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ASSESSMENT OF SEALING ABILITY OF DIFFERENT BIOCERAMICS IN REPAIR OF FURCATION PERFORATION: AN IN VITRO STUDY

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ABSTRACT

Introduction: The objective of this study was to evaluate and compare the sealing ability of MTA, Nano-MTA and Biodentine in the repair of furcation perforation by using bacterial leakage test.

Methods and Materials: Standard access cavities and furcal perforations were made in fifty extracted mandibular permanent molars. The teeth were randomly divided into five groups. Group 1: perforations were repaired with MTA, Group 2: perforations were repaired with Nano-MTA and Group 3: perforations were repaired with Biodentine. Ten teeth with unrepaired perforations were used as positive controls and ten teeth without perforations were used as negative controls. The sealing ability was evaluated using Enterococcus faecalis Bacterial leakage test. The samples were observed for 45 days for turbidity using spectrophotometer.

Results: High statistically significant difference in leakage was found between the three materials when used as furcation perforation repair materials. Nano-MTA presented the best sealing ability with the least bacterial leakage followed by MTA, while Biodentine showed maximum bacterial leakage.

Conclusion: Nano- MTA has better sealing ability than MTA and Biodentine and can be used in repair of furcation perforation.

KEYWORDS: Sealing ability, Bacterial leakage, MTA, Nano-MTA, Biodentine.

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INTRODUCTION

Perforation can be defined as pathological or mechanical communication between the root canal space and the surrounding periodontal tissues. Many etiological factors can contribute to such process, including pathological process via tooth decay, external or internal resorption, and iatrogenic causes during endodontic treatment of the teeth or intra-canal post placement. Root perforation and/or furcal perforation may lead to leakage of bacteria and their products to the periodontal and periapical tissues of the tooth. Thus, causing swelling, pain and failure of endodontic treatment. [1]

The prognosis of this complication depends on multiple factors such as size and/or location of the perforation, time-delay before perforation repair, prior contamination by microorganism, periodontium status and the sealing ability of the repair material. It has been proposed that the most favorable prognosis seems to have non-surgical repair through coronal access instantly after perforation, this will limit the contamination with micro-organism and inflammation of the area. [2]

The ideal material to be used in repair of perforation must be non-absorbable, biocompatible, radio-opaque, bactericidal or bacteriostatic. It also should induce the formation of cementum and formation of bone. The material should be dimensionally stable and tightly close the perforation against any leakage. It must also be degraded entirely during the process of repair to let new healthy bone replacement and act as barrier to which the obturation material can be placed.[3]

A lot of materials have been suggested for the repair of perforation this include amalgam, calcium hydroxide, glass ionomer, zinc phosphate cement, CEM, composite resin. None of these materials could fulfill the requirements of the most favorable repair material. [3,4]

Mineral trioxide Aggregate (MTA) was introduced by Torabinejad M in the 1990s. It has been recommended by most clinician because of its sealing ability,[5] marginal adaptation,[5,6] Biocompatibility [5] and antimicrobial action.[5,7] It has the ability to be set in the presence of moisture and blood contamination. MTA presents the disadvantages of prolonged setting time (3-4 hours) and complicated handling (depending on water/ powder ratio). [8]

Biodentine was introduced to the market to overcome these drawbacks. It appears to have comparable mechanical, physiochemical, biological properties to MTA but with decreased setting time (9-12 minutes). [9]

Nanotechnology has been used in many applications involving dentistry. It can be used in endodontics to improve the mechanical properties of materials used in root canal treatment. Moreover, it has been used to decrease the setting time of endodontic materials. [10]

Therefore, recently, researches are still carried out to develop endodontic repair materials that overcome the short coming of the current materials. Many attempts are being made to decrease the particle size of the MTA to the nano-scales and adding on some additives to this structure resulting in the formation of what's called Nano-white mineral trioxide aggregate (Nano-WMTA).[11] Because of the insufficient data in literature about its sealing ability, this study was conducted to compare the newly presented Nano-MTA with Biodentine and MTA when used as furcation perforation repair material. The null hypothesis is that there is no difference between these materials in sealing ability.

