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ORIGINAL ARTICLE

Histopathology and immunohistochemical reactions of Nano-White MTA versus NeoMTA™ Plus® and MTA Angelus® as immediate furcation perforation repair materials in a dog model

ABSTRACT

Aim: *This study compared histopathology and immunohistochemical reactions of Nano-white mineral trioxide aggregate (Nano-WMTA), Neo-mineral trioxide aggregate plus (NeoMTA™ Plus®), and mineral trioxide aggregate angelus (MTA Angelus®) as furcation perforation repair materials.*

Methodology: *Twelve premolars were used in six mongrel dogs. Seventy two teeth were divided according to post-treatment evaluation period into two main equal groups (three dogs' each/36 teeth each) including; group I: after one month and group II: after three months. Each main group was subdivided into three experimental subgroups (8 teeth each) according to the immediate perforation repair materials used, and two control groups. The perforations in subgroups 1, 2 and 3 were sealed with NeoMTATM Plus®, Nano-WMTA and MTA Angelus®, respectively. In the positive control subgroup (8 teeth), the perforations were done with no repair. Negative control subgroup (4 teeth) represented intact teeth with no perforation to show the normal histology. Pulpotomy and root canal obturation were carried out in the experimental and positive control subgroups. Inflammatory cell count and new hard tissue formation were evaluated by histopathology and immunohistochemical staining with Osteonectin. All data were statistically analyzed.*

Results: *All tested materials demonstrated significant lower inflammatory cell counts than the positive control subgroup and significant higher new bone formation than both positive and negative subgroups. There was no statistically significant difference in inflammatory cell count and new hard tissue formation among the experimental materials in both groups ($p<0.05$).*

Conclusion: *All tested materials effectively reduced inflammation and promoted hard tissue formation with no statistically significant difference.*

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Introduction

Root perforations are one of the problems that pose obstacles to successful endodontic therapy in endodontic practice (1, 2). Perforation repair prognosis is determined by its location, size, timeliness of repair, contamination, and biocompatibility of repair material (3, 4). Quick and successful perforation sealing is critical for tooth preservation, necessitating particular features in repair materials such as biocompatibility, bioactivity, non-toxicity, insolubility, radiopacity, and sealing capability (5, 6).

Mineral trioxide aggregate (MTA) and hydraulic calcium silicates are innovative dental materials with distinct properties that make them ideal for endodontic use. One of their distinguishing characteristics is their exceptional bioactivity, which means they can interact with the surrounding biological environment. This bioactivity promotes the regeneration of dental hard tissues such as dentin, making them useful for operations such as perforation repair and pulp capping (7, 8).

Furthermore, MTA and hydraulic calcium silicates have good antibacterial characteristics, inhibiting bacterial growth and reducing the risk of infection within the treated root canal (7, 9). These materials stand out due to their dual action of bioactivity and antibacterial impact, which promotes not only the structural integrity of the tooth but also the overall health of the dental tissues. MTA and hydraulic calcium silicates are indispensable in modern endodontics due to these properties, which contribute to the success and longevity of numerous dental operations (7-9).

Various materials have been used for perforation repair, MTA being a common choice due to its superior sealing and biocompatibility, albeit with drawbacks like long setting time and cost (10-12).

Neo MTA™ Plus® and Nano-White MTA have recently developed as modified alternatives with improved features such as faster setting, increased biocompatibility, and less discoloration problems (13, 14). NeoMTA™ Plus® is a modified and enhanced type of MTA that is used in endodontics to heal perforations. Its unique composition includes tricalcium silicate, dicalcium silicate, calcium aluminate, calcium sulfate, calcium hydroxide, and zirconium oxide, which enhances its handling properties and overall performance compared to traditional MTA (13-15). In comparison to MTA, NeoMTA™ Plus® has a significantly faster setting time, minimizing chair time and enhancing procedure efficiency. It has the same biocompatibility and tissue response characteristics as MTA but outperforms it in terms of handling, setting time, washout resistance, and overall formulation (14, 15). Because of these characteristics, NeoMTA™ Plus® is a favored choice for many doctors in endodontic procedures where speedy and effective perforation healing is critical for good outcomes (13-15).

Nano-White MTA is improved MTA cement used in endodontics to heal root perforations. It is distinguished by its nanoscale particle size, which allows for greater adaptability to uneven sites and facilitates a tight seal (16, 17). Furthermore, the nanoscale particles improve tissue contact, allowing the creation of a physiologically active dentin bridge and promoting spontaneous regeneration (16). Nano-White MTA uses nanotechnology to improve sealing and regenerative capacities, resulting in better results in root perforation repair and other endodontic operations (16, 17).

These materials are still being researched and developed in order to improve their properties and increase their applications in endodontic treatment. Therefore, this study compared the histopathological and immunohistochemical reactions of Nano-White MTA versus NeoMTA™ Plus® and MTA Angelus® as root repair materials in a dog model.

Materials and Methods

Ethical approval

The research proposal was approved by the Ethical Committee of Faculty of Dentistry, Ain Shams University, Egypt (number: 790-Endo). Furthermore, the authors adhered to all the recommendations outlined in the Animal Research: Reporting in Vivo Experiments (ARRIVE) guidelines.

Animal model
The sample size was determined based on earlier studies (1, 15) using the G*power software 3.1.9.2, where a large effect size of 1.38 was detected. The significance level (α-error) was set at 0.05 and the power (1-β error) was set at 0.8 using two-sided hypothesis test. The estimated sample size was 8 for each subgroup at each evaluation period. The research involved six adult mongrel dogs weighing 17-20 kg. The age of dogs ranged between one and two years and they were of both sexes. Standardized periapical radiographs were taken to confirm complete root formation and absence of dental pathologies. The study was conducted at the Faculty of Veterinary Medicine, Cairo University, Egypt where the dogs were kept individually in separate kennel and received proper nutrition, ventilation and humidity.

