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Histologic Evaluation of Furcation Perforation Treated with Mineral Trioxide Aggregate and Bioaggregate

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ABSTRACT

The aim of this work was to evaluate the healing of furcation perforation following treatment with Mineral Trioxide Aggregate (MTA) and BioAggregate by histologic examination. The present study was carried out on a total of 72 premolar teeth from 6 adult mongrel dogs. Under general anesthesia, furcation perforation was carried out by using a round bur # 4. The access cavity of all experimental and positive control teeth was left open for 4 weeks. The teeth were classified according to the observation period into three groups; I (one week), II (one month) and III (3 months) (2 dogs each). Each group was further subdivided into three subgroups according to the treatment protocol. These subgroups included; subgroup a (MTA), subgroup b (BioAggregate) and subgroup c (positive control). Inflammatory cell count, epithelial proliferation and new hard tissue formation were assessed. Statistical analysis of the results was carried out and significance of the parameters was determined in the tests at $p < 0.05$. The statistical analysis revealed that MTA and BioAggregate had a similar biological response on the periodontal tissue. There was no significant difference in inflammatory cell count, epithelial proliferation and new hard tissue formation between MTA and BioAggregate subgroups. In conclusion, both MTA and Bioaggregate show similar biological responses when used as a perforation repair material in dogs. These responses showed improved healing characteristics when compared to the control.

Key words: BioAggregate, dogs, furcation perforation, hard tissue, mineral trioxide aggregate

INTRODUCTION

Perforation can be defined as a mechanical or pathologic communication between the root canal system and the external tooth surface (AAE., 2012).

Perforations can be produced iatrogenically throughout the course of endodontic access opening due to an incorrectly directed bur, post space preparation or as a result of extension of internal resorptions into the periradicular tissues. Furcal perforation is a common cause of endodontic failure (Torabinejad and Chivian, 1999).

Factors that influence the survival of the perforated tooth include; size of the perforation, time between accident and treatment, level and location of perforation and presence of periodontal disease (Torabinejad and Chivian, 1999).

In clinical endodontics, there are several filling materials for perforation repair, such as reinforced zinc oxide eugenol cement, superEBA cement, calcium hydroxide, composite resins, glass

ionomer cements and MTA (Torabinejad *et al.*, 1995; El-Ashry *et al.*, 2013). Among these, MTA has been used successfully in endodontics for the past 10 years (Tawfik *et al.*, 2013).

Mineral trioxide aggregate is composed of dicalcium silicate, tricalcium silicate, tricalcium aluminate and tetracalcium aluminoferrite. This cement has a long setting time (~4 h) and an alkaline pH (~12.5) (Torabinejad *et al.*, 1995). MTA has been demonstrated to be a biocompatible endodontic repair material. It has several clinical applications including; apexification, pulpotomy, pulp capping, root-end filling and repair of root and furcation perforations (Torabinejad and Chivian, 1999). MTA promotes dental tissue regeneration in contact with pulp (Tawfik *et al.*, 2013; Nagy *et al.*, 2014). MTA has low cytotoxicity and antibacterial effects and promoting cementogenesis (Al-Hezaimi *et al.*, 2006; Holland *et al.*, 2007). Therefore, MTA is a new gold standard for perforation repair due to its predictable regeneration of periodontal ligament (Holland *et al.*, 2007).

More recently, new products similar to MTA have been introduced including MTA Angelus (Hashem and Hassanien, 2008), MTA-Bio (Lessa *et al.*, 2010) and BioAggregate (Zhang *et al.*, 2009). BioAggregate is a relatively new bioceramic based material which is similar to MTA. BioAggregate powder promotes cementogenesis and forms a hermetic seal inside the root canal. It has a good quality and antibacterial effect and easily manipulated when used as a root canal repair material. It is used in repair of root perforation and resorption, apexification, root end filling and pulp capping (Zhang *et al.*, 2009).