MATERIALS AND METHODS

Extracted teeth preparations

Fifty extracted mandibular permanent molar teeth with intact furcation were included in this study (from the oral surgery department at the faculty of dentistry Ain Shams University). The selected teeth were free from cracks and had fully developed roots. Any tooth with fused roots, root caries or root defects was excluded. The surfaces of all the samples were cleaned to eliminate any attached debris. All the samples were disinfected by soaking them in 5.25% sodium hypochlorite for 10 min. Then, they were rinsed and kept in daily-changed normal saline solution until they were used.

The standard access cavity was made in the fifty extracted mandibular permanent molar teeth using high-speed handpiece with water coolant. Iatrogenic furcal perforations were made in the center of the pulp chamber floor in forty teeth using round bur size #4 in high speed with water coolant. This was used to create a perforation with 1.4 mm except for the -ve control group which no perforation was done.[12]

3 mm from the root end were cut using diamond disc on a straight handpiece.[3] All the canal orifices and root ends were acid etched with 37% phosphoric acid gel (3M ESPE Dental Products, St. Paul, MN, USA) for 30 seconds. Then washed and dried. Two coats of bond (3M ESPE Dental Products, St. Paul,MN, USA) were applied on the teeth and cured using a light curing device for 20 seconds. The canal orifices and root ends were sealed with FiltekTM Z350 XT flowable composite (3M ESPE, USA). It was cured for 40 seconds according to manufacturer instructions.[3,13] Two layers of nail varnish were used to coat the entire tooth except the perforation area to avoid leakage of bacteria to any open dentinal tubule or accessory/lateral canal. Each coat of nail varnish was permitted to dry before the next coat was applied.[2] The samples used in this study (n=50) were classified into five groups according to the type of repair material used. Group 1 (n=10): Perforations were repaired with MTA, Group 2 (n=10): Perforations were repaired with Nano MTA, Group 3 (n=10): Perforations were repaired with Biodentine, Group 4 (n=10): Perforations were left unrepaired (positive control) and Group 5 (n=10): Without Perforation (negative control). All the materials were mixed according to the manufacturer's instructions. The materials were applied into perforation site using MTA carrier and then compacted gently by a plugger size 1.2 mm to ensure complete filling of the perforation site.

Method of evaluation:

The sealing ability was assessed using Enterococcus faecalis microbial leakage test.[14]

Preparation of the bacterial strain:

The bacterial strain, Enterococcus faecalis (ATCC 29212) from the microbiology laboratory * was used for biofilm formation. The bacterial strain was prepared by growing on the brain heart infusion medium for 24 hours at 37°C.

Preparation of the specimens:

Using a sterile disposable micropipette, 100μL of the culture suspension (10^6 cells/mL) was placed in the pulp chamber of each tooth every 48 hours. All the tested specimens were hanged in a broth until all the furcation area was covered by the medium by using orthodontic wire. Before fresh inoculum was placed, the existing inoculum was aspirated and randomly plated on Mac Conkey's medium to assess the viability of the bacteria and to detect contamination.

The samples were observed for **45** days for bacterial leakage in terms of:

1- Every three days, a loop of turbid broth 10μL from each tube was inoculated into Mac Conkey's medium and incubated at 37°C overnight. On the next day, the culture plates were checked for growth (viability of bacteria) and observed under the microscope.

2- Every three days, the broth turbidity (absorbance) was measured by spectrophotometer (Turbidimetric assay).

