gatón, P.; Lucisano, M.P.; Paula-Silva, F.W.; de Queiroz, A.M. Furcation perforation: periradicular tissue response to Biodentine as a repair material by histopathologic and indirect immunofluorescence analyses. *J. Endod.* **2017**, *43*, 1137-1142.
2. Noetzel, J.; Ozer, K.; Reissauer, B.H.; Anil, A.; Rössler, R.; Neumann, K.; Kielbassa, A.M. Tissue responses to an experimental calcium phosphate cement and mineral trioxide aggregate as materials for furcation perforation repair: a histological study in dogs. *Clin. Oral Investig.* **2006**, *10*, 77-83.

3. Hassanien, E.E.; Abu-Seida, A.M.; Hashem, AA.; Khanbash, S.S. Histologic evaluation of furcation perforation treated with mineral trioxide aggregate and bioaggregate. *Asian J. Anim. Sci.* **2015**, *9*, 148-156.
4. Tawfik, H.E.; Abu-Seida, A.M.; Hashem, A.A.; El-Khawlani, M.M. 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.
5. Nabeel, M.; Tawfik, H.M.; Abu-Seida, A.M.; Elgendy, A.A. Sealing ability of Biodentine versus ProRoot mineral trioxide aggregate as root-end filling materials. *Saudi. Dent. J.* **2019**, *31*, 16–22.
6. Cardoso, M.; Pires, M.; Correlo, V.; Reis, R.; Paulo, M.; Viegas, C. Biodentine for Furcation Perforation Repair: An Animal Study with Histological, Radiographic and Micro-Computed Tomographic Assessment. *Iranian Endod. J.* **2018**, *13*, 323-330
7. Patel, N.; Patel, K.; Baba, S.M.; Jaiswal, S.; Venkataraghavan, K.; Jani, M. Comparing gray and white mineral trioxide aggregate as a repair material for furcation perforation: an in vitro dye extraction study. *J. Clin. Diagn. Res.* **2014**, *8*, ZC70-73.
8. Rajasekharan, S.; Martens, L.C.; Cauwels, R.G.; Verbeeck, R.M.H. Biodentine™ material characteristics and clinical applications: a review of the literature. *Eur. Arch. Paediatr. Dent.* **2014**, *15*, 147-158.
9. Atmeh, A.; Chong, E.Z.; Richard, G.; Festy, F.; Watson, T.F. Dentin-cement interfacial interaction: calcium silicates and polyalkenoates. *J. Dent. Res.* **2012**, *91*, 454-459.
10. Alazrag, M.A.; Abu-Seida, A.M.; El-Batouty, K.M.; El Ashry, S.H. Adaptability, solubility and biocompatibility of TheraCal LC compared with MTA-Angelus and

Biodentine as a furcation perforation repair material. *BMC Oral Health*, **2020**, Accepted article.

11. Alhadainy, H.; Himel, V.; Lee, W.; Elbaghdady, Y. Use of a hydroxylapatite-based material and calcium sulfate as artificial floors to repair furcal perforations. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **1998**, *86*, 723-729

12. Al-Sherbiny, I.M.; Farid, M.H.; Abu-Seida, A.M.; Motawea, I.T.; Bastawy, H.A. Chemico-physical and mechanical evaluation of three calcium silicate-based pulp capping materials. *Saudi Dent. J.* **2020**, In Press.
<https://doi.org/10.1016/j.sdentj.2020.02.001>

13. Alhadainy, H.A.; Himel, V.T. Evaluation of the sealing ability of amalgam, Cavit and glass ionomer cement in the repair of furcation perforations. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **1993**, *75*, 362-366

14. Zhou, M.; Shen, Y.; Wang, J. In vitro cytotoxicity evaluation of novel root repair material. *J. Endod.* **2013**, *39*, 478-483

15. El-Ashry, S.; Abu-Seida, A.M.; Al-Boghdady, H.; El-Batouty, K.; Abdel-Fattah, M. The effect of different formulations of calcium hydroxide on healing of intentionally induced periapical lesions in dogs. *Pak. Vet. J.* **2013**, *33*, 48-52.