MTA and BioAggregate were found to have similar chemical composition except for the absence of aluminium and presence of significant amount of tantalum oxide in BioAggregate. Tantalum oxide acts as a radiopacifier instead of bismuth oxide which is present in MTA. The other difference between these two products is the presence of hydroxyapatite in BioAggregate (Park *et al.*, 2010). Both MTA and BioAggregate have the same bactericidal effects on *E. faecalis* (Zhang *et al.*, 2009). Moreover, Yan *et al.* (2010) found that BioAggregate may enhance PDL cell differentiation and increase mineralization.

In addition, Bioaggregate has excellent hermetic seal due to its nano-sized particles that adhere to the dentinal wall and its hydrophilic nature (Batur *et al.*, 2013).

For evaluation of the healing of furcation perforation after treatment, inflammatory cell count (Salman *et al.*, 1999), epithelial proliferation and hard tissue formation (Ford *et al.*, 1995) should be assessed.

To our knowledge, there are scarce researches describing the histologic assessment of healing of furcation perforation after treatment with MTA and Bioaggregate. Therefore, the aim of this study was to evaluate the healing of furcation perforation following treatment with MTA and BioAggregate by using histologic examination.

MATERIALS AND METHODS

The research proposal was approved by the ethical committee at Faculty of Dentistry Ain Shams University and by the Animal Use and Care committee at Faculty of Veterinary Medicine, Cairo University, Egypt.

A total of 6 healthy adult dogs, weighting 15-20 kg and approximately more than one year old were used in this study. The selected dogs were of both sexes and clinically normal. The dogs were pre-medicated with 0.05 mg kg⁻¹ atropine sulphate injected subcutaneously and 1 mg kg⁻¹ Xylazine HCl (Xylaject; ADWIA Co., Cairo, Egypt) injected intramuscularly. General anesthesia was induced by using Ketamine HCl (Keiran; EIMC pharmaceuticals Co., Cairo, Egypt) injected intravenously

using a cannula fixed in the cephalic vein at a dose of 5 mg kg⁻¹ body weight. The anesthesia was maintained by using Thiopental sodium at a dose of 25 mg kg⁻¹ body weight 2.5% injected intravenously (dose to effect).

A total of 72 premolar teeth (3 in each quadrant) were selected (12 teeth/dog). These teeth were divided according to the post-treatment evaluation period into three main equal groups (24 teeth each) including; group I (1 week), group II (1 month) and group III (3 months). Each group was further subdivided into two experimental subgroups and positive control subgroup according to the treatment protocol. The subgroups included; subgroup a (MTA), subgroup b (BioAggregate) and subgroup c (positive control). All subgroups were represented in each dog with a randomized manner.

Endodontic access cavity was prepared in all experimental and positive control teeth. Exposure of the pulp chamber was obtained through the occlusal surface using #4 round bur with conventional speed hand piece mounted on electric micro motor. All experimental and positive control teeth were perforated at the furcation area by using a #4 round bur at low speed headpiece. A 1.4 mm-diameter perforation was done at the center of the pulp chamber floor. The pulp tissue was removed and the root canals were instrumented using passive-step back technique combination of hand files and Gates-Glidden drills. A K-file #15 was placed to the apex with reaming action until the file was totally loosened, then the files #20, 25, 30 and 35 K-files were introduced into the canals. The canals were irrigated with saline after each file. A #2 Gates-Glidden drill was used to flare the coronal two third of the canal. Then Gates Glidden drills sizes 3 and 4 were used to continue planning and flaring the canal walls. After drying with paper points, the canals were obturated by Guttapercha core and Endo-fill cement (Dentsply Hero, Petrópolis, Rio de Janeiro, Brazil) as sealer in lateral condensation technique. The access cavity of all experimental and positive control teeth was left open for 4 weeks.

For pain and infection control, the dogs were given intra-muscular Cefotaxime sodium at a dose of 10 mg kg⁻¹ and diclofenac sodium at a dose of 1.1 mg kg⁻¹ once/day for 5 days after surgery (Abu-Seida, 2012).