Animal model classification
Twelve premolar teeth were used in each selected dog. A total of 72 teeth were divided according to post-treatment evaluation period into two main equal groups (three dogs' each/36 teeth each) including; group I: evaluated after one month and group II: evaluated after three months. Each main group was subdivided into three experimental subgroups (8 teeth each) according to the perforation repair materials used, and two control groups. The perforations in subgroups 1, 2 and 3 were sealed with NeoMTA™ Plus® (Avalon Biomed Inc. Bradenton, FL, USA), Nano-White MTA (Tooth-colored MTA, DENTSPLY, Tulsa Dental, Tulsa, OK, USA) and MTA Angelus® (Londrina, Brazil), respectively. The details of materials used in this study are shown in Table 1.
In the positive control subgroup (8 teeth), the perforations were done with no repair. Negative control subgroup (4 teeth) represented intact teeth with no perforation to show the normal histology (18, 19).

Anesthetic procedure
Each dog was anesthetized with general anesthesia after fasting for 12 hours. The dogs were pre-medicated with 0.05 mg/kg body weight Atropine sulphate (Atropine 1%®, ADWIA, Egypt) injected subcutaneously and 1 mg/kg body weight Xylazine HCl (Xylaject HCl 2%®, ADWIA, Egypt) injected intramuscularly. General anesthesia was induced by using Ketamine HCl (Ketamine 5%®, EPICO, Egypt) injected intravenously using a cannula fixed in the cephalic vein at a dose of 5mg/kg body weight. The anesthesia was maintained by using Thiopental sodium (Thiopental Sodium®, EPICO, Egypt) at a dose of 25 mg/kg body weight 2.5% injected intravenously (dose to effect).

Teeth instrumentation
Prophylaxis disinfection of the operative field was done using 2% chlorhexidine gluconate solution (JK Dental Vision CO., India). After general anesthesia, access cavity was done in all experimental and positive control teeth. Exposure of the pulp chamber was obtained through the occlusal surface using No.4 round bur with high-speed hand piece mounted on a portable air motor (X-Smart; Dentsply Tulsa Dental Specialties). Pulp extirpation was performed using large spoon sterile excavator. Working length was determined with an electronic apex locator (NSK Inc., Kanuma, Japan). Root canals were instrumented with ProTaper Next (Dentsply Maillefer, Ballaigues, Switzerland) to size X4 (40/06) mounted on handpiece of an electric motor under irrigation with 3.6 mL 1% sodium hypochlorite (NaOCl). After drying the canals with paper points (Meta Biomed CO., LTD, Korea), root canals were obturated by lateral condensation of gutta-percha cones (META, China) and AD Seal sealer (Meta Biomed CO., LTD, Korea).

Perforation creation
Perforation was induced in the central region of the pulpal chamber floor with #4 round diamond bur (KG Sorensen, Sao Paulo, SP, Brazil). The diameter of the perforations was standardized as being the diameter of the bur used. A 1.4 mm-diameter furcation perforation was done at low speed hand piece in both experimental and positive control subgroups until the hemorrhage was observed. The perforation depth was limited to 2 mm into the alveolar

Table 1

Materials used for perforation repair in this study

Brand name	Lot number	Expiration date	Composition	Setting time (Minutes)	Manufacturer
NeoMTA Plus®	2023-NMTA-1	31/8/2024	Tricalcium silicate, Dicalcium silicate, Tantalum pentoxide	20	Avalon Biomed Inc. Bradenton, FL, USA
Nano White MTA®	NWMTA- 789	30/9/2024	Tricalcium silicate, Bismuth oxide	30	Tooth-colored MTA, DENTSPLY, Tulsa Dental, Tulsa, OK, USA
MTA Angelus®	ANG-4567	15/7/2024	Tricalcium silicate, Zirconium dioxide	15	Londrina, Brazil

bone by a rubber stopper (1). A new bur was used for every 3 perforations. Hemostasis was achieved with abundant sterile saline (NaCl 0.9%) and gentle pressure with sterile cotton pellets (1, 18).

Perforation repair

The perforations were immediately sealed according to the subgroups. In subgroups 1, 2 and 3, the materials were mixed according to the manufacturer's instructions, carried into the perforation sites by small MTA carrier and compacted with a suitable sized plugger. A sterile wet cotton pellet was then placed in the access cavity. Radiographs were taken to confirm the perforation repair with the intended cements. Positive control subgroup was cleaned by saline (NaCl 0.9%) and no repair material was used and the defect was sealed by Teflon (Chemours Company, USA) (15). No perforations were performed in the negative subgroup.

All experimental and control subgroups were represented in each dog. The coronal access cavities of all experimental and positive control teeth were sealed with chemical cured glass ionomer filling material (Medifil, Promedica, Germany).

All dogs were given intra-muscular injections of Cefotaxime sodium at a dose of 10 mg/kg (Cefotax 250 mg vial®, T3A Co., Egypt) and Diclofenac sodium at a dose of 1.1 mg/ kg (Voltaren 75 amp®, Novartis Co., Egypt) once daily for five postoperative days (20). The dogs were kept under continuous monitoring for any changes in habits, body weight and food intake during the post treatment evaluation periods. Then, all animals were sacrificed according to the designated observation period by an overdose of Thiopental sodium.