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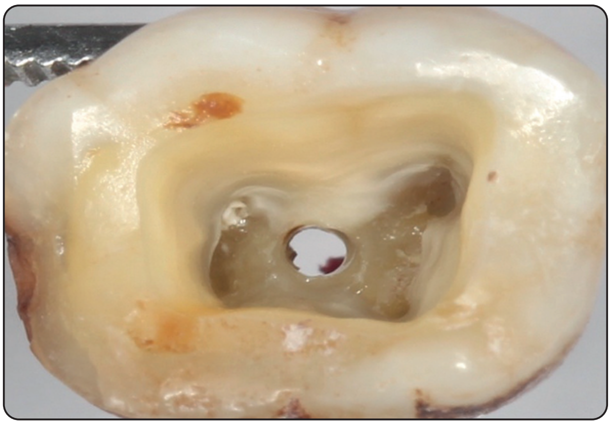

Fig. (1) Shows Standard access cavity with furcation perforation

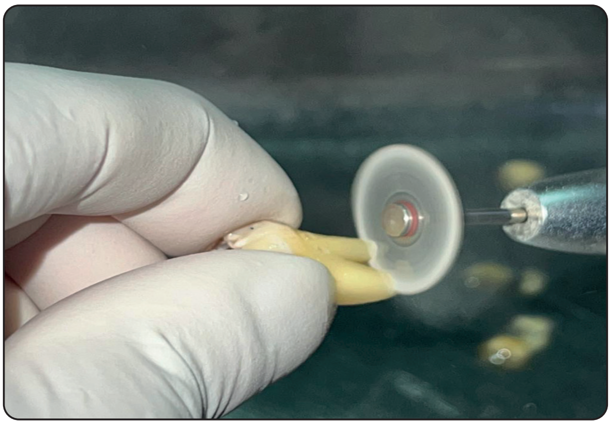

Fig. (2) Shows Root end prepararion of the specimen

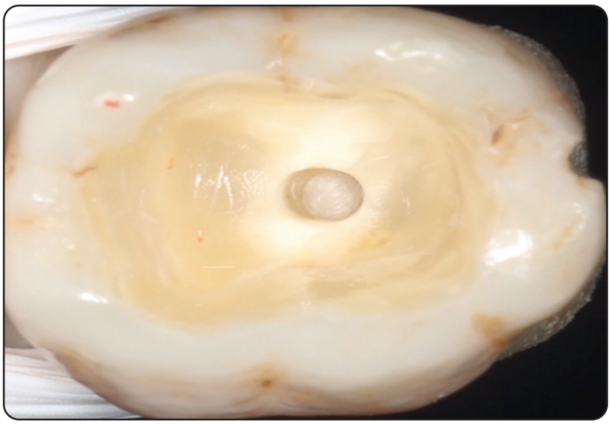

Fig. (3) Showing all the canals were sealed with flowable composite

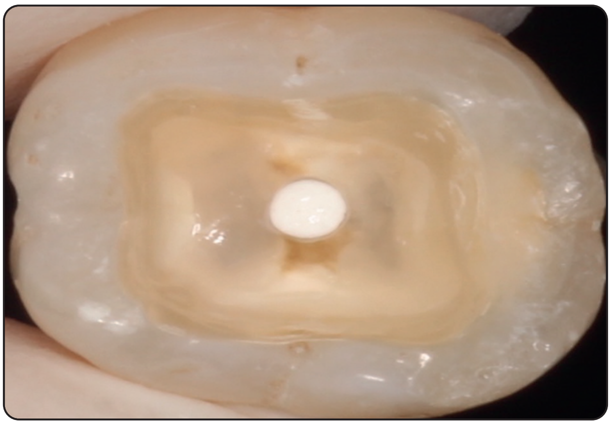

Fig. (4) Photograph showing application of the material

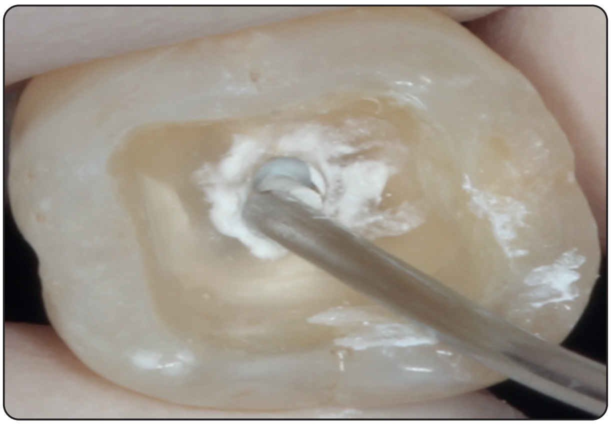

Fig. (5) Showing the material after condensation with plugger from coronal aspect

