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Evaluation of Sealing Ability of Nano-Hydroxyapatite and Nano-MTA as Perforation Repair Material: (In-Vitro Study)

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ABSTRACT

Background: Furcal perforation is one of the reasons for endodontic treatment failure, and repairing it is a challenge. Studying new repairing materials, as opposed to conventional White - Mineral Trioxide Aggregate (MTA) contributes to the success of the endodontic treatment. ***Objectives:*** to evaluate the sealing ability of nano-hydroxyapatite and nano-MTA as a repair material for bifurcation perforation compared to conventional MTA. ***Materials and methods:*** Fifty extracted human mandibular lower permanent molars with intact furcation area were randomly distributed into five groups: negative control group without perforation (n=10), positive control group where the perforation is unrepaired (n=10), and three groups according to the perforation repair material used with (n=10 for each): Group A: nano-sized Hydroxyapatite, Group B: nano-sized MTA and Group C: conventional MTA (ProRoot MTA). Perforation was created in the bifurcation area, then repaired using the three materials. Dye extraction method with spectrophotometer UV used for sealing ability evaluation. ***Results:*** there is a statistically significant difference in results between all repair materials used. Conclusion: Nano-MTA showed promising results in comparison with conventional MTA (ProRoot MTA).

Keywords: nano-hydroxyapatite, nano-MTA, perforation repair, bifurcation area, sealing ability.

INTRODUCTION

The objective of endodontic treatment is to disinfect and isolate the root canal system from the surrounding periradicular tissues and the oral environment. Accidental events such as iatrogenic perforation, which is defined as an artificial communication between the pulp space and the supporting tissues of the tooth or oral cavity, can compromise the objective and prognosis of endodontic treatment.[1] The incidence probability of this event is 2-12% of endodontic-treatment cases.[2] It is the pulpal floor forms a natural barrier between the periodontium and the pulp space. Therefore, perforation of the bifurcation area becomes a challenging treatment location as it is

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communicating area with the coronal part of the tooth, the periodontal tissues (PDL and bone), and the pulp as well as is considered a stress-bearing area. The prognosis of this perforation depends on many factors such as the time of treatment, size, and shape of perforation, and the material used in repair.[3]

Many materials have been used as perforation repair materials in literature: Indium foil, zinc oxide eugenol, calcium hydroxide, zinc oxide eugenol cement reinforced with ethoxy benzoic acid (Super EBA), Cavit, amalgam, glass ionomer, Metal-Modified Glass ionomer Cement, IRM, Portland Cement, Gutta Percha composite resin, Decalcified Freezed Dried Bone (DFDB) mineral trioxide aggregate (MTA) Calcium Phosphate Cement (CPC), Tricalcium Phosphate, and calcium-enriched mixture (CEM) cement.[4]

Mineral Trioxide Aggregate (MTA) has become the gold standard and an ideal choice as a root repair material. It is composed of calcium, silicon, and aluminum, with the addition of bismuth oxide, added for radiograph detection. The key entity phases are tricalcium and dicalcium silicate and tricalcium aluminate.[5] Its main physical properties offer biocompatibility, dimensional stability, and tolerance to moisture. It can stimulate osteogenesis and cementogenesis – being both, an osteoinductive agent and an osteoconductive agent. Finally, its initial pH is 10.2 which increases to 12.5 after 3 hours of mixing which increases its antimicrobial effect.[6]

Hydroxyapatite, on the other hand, is the main component of the enamel and bone; most biomaterial studies proved its biocompatibility, remineralization of the enamel, and stimulation of osteogenesis and cementogenesis.[7] Hydroxyapatite is used as an internal matrix to avoid extrusion of other repair material(s) and it showed stability as a matrix. It is also used as a direct perforation repair material to repair the furcation bone loss because of its conductivity of osteogenesis.[8]

The innovation of nanotechnology has been recently introduced to the dental field and thus will be relevantly applicable to the future of dental Nanomaterials. Advancements in nano-sized materials have contributed to the improvement of the physicochemical and biological properties of dental materials. Intrinsically, nano-sized materials have increased compressive strength, provide greater surface area, and fill the gaps of the micro-sized materials thus decreasing porosity.[9] Therefore, nano-sized particles of SiO2, TiO2, and Al2O3 have been incorporated in the composition of different dental materials, and have been introduced as nano-hybrid materials in the literature.[10,11] In contrast to the nano-hybrid materials which add heterogeneous nano-sized particles to existing materials, existing materials like bioceramics have been formed in a nano-sized form such as MTA and hydroxyapatite to improve their physicochemical properties. The sealing ability property resists the micro-leakage of fluids and penetration of bacteria into the material itself and to the surrounding tissues.[12] In this study, the sealing ability of the material will be evaluated with dye extraction using UV Spectrophotometer.

MATERIAL AND METHODS

Fifty sterilized extracted mandibular human permanent molars with minimal restoration, sound furcation area, and diverged roots were collected from Misr International University teeth bank and approved by the ethical committee (IRB 800010118). Teeth examined under magnification for cracks, vertical fracture, perforation or resorption to be excluded from selected teeth. Samples were randomly divided into:

Negative control group: (n=10 without perforation). Positive control group (10 samples unrepaired perforation).