Histological evaluation

The maxillae and mandibles were surgically dissected and cut into four quadrants to accelerate the decalcification time. Specimens were fixed in 10% neutral buffer formalin for 14 days then EDTA solution 17% MD- (ChelCream, Meta Biomed, Korea) for 120 days. During that period the decalcifying solution was changed by a fresh mix every 2 days. Decalcification was confirmed clinically by confirming that a surgical lancet can pass without resistance through cortical bone. Each block was trimmed 1 mm away from the edge of perforation in mesiodistal direction in each sample. The specimens were washed in running water for 24 hours. A code number was given for each specimen. The specimens were processed by using an open processing system in which the specimens were dehydrated in ascending grades of ethyl alcohol 70%, 95%, and absolute alcohol in 18 hours, cleared in xylene and embedded in paraffin wax. Thin sections (4-6µ) of each block were cut using a microtome through the area of

the furcal perforation. Slides were stained with hematoxylin and eosin stain. Under the light microscope (BX60, Olympus, Japan), the sections were examined and the inflammatory cell count was evaluated. It was assessed according to Salman et al. (21). 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, 1.41a, NIH, USA). 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 10^3.

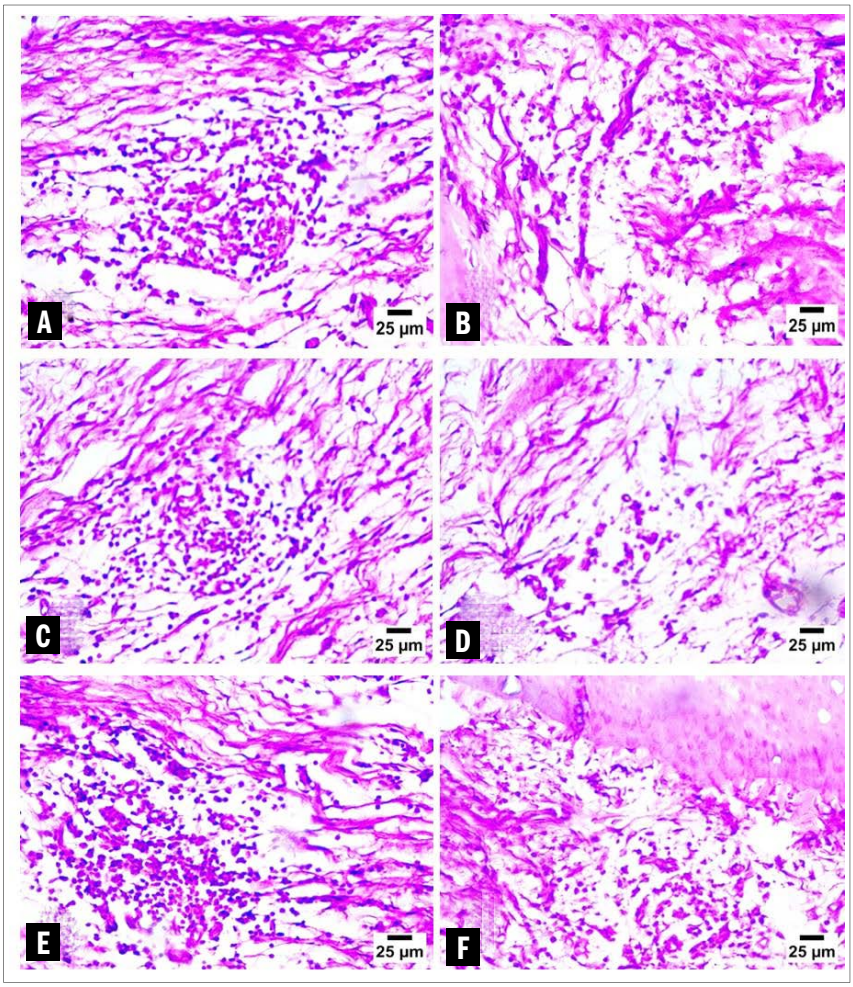

Figure 1
Photomicrographs showing inflammatory cell count for Neo MTA plus **(A),** Nano-WMTA **(C)** and MTA Angelus **(E)** at one month evaluation period. Photomicrographs showing inflammatory cell count for NeoMTA plus **(B),** Nano-WMTA **(D)** and MTA Angelus **(F)** at three months evaluation period (magnification, x400).

Immunohistochemical evaluation
Immunohistochemical staining was performed using Osteonectin antibody to identify new hard tissue formation. Paraffin sections were mounted on positively charged slides by using avidin-biotin-peroxidase complex (ABC) method. Sections from each subgroup were incubated with primary antibodies and then the reagents required for ABC method were added (Vectastain® ABC-HRP kit, Vector laboratories, USA). Marker expression was labeled with peroxidase and colored with diaminobenzidine (DAB, Sigma, Japan) to detect antigen-antibody complex. Negative control samples were included using non-immune serum in place of the primary or secondary antibodies. The stained sections were examined via using Olympus microscope (BX-53). The sections were graded according to Al-Hadainy et al. (22) scoring system as follows: Score 0: no bone formation, Score 1: slight bone formation, Score 2: moderate bone formation and Score 3: heavy bone formation.

Statistical analysis
All data were expressed as mean and standard deviation values. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. One-way ANOVA followed by tukey post hoc test was used to compare between more than two non-related samples. Repeated measure ANOVA followed by Paired sample t-test was used to compare between more than two related samples. Independent sample t-test was used to compare between two non-related samples. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

Results

Histological findings
In both groups, positive control subgroup showed the highest mean value of inflammatory cell count while the least mean value of inflammatory cell count was found in negative control subgroup. Representative photomicrographs of all experimental subgroups in both groups are shown in Figure 1.