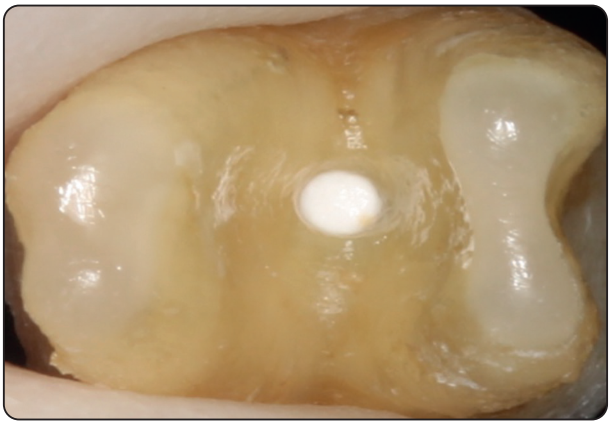

Fig. (6) Showing filling of the furcation perforation with test material and root end with flowable composite

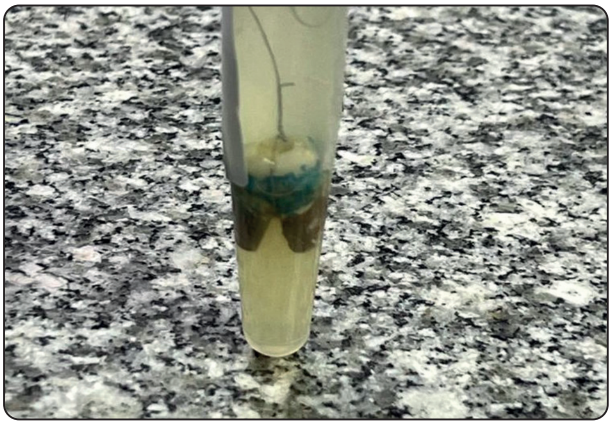

Fig. (7) Shows specimen hanged in broth using orthodontic Wire

Microleakage between different groups were compared by analyzing the data statistically. One-Way ANOVA test was used for statistical analysis and Tukey's test was used in case of significance. Statistical significance was considered when $P< 0.05$. Statistical analysis was made with SPSS 20 (Statistical Package for Social Science, IBM, NY, USA).

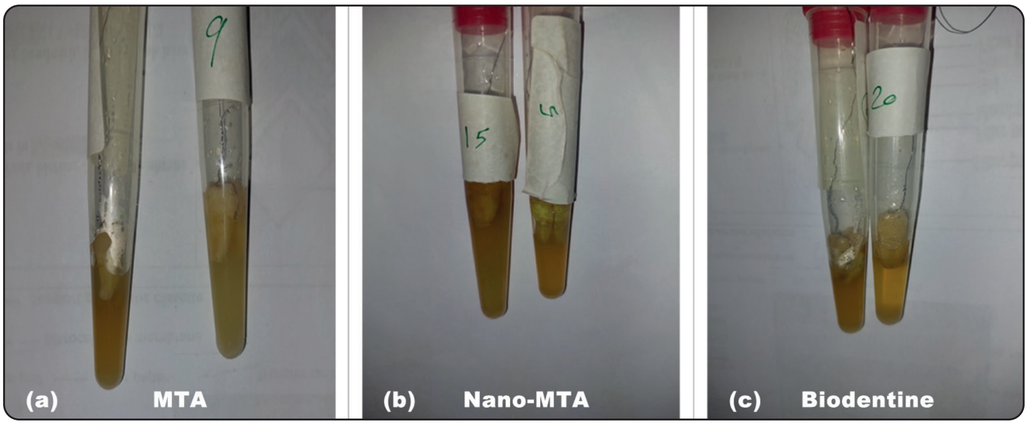

Fig. (8) a) image shows 1st day of turbidity in MTA. b) image shows 1st day of turbidity in Nano-MTA. c) image shows 1st day of turbidity in Biodentine.