After the infection period, the dogs were re-anesthetized and preoperative radiographs were taken to confirm the formation of the furcal lesion. Under complete aseptic condition by using the dental dam, the perforation site was curetted by a small spoon excavator to remove the debris and inflamed tissues, cleaned with normal saline and dried with paper points. Treatment of furcal perforations was carried out according to the subgroups as follow:

Subgroups: In subgroup (a), the perforations were treated by Angelus MTA cement (MTA Angelus, Londrina, Brazil); in subgroup (b), the perforations were treated by BioAggregate cement (Innovative Bioceramix, Vancouver, BC, Canada) and in subgroup (c), the furcation perforations were left open without filling as a positive control.

In subgroups (a) and (b), the materials were mixed according to the manufacturer's instructions, carried out into the perforation sites by small amalgam carrier and compacted with a suitable size plugger. A sterile wet cotton pellet was then placed in the access cavity and then the coronal access cavity was filled with chemical cured Glass Ionimer cement. Radiographs were taken to confirm the perforation repair with MTA and BioAggregate cement.

For pain and infection control, the dogs were given aforementioned drugs. The dogs were kept under continuous monitoring for any changes in habits, body weight and food intake during the post treatment evaluation periods.

Histological evaluation: The dogs were sacrificed at the end of each evaluation period using an overdose of Thiopental sodium. Teeth and surrounding periapical tissues were fixed in 10% buffered formalin solution and decalcified using 17% EDTA solution for 120 days. Decalcified bone blocks were sectioned in bucco-lingual at 6 µm thickness. Sections were stained using haematoxylin and eosin. The following histopathological findings were evaluated.

Inflammatory cell count: It was assessed according to Salman *et al.* (1999). For each slide, 3 representative fields were analyzed. Fields were characterized by well-preserved tissue with good architecture and intense inflammatory cell infiltration and without artifacts. Total inflammatory cell number was counted using image analysis software 'Image-J software'. The color-coding threshold was adjusted to select the perimeter of the whole range of inflammatory cells to exclude other non desired structures. Then, binary thresholds of the selected color coded inflammatory cells were completed prior to calculation. The total number of cells was then counted as a factor of 10³.

Epithelial proliferation: The epithelial response was classified as score 0, absence of epithelial proliferation and score 1, presence of epithelial proliferation (Ford *et al.*, 1995).

Hard tissue formation: It was classified as score 0, absence of new hard tissue formation and score 1, presence of new hard tissues formation (Ford *et al.*, 1995).

Statistical analysis: Data were analyzed using SPSS (Statistical Packages for the Social Sciences 19.0, IBM, Armonk, NY, USA). Numerical data were presented as mean and Standard Deviation (SD) values. One-way ANOVA test was used to compare the inflammatory cell counts among different groups and subgroups. For non-parametric data, Kruskal-Wallis test was used to compare the different groups and subgroups. A p-value <0.05 was considered significant.

RESULTS

The follow up of the operated dogs showed no changes in their habits. All dogs ate and drank well next day of the operations and no signs of pain were noticed. No dog was discarded from the study.

Histological findings

Inflammatory cell count: The results were collected on Table 1 and Fig. 1.

There was no significant difference between MTA and BioAggregate subgroups in groups I, II (p<0.001) and III (p<0.002). Both MTA and BioAggregate subgroups showed lower mean inflammatory cell count than positive control subgroup.

Epithelial proliferation: The results were shown on Table 2 and Fig. 2.

In group I (p = 0.001), group II (p = 0.161) and group III (p = 0.076), there was no statistically significant difference between the prevalence of epithelial proliferation in subgroups a, b and c.

Table 1: Mean of inflammatory cell counts among different groups and subgroups

Time duration	MTA	Bio aggregate	Positive control	p-value
One week	770.5±23.1 ^b	703.9±22.2 ^b	840.4±32.2 ^a	<0.001*
One month	554.4±18.7 ^b	526.7±20.7 ^b	912.0±42.3 ^a	<0.001*
Three months	305.9±22.1 ^b	315.4±27.2 ^b	960.8±28.2 ^a	0.002*

Different letters in the same row are statistically significantly different, *Significant at p<0.05, MTA: Mineral trioxide aggregate