16. Pitt Ford, T.R.; Torabinejad, M.; McKendry, D.J.; Hong, C.; Kariyawasam, S.T. Use of mineral trioxide aggregate for repair of furcal perforations. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **1995**, *79*, 756-762

17. Holland, R.; Filho, J.A.; de Souza, V.; Nery, M.J.; Bernabe, P.F.; Junior, E.D. Mineral trioxide aggregate repair of lateral root perforations. *J. Endod.* **2001**, *27*, 281-284.

18. Hartwell, G.R.; England, M.C. Healing of furcation perforations in primate teeth after repair with decalcified freeze-dried bone: a longitudinal study. *J. Endod.* **1993**, *19*, 357-361.
19. Jew, R.; Weine, F.; Keen, J. A histologic evaluation of periodontal tissues adjacent to root perforations filled with Cavit. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **1982**, *54*, 124-135
20. Schulz, A.; Jundt, G. Immunohistological demonstration of osteonectin in normal bone tissue and in bone tumors. *Curr. Top. Pathol.* **1989**, *80*, 31-54
21. Jain, P.; Raj, J.D. Dentin substitutes: A Review. *Int. J. Pharm. Bio. Sci.* **2015**, *6*, 383-391.
22. Torabinejad, M.; Hong, C.; McDonald, F.; PittFord, T. Physical and chemical properties of a new root end filling material. *J. Endod.* **1995**, *21*, 349-353
23. Alhadainy, H.A. Root perforations. A review of literature. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **1994**, *78*, 368-374
24. Al-Daafas, A.; Al-Nazhan, S. Histological evaluation of contaminated furcal perforation in dogs' teeth repaired by mineral trioxide aggregate with or without internal matrix. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* **2007**, *103*, 92-99
25. Broon, N.; Bramante, C.; DeAssis, G.; Bortoluzzi, E.; Bernardineli, N.; De Moraes, I.; Garcia, R. Healing of root perforations treated with mineral trioxide aggregate and Portland cement. *J. Appl. Oral Sci.* **2006**, *5*, 305-311
26. Schwartz, R.S.; Mauger, M.; Clement, D.J.; Walker, W.A. Mineral trioxide aggregate: a new material for endodontics. *J. Amer. Dent. Assoc.* **1999**, *130*, 967-975.

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)		
	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 ^b	511.67±14.3 ^b	296.67±6.41 ^b	755.67±10.23 ^b	546.00±10.64 ^b	311.67±9.33 ^b
BD	702.50±16.33 ^b	504.50±7.89 ^b	292.83±8.95 ^b	735.17±10.80 ^b	536.17±9.64 ^b	317.67±5.20 ^b
Positive	842.50 ± 3.54 ^a	922.00±14.14 ^a	962.50±3.54 ^a	842.50 ± 3.54 ^a	922.00±14.14 ^a	962.50±3.54 ^a
Negative	61.02 ± 1.42 ^c	69.00 ± 1.41 ^c	64.03 ± 1.44 ^c	61.02 ± 1.42 ^c	69.00 ± 1.41 ^c	64.03 ± 1.44 ^c
P-value	≤0.001*	≤0.001*	≤0.001*	≤0.001*	≤0.001*	≤0.001*

Means with different letters in the same column indicate significance difference

*: Significant at P<0.05.

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)		
	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 ^a	2.50±0.55 ^a	3.00 ±0.00 ^a	1.00 ±0.00 ^a	1.67±0.52 ^a	2.17±0.41 ^a
BD	1.67±0.52 ^a	2.83±0.41 ^a	3.00±0.00 ^a	1.00±0.00 ^a	2.00 ±0.63 ^a	2.33 ±0.52 ^a
P-value	1 Ns	0.241 Ns	1 Ns	1 Ns	0.336 Ns	0.523 Ns

Means with different letters in the same column indicate significance difference

*: Significant at P<0.05); Ns: non-significant at P>0.05.