RESULTS

Our results revealed that Nano-MTA presented the best sealing ability with the least bacterial leakage, showing a change in turbidity at day 28, compared to 33 days in the negative control group followed by MTA. While Biodentine was the earliest material showing bacterial leakage at day 19, as listed in table (1).

Comparative analysis between the mean value for the day of starting bacterial leakage in the five tested groups as detected by broth turbidity was performed using one-way ANOVA test which revealed a high significant difference between the sealing ability of the three tested materials compared to the unperforated teeth (negative control) ($p<0.0001$). Furthermore, a Tukey's test was conducted to detect if there was a significant difference between the sealing ability of each material compared to unperforated teeth (NC), unsealed perforated teeth (PC) as well as other tested materials. A high significant difference was detected for the day at which leakage start between teeth sealed with Nano.MTA, MTA and Biodentin, compared to unperforated teeth (NC) and unsealed perforated teeth (PC), P-value <0.0001.

TABLE (1) Mean and standard deviation values for change in turbidity as measured in days of MTA, Nano-MTA, Biodentine, PC and NC:

	Mean±SD
Group 1: MTA	23.4[a] ±1.52
Group 2: Nano-MTA	28.6[b]±1.14
Group 3: Biodentine	19.2[c] ±1.30
Group 4: PC	2.4[d] ±1.14
Group 5: NC	33.0[e] ±1.58
P-value	<0.0001*

Means with different superscript letter in the same column were significantly different

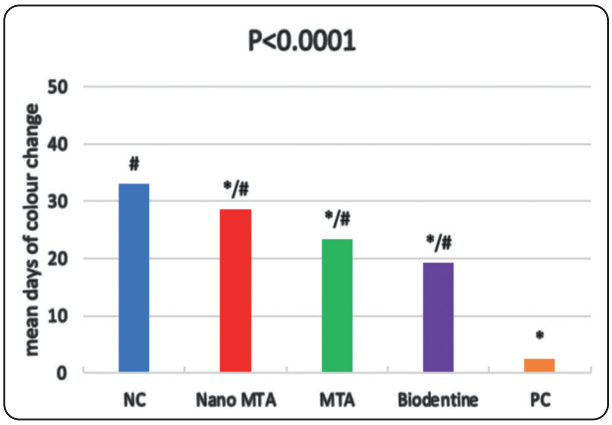

Figure (9) Bar chart showed a significant difference between the changes in turbidity of broth in teeth sealed with the three tested materials. *: significant difference compared to negative control, #: significant difference compared to the positive control group.

DISCUSSION

Perforation is the second most reported cause for Endodontic failure, accounting for 9.6% of all Endodontic failures. [2] Multiple factors influence the success and long-term prognosis of furcation perforation such as the size, location of the perforation, time-delay between the occurrence of the perforation and repair, and the ability of the material to seal perforation.[3] Therefore, A suitable furcation perforation material should be used to reduce irritation and improve PDL (periodontal ligament) attachment. This leads to the present study's aim of evaluating newer bioceramic perforation repair materials.

MTA was used in this study since it was approved as a gold standard repairing material [12,15]. MTA has several favorable properties such as biocompatibility, excellent sealing ability, antibacterial activity, radio-opacity and insolubility in fluids. Moreover, it can produce hard tissue formation. Nevertheless, it has many disadvantages such as difficult manipulation, discoloration and long setting time.[16]

Biodentin is a new bioactive calcium silicate-based cement that has been introduced to the dental market as a 'dentin substitute'. It was presented by Gilles and Olivier in 2010. It has decreased setting time, good compressive strength, easy handling and it's cost-effective. But it has lots of disadvantages such as reduced radio-opacity, low wear resistance and poor flexural strength when compared with MTA.[17]

Nanotechnology has been introduced to the dental field, presenting nano-sized materials with enhanced physicochemical and biological properties [12,18]. The manufacturers state that it has comparable composition of MTA. But it has reduced particle size to gain increased specific powder surface area that gives rise to achieve enhanced and quicker hydration process. It's assumed that in mixing phase, the number of particles that are involved in the reaction in the hydration phase is increased. This leads to formation of a material with less porosity. [19] Komabayashi and Spangberg [20] stated that particle size of MTAhas a huge influence on the extent of particles that penetrate the dentinal tubules. Because of the insufficient data in literature about its sealing ability, this study was conducted to compare the newly presented Nano-MTA with Biodentine and MTA.