Table 2

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

Subgroups	Group I (After one month)		Group II (After three months)		p-value
	Mean	SD	Mean	SD	
Subgroup 1 (NeoMTA Plus)	558.63^{bA}	46.51	445.13^{bB}	17.14	<0.001*
Subgroup 2 (Nano-WMTA)	539.63^{bA}	33.76	406.63^{bB}	53.71	0.001*
Subgroup 3 (MTA Angelus)	565.50^{bA}	29.20	455.00^{bB}	56.13	<0.001*
Positive control subgroup	866.63^{aA}	35.04	862.63^{aA}	43.91	0.851ns
Negative control subgroup	69.00^{cA}	6.19	68.00^{cA}	6.00	0.755ns
p-value	<0.001*		<0.001*		

*Mean with different small letters in the same column indicate statistically significance difference and mean with different capital letters in the same row indicate statistically significance difference. *Significant at p<0.05. ns: non-significant at p>0.05.*

Regarding effect of time periods on inflammatory cell count (Figure 2), there were statistically significant differences between group I (After one month) and group II (After 3 months) in all experimental subgroups (p≤0.001). Group I had significantly higher inflammatory cell count than group II. Regarding the effect of experimental materials on inflammatory cell count: there was no statistically significant difference between NeoMTA™ Plus®, Nano-WMTA and MTA Angelus® in both groups I and II (p<0.05). In both groups, the highest mean value was found in MTA Angelus® while the least mean value was found in Nano-WMTA (Table 2).

Immunohistochemical findings

In groups I and II, there were statistically significant differences between positive control subgroup and each of experimental subgroups (p≤0.001). Also, a statistical-

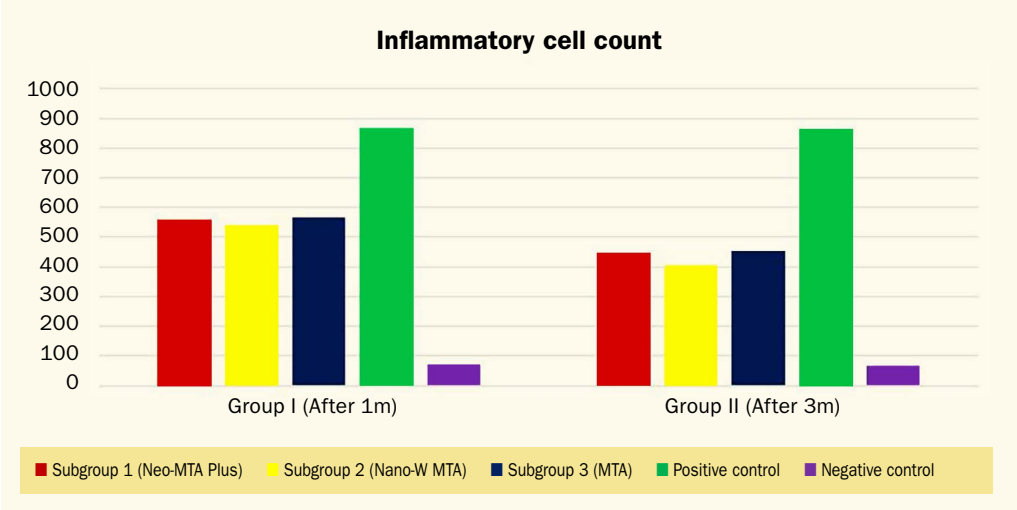

Figure 2
Bar charts representing effect of time periods on inflammatory cell count in different groups.

Table 3

The mean, standard deviation (SD) values of bone formation at all groups and subgroups

Subgroups	Group I (After one month)		Group II (After three months)		p-value
	Mean	SD	Mean	SD	
Subgroup 1 (Neo-MTA Plus)	1.50^{aB}	0.53	2.63^{aA}	0.52	0.024*
Subgroup 2 (Nano-WMTA)	1.63^{aB}	0.52	2.7 ^{aA}	0.46	0.020*
Subgroup 3 (MTA Angelus)	1.38^{aB}	0.52	2.25^{aA}	0.46	0.038*
Positive control subgroup	0.00^{bA}	0.00	0.00^{bA}	0.00	1ns
Negative control subgroup	0.00^{bA}	0.00	0.00^{bA}	0.00	1ns
p-value	<0.001*		<0.001*		

*Mean with different small letters in the same column indicate statistically significance difference and mean with different capital letters in the same row indicate statistically significance difference. *Significant at p<0.05. ns: non-significant at p>0.05.*

ly significant difference was found between negative control subgroup and each of experimental subgroups (p≤0.001) as shown in Table 3. Both positive and negative control subgroups showed 0 score in hard tissue formation. Representative photomicrographs of all experimental subgroups in group II are shown in Figure 3.

Effect of time periods on bone formation

Regarding the mean value of bone formation, there were statistically significant differences between group I and group II in all experimental subgroups (Table 3). Group II exhibited higher mean value of bone formation than group I in all experimental subgroups.

Effect of experimental materials on bone formation

There were no statistically significant differences in mean value of bone formation between NeoMTA™ Plus®, Nano-WMTA and MTA Angelus®. The highest mean value of bone formation was found in subgroup 2 (Nano-WMTA) while the least mean value was found in subgroup 3 (MTA Angelus®).