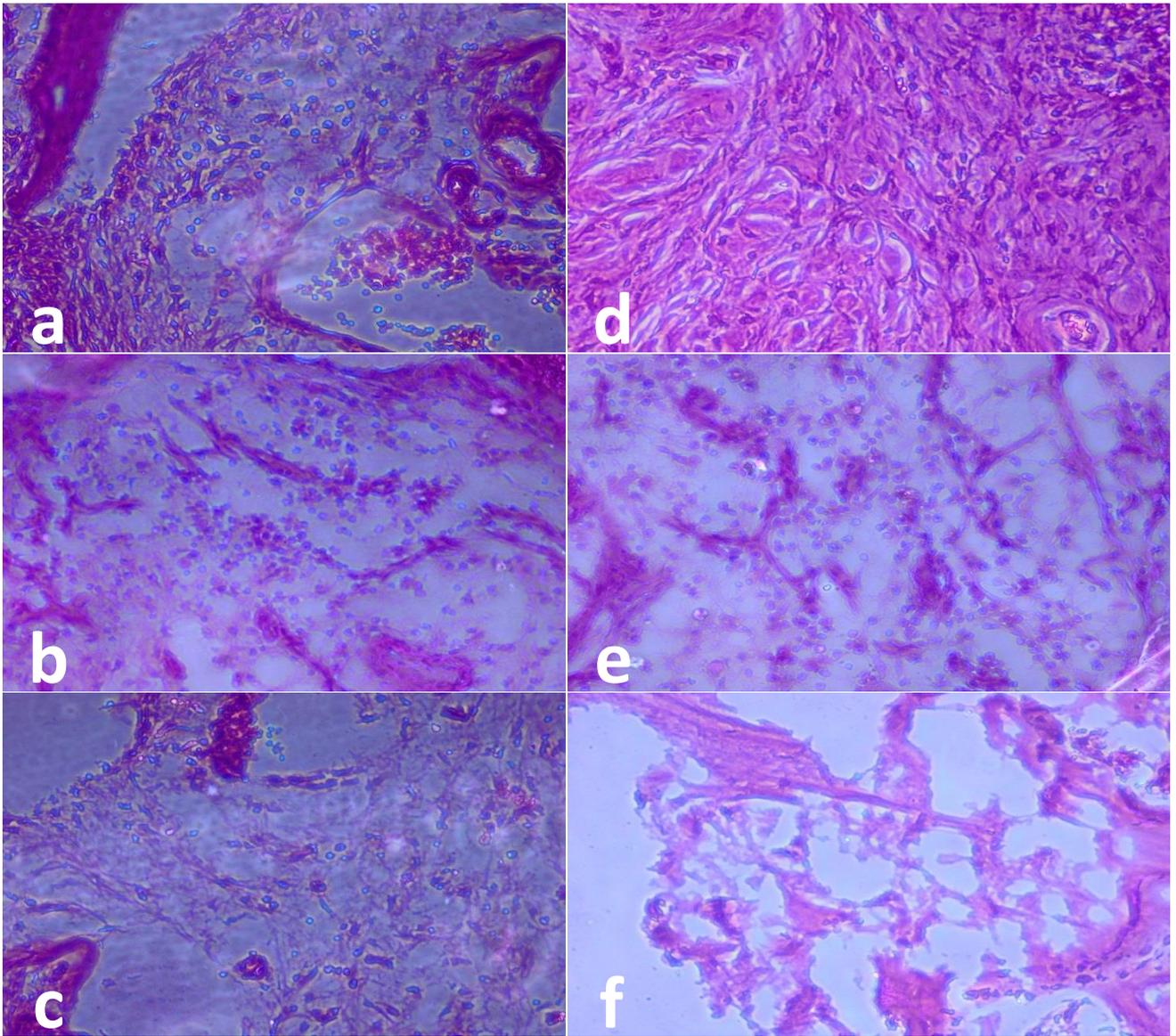


Figure 1. Representative photomicrographs of group II showing mild (a), moderate (b) and severe (c) inflammatory reaction in the MTA subdivision after 1, 2 and 3 months, respectively. Representative photomicrographs of group II showing mild (d), moderate (e) and severe (f) inflammatory reaction in the Biodentine subdivision after 1, 2 and 3 months, respectively. (H&E, X 200)