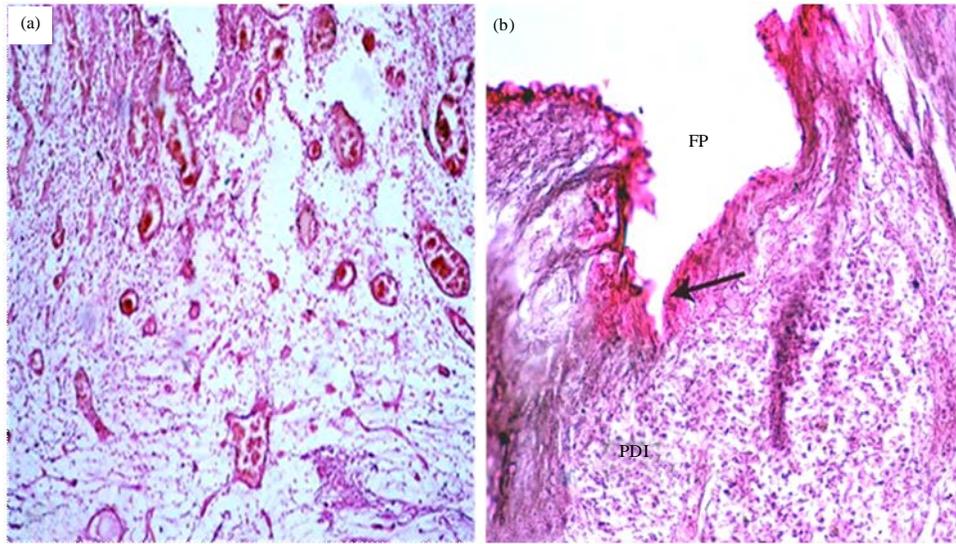


Fig. 1(a-b): (a) Photomicrograph showing moderate inflammatory cell infiltration and engorged blood vessels in subgroup Ib (Bioaggregate) (H and E×200) and (b) Photomicrograph showing severe inflammatory cells infiltration in subgroup Ic (positive control) (H and E×40). FP: Furcation perforation. PDL: Periodontal ligament

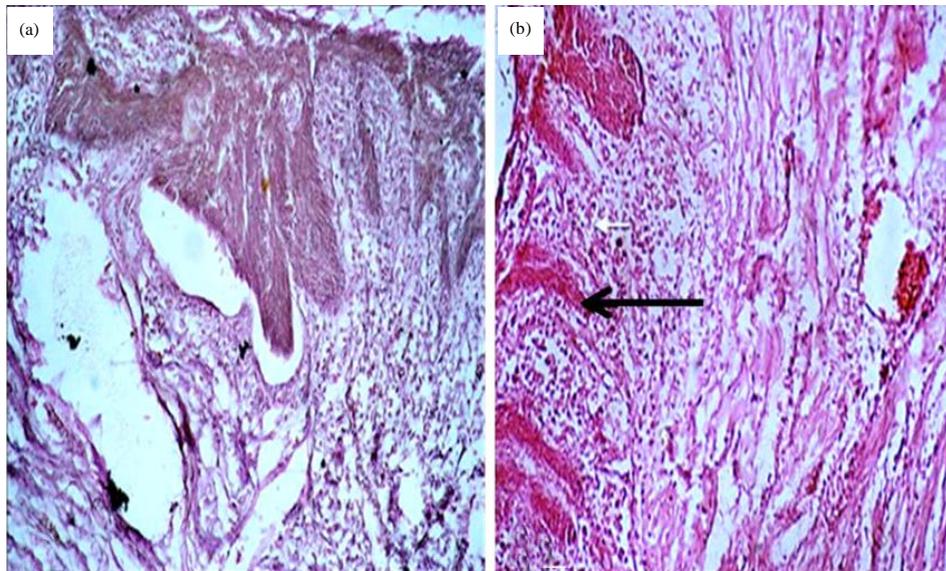


Fig. 2(a-b): (a) Photomicrograph showing dense epithelial proliferation in subgroup IIIc (positive control) (H and E×200) and (b) Photomicrograph showing epithelial proliferation (black arrow) in subgroup IIb (Bioaggregate) (H and E×200)

Hard tissue formation: The results were shown on Table 3 and Fig. 3.