Discussion

Perforation repair materials should have high flowability to adapt to the complicated anatomy of root canal systems, strong biocompatibility to minimize unfavorable re-

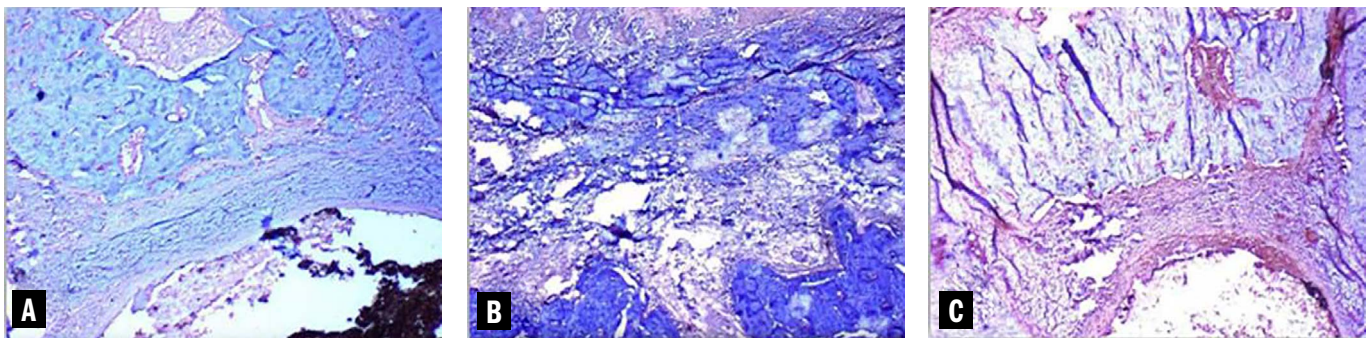

Figure 3
Photomicrographs showing positive response to Osteonictin antibody at furcation area **(A)** NeoMTA plus, **(B)** Nano-WMTA and **(C)** MTA Angelus.

actions with surrounding tissues, and radiopacity to permit monitoring during post-treatment assessments (1-3).The location and size of the perforation, the clinician's experience and preference, and the patient's overall oral health all influence the choice of perforation repair material (23).

MTA is commonly considered as the gold standard perforation repair material in dentistry (1-3). This is because of its extraordinary qualities, such as high biocompatibility and sealing ability (24, 25). Nevertheless, there are some drawbacks of MTA such as long setting time, technique sensitive and washout during initial setting (26, 27).

In endodontics, premixed bioceramic sealers and standard MTA powder-liquid formulations have significant advantages. Bioceramic sealers are pre-mixed for uniformity, have high biocompatibility and antibacterial qualities, and may aid in tissue healing. MTA has a solid track record for numerous endodontic operations, as well as long-term stability. MTA is adaptable and well-established, whereas bioceramic sealers are consistent and eliminate mixing errors (28-30). Their selection is determined by clinical preferences, case requirements, and physician experience. To increase the consistency of MTA powder-liquid formulations, putty formulations have been produced.

One of the improved versions of traditional MTA is NeoMTA™ Plus®. In comparison to MTA, NeoMTA™ Plus® has various components that provide several benefits in endodontics (31, 32).

The inclusion of radiopacifiers in endodontic root restoration materials is required for these materials to be visible on radiographs. However, the radiopacifier used can have a considerable impact on the biological features of these materials, such as cell inflammation and biocompatibility. Common radiopacifiers include bismuth oxide, zirconium dioxide, and tantalum pentoxide, which have diverse effects on materials and biological responses (33). Bismuth oxide (found in ProRoot MTA) has been linked to increased inflammation in certain studies, but zirconium dioxide (found in MTA Angelus®) and tantalum pentoxide (found in NeoMTA™ Plus®) are commonly thought to be superior solutions for limiting tissue irritation (33). Individual patient characteristics, material composition, and clinical approach can all have an impact on the overall biocompatibility of endodontic materials.

Nano-WMTA is a cutting-edge dental material that is a new advancement of classic MTA used in endodontics and restorative dentistry. Nano-WMTA has superior handling features, smoother consistency, and shorter setting times when compared to MTA, shortening treatment duration and potentially saving important chair time for both patients and dentists (34, 35).

The objective of this study was to assess the effectiveness of Nano-WMTA versus NeoMTA™ Plus® and MTA Angelus® when used for immediate furcation perforation repair. The current findings demonstrated that Nano-WMTA exhibited lower inflammatory cell count and higher new bone formation than both NeoMTA™ Plus® and MTA Angelus® in both groups with none statistical significant differences.

This study used an animal model to establish and control surgical circumstances that would be impossible to perform in a clinical situation, allowing for the creation of perforations and the gathering of histologic data that would be difficult to get in human beings. Because of their well-developed root structures and the accessibility and visibility afforded by their furcation area, dogs were chosen as the animal model (1-3). Furthermore, due to their faster growing rate, dogs have comparable apical healing mechanics with humans in a shorter duration (average 1/6 of human). They have the same mineral structure and organic responses as humans and can survive long periods of research under general anesthesia (18). The furcation in dog premolars is 1 to 2 mm from the cement enamel junction, but the furcation in human teeth is deeper within the alveolus (15, 20, 36). As a result, any technique or cement that has been proved to create beneficial effects in dogs may produce a more favorable reaction in humans, since the distance between the CEJ and the furcation area is greater (18, 37). According to

earlier research, the standardized perforation size was fixed at 1.4 mm, and the bur penetration depth was limited to 2 mm in order to trigger the inflammatory response (1, 38).