In the present study, sealing ability was tested using bacterial leakage test. This is because microbiological investigations are more clinically based methods for evaluating leakage. They give more reliable results.[14] All peri-radicular pathosis is associated with bacterial leakage and the majority of bacteria that cause endodontic infections are anaerobes. [2, 21] *Entero- coccus faecalis* (ATCC-29212) was selected as a commonly adopted model to be used in bacterial leakage methods. [22] Using one species of bacteria may be considered as a disadvantage of the test. This may be because of the wide variation of bacteria exists in root canal structure. But the purpose of this choice was to offer standardization among the groups.

Three-millimeter from root end was cut since most of the lateral/accessory canals and ramifications present in this area. This was done to avoid leakage of bacteria to these regions and confine bacteria to furcation area only. [3] Two layers of nail varnish was used to coat the entire tooth except the perforation area. This was done to avoid leakage of bacteria to any open dentinal tubules or accessory/lateral canals.

Nano-MTA showed the best sealing ability with the least bacterial leakage when compared with MTA and Biodentine.This could be due to the small particle size of Nano-MTA.

These results were different from previous study done by Askoura et al. [12] who reported that sealing ability between Nano-MTA and MTA was insignificantly different when used as furcation perforation repair material. The difference between our results and other controversial results is probably due to difference in method of evaluation, sample preparation and the materials used.

According to the results of this study, The positive control group showed the maximum bacterial leakage of all the samples and the negative control group showed the least bacterial leakage which indicated the method's validity. This in agreement with Shaheen et al.[3] and Bellam et al.[14]. Nano-MTA showed better sealing ability with least bacterial leakage when compared to MTA and Biodentine. This was in agreement with Askoura et al. [12] who stated that Nano-MTA has better sealing ability when compared with Nano-hydroxyapatite. This may be attributed to the particle size of Nano-MTA. Several researches proved that smaller particle size can reduce the space between particles, increase surface area and better interlocking of powder particles to enhance integrity, making the material more resistant to liquid penetration [19,,23]

MTA showed better sealing ability than Biodentine. This was in agreement with Kamal et al. [24]. This may be because MTA is hydrophilic and undergoes expansion during setting. Moreover, formation of an interfacial layer (mineral infiltration zone) between the material and dentin which supports its adaptation to dentin. [19] MTA binds chemically to the dentine wall. Calcium hydroxide that is released from the reaction will react with phosphate ions. A hydroxyapatite-like precipitate (amorphous calcium phosphates) will be produced [25] and bind to dentin structure. It was reported that the additional hydration of MTA powder by moisture can cause an increase in the compressive strength and decrease leakage. [19]

On the other hand, findings by Francis et al. [2], reported that Biodentine had superior sealing ability than MTA. This may be owing to the presence of fast setting time (9-12 min) of Biodentine, due to presence of calcium chloride which acts as a reaction accelerator. The fast-setting time of Biodentin leads to less bacterial contamination and less material loss in the interface during the final processing stages which makes the crystals in Biodentine are more stably linked to the dentine surface.[9] The difference in the reported outcomes between the previously mentioned studies may be attributed to the heterogenicity noted between them in terms of methodological differences and lack of standardized tools of assessment.

CONCLUSION

Under the circumstances of this study, it can be concluded that:

1- Conventional MTA remains the material of choice for repair of furcation perforation.

2- Reducing the particle size of Nano-MTA has played an important role in enhancing its sealing ability

3- Nano-MTA showed promising results when compared with MTA and Biodentine in repair of furcation perforation.

Further research needs to be carried out to evaluate sealing ability of Nano-MTA in repair of furrcation perforation.

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