There was no significant difference between MTA and BioAggregate subgroups in group I ($p = 0.545$), group II ($p = 0.061$) and group III ($p = 0.014$). Positive control subgroup showed lower mean prevalence of new hard tissue formation than MTA and BioAggregate subgroups.

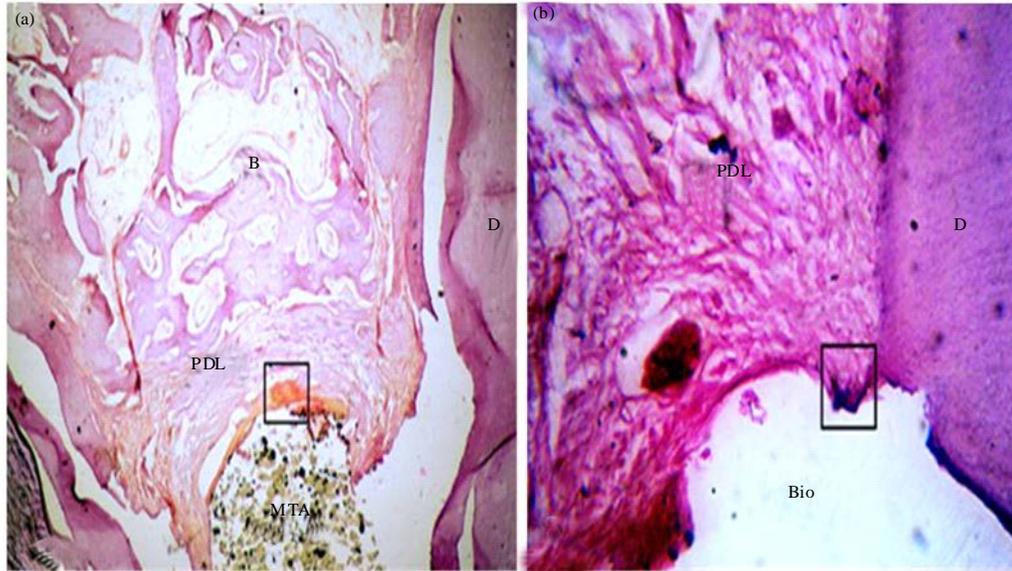


Fig. 3(a-b): (a) Photomicrograph of subgroup IIIa (MTA) showing new hard tissue over the MTA (H and E×40). B: Bone, D: Dentine, PDL: Periodontal ligament, MTA: Mineral trioxide aggregate and (b) Photomicrograph of subgroup IIIb (bioaggregate) showing new hard tissue over the bioaggregate (H and E×40). D: Dentine, PDL: Periodontal ligament, Bio: Bioaggregate

Table 2: Frequencies and percentages of epithelial proliferation in the different groups and subgroups

Groups/Subgroups	One week		One month		Three months	
	Frequency	%	Frequency	%	Frequency	%
MTA	7.0	87.5	4.0	50.0	2.0	25.0
Bioaggregate	8.0	100.0	3.0	37.5	1.0	12.5
Positive control	5.0	62.5	3.0	37.5	3.0	37.5
p-value	0.001		0.161		0.076	NC**

*Significant at $p \leq 0.05$, NC: **Not Computed, MTA: Material trioxide aggregate

Table 3: Frequencies and percentages of prevalence of new hard tissue formation among different groups and subgroups

Groups/Subgroups	One week		One month		Three months	
	Frequency	%	Frequency	%	Frequency	%
MTA	1.0	12.5	3.0	37.5	4.0	50
Bioaggregate	1.0	12.5	3.0	37.5	4.0	50
Positive control	0.0	0.0	0.0	0.0	0.0	NC**
p-value	0.545	0.061	0.014*			

*Significant at $p \leq 0.05$, NC: **Not computed, MTA: Material trioxide aggregate

DISCUSSION

Furcation perforations have been considered one of the major complications leading to failure of endodontic treatment. Besides being iatrogenic in origin, root perforations are also created pathologically by resorption and caries (Torabinejad and Chivian, 1999). Furcal perforations might be sealed either intracoronally or with external surgical access using different materials. Among these materials, MTA has been applied with good treatment outcomes due to its biocompatibility, low provocation of inflammation, good sealability and high pH (12.5) which promotes growth of cementum and regeneration of periodontal ligament (Roberts *et al.*, 2008; Nagy *et al.*, 2014). More

recently, new products similar to MTA have been introduced into the market including MTA Angelus and MTA Bio and BioAggregate (Zhang *et al.*, 2009). BioAggregate appears to be a modified version of MTA; it is a new retrograde filling and root canal perforation repair material (Park *et al.*, 2010).