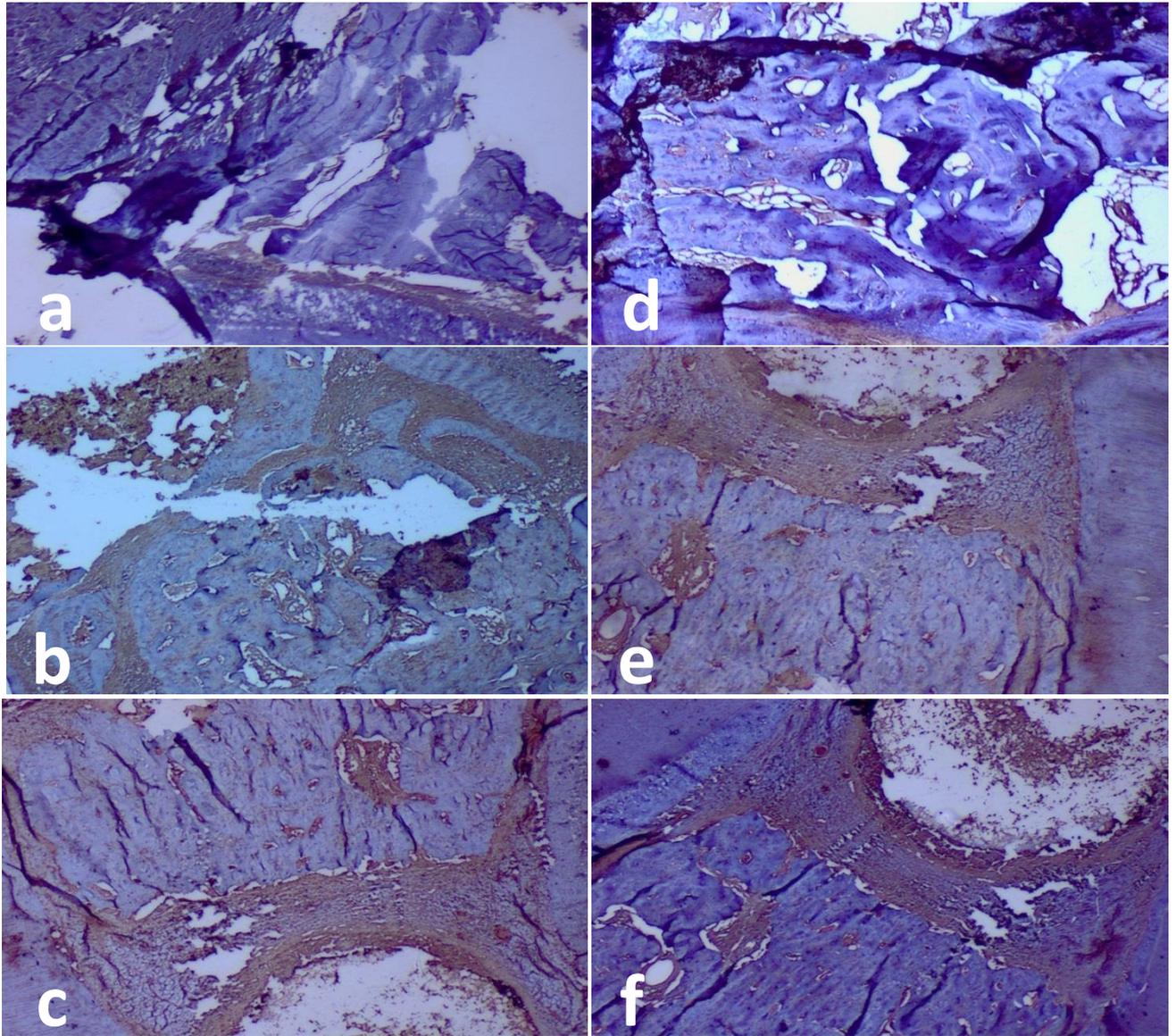


Figure 2. Representative photomicrographs of Osteonectin section of group I showing mild (a), moderate (b) and dense (c) new bone formation at the FP site in the MTA subdivision after 1, 2 and 3 months, respectively. Representative photomicrographs of Osteonectin section of group I showing mild (d), moderate (e) and dense (f) new bone formation at the FP site in the Biodentine subdivision after 1, 2 and 3 months, respectively.