The current study used immediate seal of perforation because it is critical in preventing potentially serious consequences and guaranteeing satisfactory treatment outcomes. Dental practitioners can avoid bacterial entry into the root canal system and surrounding tissues by sealing the perforation as soon as possible, lowering the risk of infection, inflammation, and potential tooth loss. Furthermore, the initial seal contributes to the preservation of the natural barrier between the pulp space and the periapical tissues, promoting good healing responses and assisting in the retention of tooth vitality (39).

In the current study, two evaluation periods were chosen: one and three months. The inflammatory phase is usually over after one month. However, the second evaluation time of three months is required for the procedure's ultimate judgment. The majority of research that used animal models to evaluate histopathological tissue reactions for various endodontic procedures and/or materials used the same time frame (1, 15, 18).

Histological evaluation following perforation repair is critical for determining the healing response and biocompatibility of the repair materials utilized. It enables quantitative examination of characteristics such as mineralized tissue creation and the presence of inflammatory cells, providing objective data for assessing the success of perforation repair (18, 40).

Immunohistochemical analysis is a specialized and powerful approach that involves the use of specific antibodies that target certain proteins or cellular markers in repair tissue samples. Researchers and doctors can use immunohistochemistry to identify and quantify specific cell types, inflammatory indicators, growth factors, and tissue-regenerating chemicals found in healed tissues (41). Osteonectin is a glycoprotein that is found in the extracellular matrix of numerous tissues such as bone, cartilage, and connective tissues. It is essential for tissue remodeling, cell-matrix interactions, and cell behavior modulation. As a result, it is frequently employed to identify and investigate bone regrowth. When osteonectin antibody is given to tissue sections, it binds to osteonectin if it is present. A chromogenic or fluorescent marker is then used to visualize the attached antibody, allowing researchers to establish the location and distribution of osteonectin inside the tissue (42).

Regarding the histologic evaluation, inflammatory cell count in the three experimental materials at both time periods showed that, the highest amount of inflammatory cell count was at the positive control group due to presence of microorganisms in furcation site and its direct contact with the oral cavity (13). On the other hand, MTA Angelus®, NeoMTA™ Plus® and Nano-WMTA showed statistically significant lower amount of inflammatory cell count than the positive control due to good sealing ability, biocompatibility, alkaline pH on setting (15, 17), and the closure of the access cavity that prevents further infection at the furcation perforation (6). The three experimental groups had no statistically significant difference in the inflammatory cell count; this is probably due to the high similarity in the chemical composition between the materials.

Regarding the effect of time periods on inflammatory cell count, for all tested materials, a higher inflammatory reaction was found in the one-month period and decreased at three months. This might be attributed to the time factor that when increased, the inflammatory reaction decreased upon the application of the biocompatible materials. Similar findings were reported by earlier authors (1, 37, 43, 44).

Results of immunohistochemical analysis showed that, positive control subgroup recorded significantly lowest mean value with no scores for new hard tissue formation at both time periods. This was due to persistence of chronic inflammation throughout the periods of the study and continued release of inflammatory mediators which prevent regeneration ability of the tissues. Similar finding was recorded before (1).

There were no statistically significant differences in new hard tissue formation between the three experimental materials within both groups (13). Results showed that after three-month evaluation period, there were statistically significant high scores of hard tissue formation in all experimental subgroups. This could be explained by the sealing ability, biocompatibility and alkaline pH on setting that accelerates new hard tissue formation (45). Calcium ions produced by the three materials may be the source of calcification, since they can react with phosphate ions in tissue fluids, resulting in the production of an amorphous calcium-phosphate layer, which is a precursor to calcification, and the formation of hard tissue barriers. The one-month evaluation period yielded the lowest grade for hard tissue development. This is due to the increased inflammatory response during this time period. This is consistent with past research (1-3, 46).

Conclusions

The Nano-White MTA, NeoMTA™ Plus® and MTA Angelus® were able to reduce inflammation and induce new hard tissue formation over time. Although Nano-WMTA exhibited lower inflammatory cell count and more new bone formation values than NeoMTA™ Plus® and MTA Angelus®, there were no statistically significant differences between the tested materials in terms of inflammatory cell count and new bone formation.

Clinical Relevance

Nano-WMTA can alternate both NeoMTA™ Plus® and MTA Angelus® when used as a root repair material.

Conflict of Interest

There are no conflicts of interest.

References

1 *Badr M, Abu-Seid AM, Hashem AA, El Ashry SH. Immunohistochemical analysis of Biodentine versus MTA in repair of furcation perforation: an animal study. G Ital Endod. 2023; 37(1): 85-93. doi: 10.32067GIE.2023.37.01.09.*

2 *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 Pathol. 2022; 103(4): 156-63. doi: 10.1111/iep.12439.*

3 *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. doi: 10.1016/j.sdentj.2018.08.001.*

4 *Özçopur B, Akman, M, Hakkı, S, Belli S. Quality and leakage of perforation repair-materials: A comparison of intracoronal and retrograde techniques. Selcuk Dent J. 2014; 3: 92-100.*

5 *Parirokh M, Torabinejad M. Mineral trioxide aggregate: a comprehensive literature review–Part III: Clinical applications, drawbacks, and mechanism of action. J Endod. 2010; 36 (3): 400–13. doi: 10.1016/j.joen.2009.09.009.*

6 *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: 148-56.*

7 *Agnes A, Long A, Best S, Lobner D. Pulp capping materials alter the toxicity and oxidative stress induced by composite resins in dental pulp culture. Eur Endod J. 2017; 2(1):1-6. doi: 10.5152/eej.2017.17001.*

8 *Spagnuolo G, De Luca I, Iaculli F, Barbato E, Valletta A, Calarco A, Valentino A, Riccitiello F. Regeneration of dentin-pulp complex: Effect of calcium-based materials on hDPSCs differentiation and gene expression. Dent Mater. 2023; 39(5):485-91. doi: 10.1016/j.dental.2023.03.017.*