The dog is a demanding experimental model, having two rooted premolars that often furcate as close as 1-2 mm from the cemento-enamel junction. As a result, epithelialization and the formation of connective tissue at a furcation perforation should be more likely than in humans, where the furcation lies deeper within the alveolus (Ford *et al.*, 1995).

The size of perforations in this investigation was standardized at 1.4 mm which is similar to several previous studies (Aguirre *et al.*, 1986; Ford *et al.*, 1995). The timing of repair is an important variable in the treatment of furcation perforations. The poor prognosis of furcal perforations is owing to the role of infection; this has been particularly marked in unfilled controls in previous studies (ElDeeb *et al.*, 1982).

While radiographic evaluation was not able to detect tissue response to different treatment modalities after one week, the histologic evaluation demonstrated variable degrees of osteoplastic and osteoclastic activities that reflect a bony reaction to different treatment modalities (Holland *et al.*, 2007). Therefore the present study depended upon histological evaluation of healing of the furcation perforation.

Regarding the histological evaluation of inflammatory cell count in group I, the positive control subgroup showed severe inflammatory cell infiltration due to persistent infection and chronic inflammation resulted from the direct contact between the furcation perforation and oral cavity. In addition, both MTA and BioAggregate subgroups showed high inflammatory cell count because the time is not enough to repair the defect. This agrees with previous studies (Noetzel *et al.*, 2006; Al-Daafas and Al-Nazhan, 2007).

As regards groups II and III (one and three months), positive control subgroup showed a significant higher mean inflammatory cell count than MTA and BioAggregate subgroups. This could be attributed to the persistent infection and chronic inflammation. Both MTA and BioAggregate subgroups showed a significant lower mean inflammatory cell count than positive subgroup due to sealing ability, biocompatibility and alkaline pH on setting (Schwartz *et al.*, 1999; Park *et al.*, 2010). In addition, the closure of the access cavity prevents further infection at the furcation perforation. In contrast to our results, Batur *et al.* (2013) found that BioAggregate had a significantly better inflammatory reaction and foreign body reaction than the MTA.

Regarding epithelial proliferation in group I (one week), no significant difference between MTA, BioAggregate and positive control subgroups was seen. All subgroups showed high prevalence of epithelial proliferation due to the furcation is often as close as 1-2 mm from the cemento-enamel junction in the dogs. Similar findings were mentioned by Salman *et al.* (1999). In groups II and III (one and three months), no significant difference in prevalence of epithelial proliferation between MTA, BioAggregate and positive control subgroups was observed. This might be due to the small size of samples.

As regards new hard tissue formation in group I, no significant difference was noticed between MTA and BioAggregate subgroups. Deposition of new hard tissue was evident in very little samples treated with MTA and BioAggregate. This might be due to the relatively short period of time which was not enough for new hard tissue formation. This is in agreement with the results of a previous study (Sluyk *et al.*, 1998). In positive control subgroup, no scores of new hard tissue formation were noticed due the presence of infection. In groups II and III, there was no significant difference in

new hard tissue formation between MTA and BioAggregate subgroups. Both subgroups showed a significant higher mean prevalence of new hard tissue formation than positive control subgroup. Half of specimens in MTA and BioAggregate subgroups showed deposition of new hard tissue especially in groups II and III. This could be attributed to some factors such as sealing ability, biocompatibility and alkaline pH on setting that accelerate the new hard tissue formation. Similar findings were recorded in many previous studies (Ford *et al.*, 1995; Al-Daafas and Al-Nazhan, 2007).

CONCLUSION

In conclusion, both MTA and Bioaggregate show similar biological responses when used as a perforation repair material in dogs. These responses showed improved healing characteristics when compared to the control.

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