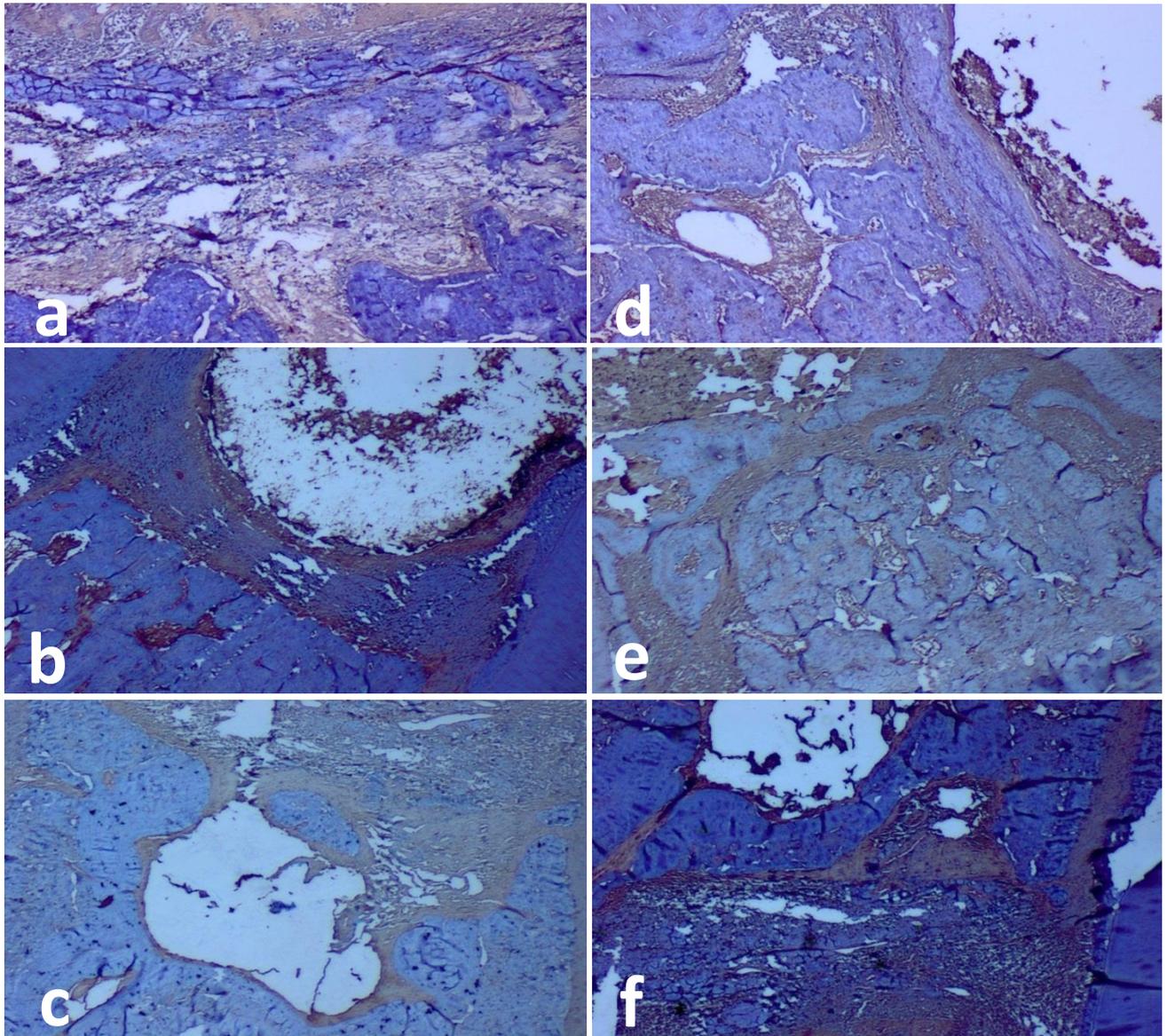


Figure 3. Representative photomicrographs of Osteonectin section of group II showing mild (a), moderate (b) and dense (c) new bone formation at the FP site in the MTA subdivision after 1, 2 and 3 months, respectively. Representative photomicrographs of Osteonectin section of group II showing mild (d), moderate (e) and dense (f) new bone formation at the FP site in the Biodentine subdivision after 1, 2 and 3 months, respectively.

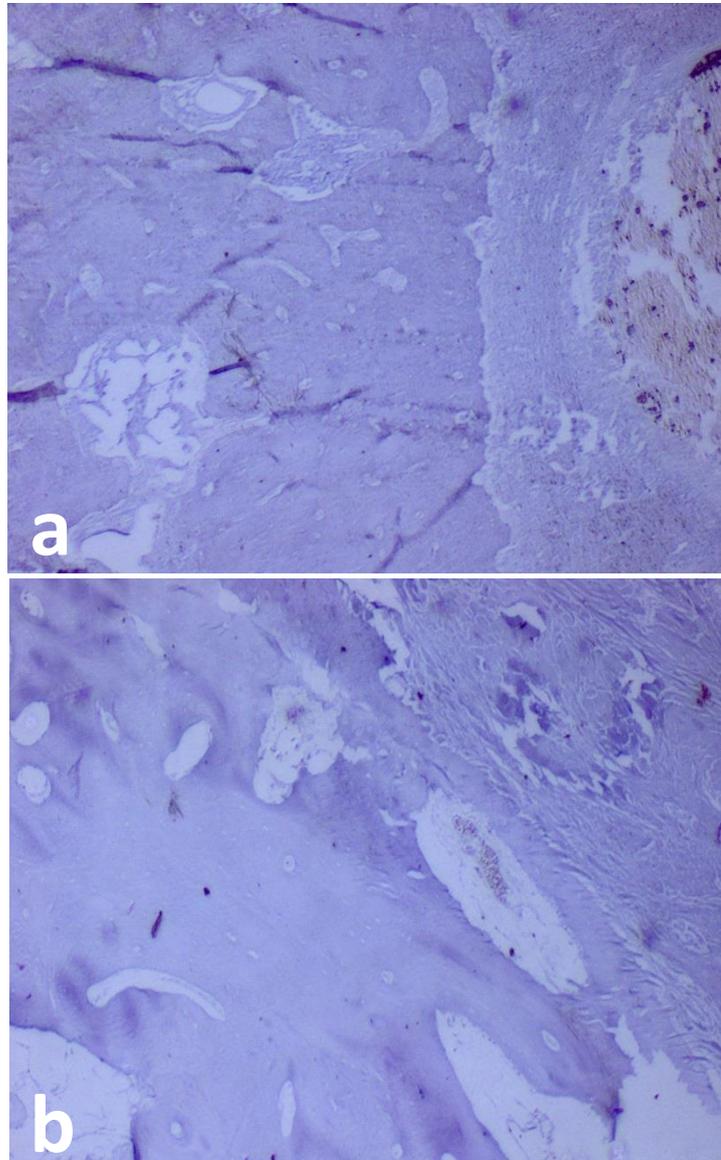


Figure 4. Representative photomicrographs of Osteonectin section showing no bone formation at the FP site in the control positive subdivision in group I (a) and group II (b).