9 *Arvelaiz C, Fernandes A, Graterol V, Gomez K, Gomez-Sosa JF, Caviedes-Bucheli J, Guilarte C. In vitro comparison of MTA and BC RRM-Fast set putty as retrograde filling materials. Eur Endod J. 2022; 7(3):203-9. doi: 10.14744/eej.2022.36036.*

10 *Mir A, Misgar OH, Farooq PR, Purra AR, Ahanger FA. Comparison of sealing ability of biodentine, bioactive bone cement and MTA as furcation repair materials. IOSR- J Dent Med Sci. 2017; 16 912): 82-6 (2017). DOI: 10.9790/0853-1612048286.*

11 *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: 321-7. doi: 10.1016/j.etp.2016.03.004.*

12 *Nikoloudaki GE, Kontogiannis T, Meliou HA, Kerezoudis NP. A comparative in-vitro study of sealing ability of four different materials used in furcation perforation. Open J Stomatol. 2014; 4: 402-11. DOI: 10.4236/ojst.2014.48054.*

13 *Walsh RM, Woodmansey KF, He J, Svoboda KK, Primus CM, Opperman LA. Histology of NeoMTA Plus and Quick-Set2 in contact with pulp and periradicular tissues in a canine model. J Endod. 2018; 44: 1389-95. doi: 10.1016/j.joen.2018.05.001.*

14 *Quintana, RM, Jardine AP, Grechi TR, Grazziotin-Soares R, Ardenghi DM, Scarparo RK, Grecca FS, Kopper PM. Bone tissue reaction, setting time,*

solubility, and pH of root repair materials. Clin Oral Investig. 2019; 23: 1359-66. doi: 10.1007/s00784-018-2564-1.
15 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. doi: 10.1186/s12903-021-01552-w.
16 Saghiri MA, Asatourian A, Orangi J, Lotfi M, Soukup JW, Garcia-Godoy F, Sheibani N. Effect of particle size on calcium release and elevation of pH of endodontic cements. Dent Traumatol. 2015; 31(3): 196-201.
17 Mammen J. Shetty HS, Jayasheelan N. Nano-White MTA: A review. Int J Adv Res. 2018; 6: 1564-71. DOI: 10.21474/IJAR01/6592.
18 Alazrag MA, Abu-Seida AM, El-Batouty KM, El Ashry SH. Adaptability, solubility and biocompatibility of TheraCal LC compared with MTA-Angelus and Biodentine as a furcation perforation repair material. BMC Oral Health. 2020; 20: 298. doi: 10.1186/s12903-020-01289-y.
19 Askoura AM, Elgendy AA, Khalaf AH. Evaluation of sealing ability of Nano-Hydroxyapatite and Nano-MTA as perforation repair material: (In-vitro study). J Funda Clin Res. 2022; 2: 129-40.
20 Abu-Seida AM. Efficacy of diclofenac sodium, either alone or together with cefotaxime sodium, for control of postoperative pain, in dogs undergoing ovariohysterectomy. Asian J Anim Vet Adv. 2012; 7:180-6.
21 Salman MA, Quinn F, Dermody J, Hussey D, Claffey N. Histological evaluation of repair using a bioresorbable membrane beneath a resin-modified glass ionomer after mechanical furcation perforation in dogs' teeth. J Endod. 1999; 25: 181-6. doi: 10.1016/S0099-2399(99)80138-1.
22 Alhadainy HA, Himel VT, Lee WB, Elbaghda YM. 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: 723-9. doi: 10.1016/s1079-2104(98)90211-6.
23 Mitthra S, Karthikeyan K, Shobhana R, Mahalaxmi S. Mid-root perforation repair with Mineral Trioxide Aggregate and andodontic rehabilitation with fibre reinforced post- A case report. J. Posit Sch Psychol. 2022; 641-9.
24 Mohamed M, Hashem AA, Obeid MF, Abu-Seida A. Histopathological and immunohistochemical profiles of pulp tissues in immature dogs' teeth to two recently introduced pulpotomy materials. Clin Oral Investig. 2023; 27(6): 3095-103. doi: 10.1007/s00784-023-04915-5.
25 Gonçalves LA, Moreira GE, Casque KC, Frozoni MR, Amaral FL. Chemo-mechanical properties of the new bioceramic cement PBS CIMMO® compared to MTA REPAIR HP Angelus® cement. J Dent Heal Oral Disord Ther. 2022; 13: 109-13. DOI: 10.15406/jdhodt.2022.13.00583.
26 El-Bialy MM, Ali MM, Kataia EM, El Nemr RS, Hassan R. Comparison of biocompatibility of experimental tricalcium phosphate cement versus biodentin and mineral trioxide aggregate used for furcation perforation repair (in vivo study). Open Dent J. 2021; 15: 532-8. DOI: 10.2174/1874210602115010532.
27 Seif H, Elbanna A, Abu-Seida AM, El-Korashy DI. Regenerative potential of a novel Aloe vera modified tricalcium silicate cement as a pulp capping material: an animal study. Dent Mat J. 2023; 42 (6): doi:10.4012/dmj.2023-129.
28 Gonzalez LC, Vargas-Sanchez PK, Bombonato-Prado FK, Delgado-Mejia E, Rodriguez CT. Response of non-differentiated pulp cells (OD-21) to a novel bioceramic for dental pulp capping. G Ital Endod. 2023; 37(1): 28-36. https://doi.org/10.32067/GIE.2023.37.01.12.
29 Debelian G, Trope M. The use of premixed bioceramic materials in endodontics. G Ital Endod. 2016; 30 (2): 70-80. http://dx.doi.org/10.1016/j.gien.2016.09.001.
30 Zamparini F, Prati C, Taddei P, Spinelli A, Di Foggia M, Gandolfi MG. Chemical-physical properties and bioactivity of new premixed calcium silicate-bioceramic root canal sealers. Int J Mol Sci. 2022; 23(22):13914. doi: 10.3390/ijms232213914.
31 Alghamdi F, Biology O. The biological effect of the endodontic bioactive cements fast set NeoMTA plus and ProRoot-MTA on osteogenic differentiation of mesenchymal stem cells: A systematic review. Int Dent J Student Res. 2019; 7: 24-8.
32 Kunjan Anju P, Purayil TP, Ginjupalli K, Ballal NV. Effect of chelating agents on push-out bond strength of NeoMTA Plus to root canal dentin. Pesqui Bras Odontopediatria Clin Integr. 2022; 22: 210058. doi.org/10.1590/pboci.2022.005.
33 Antonijević D, Despotović A, Bioč anin V, Miloš ević M, Trišić D, Lazović V, Zogović N, Milašin J, Ilić D. Influence of the addition of different radiopacifiers and bioactive nano-hydroxyapatite on physicochemical and biological properties of calcium silicate based endodontic ceramic. Ceram Int. 2021; 47(20): 28913-23. https://doi.org/10.1016/j.ceramint.2021.07.052.
34 Carvalho P, Duarte T. Biocompatibility and biomineralization of the experimental nanoparticulate mineral trioxide aggregate (MTA). Res Soc Devel. 2021; 10 (5): e27710514866. DOI: http://dx.doi.org/10.33448/rsd-v10i5.14866.
35 Tawfiq N, Mohamed Z, Mahran AH, Elsewefy T. Effect of Neo-MTA and hydroxyapatite nano-particles on proliferation and osteogenic differentiation of apical papilla stem cells (Scaps) (in Vitro Study). J Pharmaceut Negat Resu. 2023; 14: 2882-907. DOI: https://doi.org/10.47750/pnr.2023.14.02.344.
36 Roberts HW, Toth JM, Berzins DW, Charlton DG. Mineral trioxide aggregate material use in endodontic treatment: A review of the literature. Dent Mater. 2008; 24: 149-64. doi: 10.1016/j.dental.2007.04.007.
37 Rafter M, Baker M, Alves M, Daniel J, Remeikis N. Evaluation of healing with use of an internal matrix to repair furcation perforations. Int Endod J. 2002; 35: 775-83. doi: 10.1046/j.1365-2591.2002.00566.x.
38 Gomes-Filho JE. Rodrigues G, Watanabe S, Bernabé PFE, Lodi CS, Gomes AC, Faria MD, Dos Santos AD, Moraes JCS. Evaluation of the tissue reaction to fast endodontic cement (CER) and Angelus MTA. J Endod. 2009; 35: 1377-80. doi: 10.1016/j.joen.2009.06.010.
39 El-Tahan AI, Tewfik HM, Diab AH, Bayoumi AA. Seal-

ing ability and dislodgement resistance of three materials for the repair of furcation perforation (In-vitro Study). J Fundam Clin Res. 2021; 1 (2): 121-37. DOI: 10.21608/JFCR.2021 .211107.

40 Bakhtiar H, Mirzaei H, Bagheri MR, Fani N, Mashhadiabbas F, Baghaban Eslaminejad M, Sharifi D, Nekoofar MH, Pmh Dummer. Histologic tissue response to furcation perforation repair using mineral trioxide aggregate or dental pulp stem cells loaded onto treated dentin matrix or tricalcium phosphate. Clin Oral Investig. 2017; 21 (5): 1579-88. doi: 10.1007/s00784-016-1967-0.

41 Ramli R, Mohd Naf SN, Ahmad Tarmidzi NA, Hasbullah N, Ghani N. Immunohistochemistry as a detection tool for ion channels involved in dental pain signaling. Saudi Dent J. 2022; 34: 155-66. doi: 10.1016/j.sdentj.2022.02.004.

42 Schulz A, Jundt G. Immunohistological demonstration of osteonectin in normal bone tissue and in bone tumors. Curr Top Pathol. 1989; 80: 31-54. doi: 10.1007/978-3-642-74462-4_2.

43 Broon NJ, Bramante CM, de Assis GF, Bortoluzzi EA, Bernardineli N, de Moraes IG, Garcia RB. Healing of root perforations treated with Mineral Trioxide Aggregate (MTA) and Portland cement. J Appl Oral Sci. 2006; 14: 305-11. doi: 10.1590/s1678-77572006000500002.

44 Holland R. Ferreira LB, de Souza V, Filho JAO, Murata SS, Dezan Jr E. Reaction of the lateral periodontium of dogs' teeth to contaminated and noncontaminated perforations filled with mineral trioxide aggregate. J Endod. 2007; 33 (10): 1192-7. doi: 10.1016/j.joen.2007.07.013.

45 Siboni F, Taddei P, Prati C, Gandolfi MG. Properties of NeoMTA plus and MTA plus cements for endodontics. Int Endod J. 2017; 50: e83–e94. doi: 10.1111/iej.12787.

46 Al-Daafas A, Al-Nazhan S. Histological evaluation of contaminated furcal perforation in dogs' teeth repaired by MTA with or without internal matrix. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007; 103(3): e92-9. DOI: 10.1016/j.tripleo.2006.09.007.