Group A: (n=10 perforation repaired with nano-sized Hydroxyapatite).

Group B: (n=10 perforation repaired with nano-size MTA).

Group C: (n=10 perforation repaired with conventional MTA).

Teeth preparation:

All procedures have been done using Dental Surgical Microscopy with a magnification of 10X (M320, Leica microsystem, Germany). Calculus and soft tissue were removed from all collected teeth using ultrasonic scaler tip G8 (NSK, Japan). To facilitate preparation, teeth were decoronated and amputated 3mm below the furcation area using tapered diamond stone **(Figure 1)**.

Figure (1): Root amputation using tapered diamond stone.

Standard coronal access prepared using round bur size #4 (#4RC; SybronEndo Europe, The Netherlands) for initial entry followed by tapered diamond stone for lateral extension and finishing the walls, all pulp tissues removed, orifices located then irrigated with Sodium Hypochlorite (NaOCL) 2.5% and dried **(Figure 2)**.

Figure (2): Decoronation and access preparation.

All orifices and apically sealed with a temporary filling (Coltosol Coltene/whaledent AG, Altstatten, Switzerland) **(Figure 3)**.

Figure (3): Orifice and apex sealed with temporary filling.

Cavity walls, pulpal floor, and sealed orifices covered with two successive layers of nail varnish and allowed to air dry. A marker was used to locate a perforation at the center of the pulpal floor (furcation area) then, intentionally created the perforation with a long shank round bur size #4 with a high-speed headpiece in the middle of the bifurcation area perpendicular to the long axis of the tooth to create a perforation with a diameter of 1.4, except for negative control group samples (n=10) that are kept without perforation. Final irrigation with sodium hypochlorite and drying of the samples with a cotton pellet then molars divided into their groups.[13,14]

Perforation repair:

Perforations in this study were repaired and sealed using three different materials in powder liquid form to be mixed:

1.ProRoot white MTA (conventional MTA). (Dentsply Sirona, US)

2.Nano- MTA. (Nano Gate, Egypt)

3.Nano-hydroxyapatite. (Nano Gate, Egypt)

Using a surgical microscope (M320, Leica microsystem, Germany) with X16 magnification, each group was repaired with selected material as mentioned in sample classification. Each material was mixed with Manufacture instructions. The powder was mixed with distilled water on a glass slab in a 3:1 powder/water ratio; after 30 seconds of mixing the mixture exhibited putty-like consistency for the MTA and Nano-MTA. Mixing of 4:2 powder/liquid ratios for Nanohydroxyapatite a granular-like mixture occurred after 1 minute for both materials immediately after that it was placed into the perforation using the Microapical Placement System (MAP, Produits Dentaires SA, Vevey, Switzerland) and condensed with Buchanan pluggers followed by a wet cotton pellet. The excess material was removed gently by a sharp small excavator and sharp endodontic prob. A moistened cotton pellet was placed on the pulp chamber to avoid dehydration of the repair material, and samples were allowed to be set for 24 hours in 100% humidity.[13,14]

Sealing ability evaluation using UV spectrophotometric analysis:

Each group of samples were placed in silicon impression material (Zeta plus intro kit, Italy) mixed according to the manufacturer's instructions to obtain a matrix simulating the bony socket. Teeth are painted with 2% methylene blue that covers the entire pulp chamber using a micro-brush followed by their storage for 24 hours **(Figure 4)**. Teeth were rinsed from the dye with running water for 30 minutes to remove all residues of methylene blue. Varnish was removed with Parker blade #15 then, and Samples were stored in vials containing 1 ml of 65% nitric acid for 3 days **(Figure 5)**. The solution obtained was centrifuged at 14,000 for 5 minutes **(Figure 6)**. Two hundred

Figure (4): Methylene blue dying with micro-brush to teeth in silicon impression that simulate the bony socket.

Figure (5): Vials containing samples in 1 ml 65% nitric acid.

Figure (6): After 3 days solution centrifuge at 14,000 rpm for 5 min.

microliters of the supernatant liquid of each group were transferred to a cuvette (Cole-Parmer Fluorimeter cuvettes with a cover of PTFE resin; quartz) to be analyzed in a spectrophotometer (Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer; 115 VAC, Japan) at 550 nm wavelengths with the concentrated nitric acid as the blank and the reading will be measured in absorbance units **(Figure 7)**.[13,14]

Figure (7): Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer; 115 VAC, Japan.

Statistical analysis:

Numerical data were presented as mean and standard deviation values. They were explored for normality by checking the data distribution and by using the Shapiro-Wilk test. Data showed parametric distribution and were analyzed using one-way ANOVA followed by Tukey's post hoc test. The significance level was set at p ≤0.05 within all tests. Statistical analysis was performed with R statistical analysis software version 4.1.3 for Windows[1].

RESULTS

Mean and standard deviation (SD) values of sealing ability for different groups were presented in **table (1) and figure (8)**

Table (1): Mean ± standard deviation (SD) of sealing ability for different groups.

Sealing ability (mean±SD)					f-value	p-value
Group (A)	Group (B)	Group (C)	Positive control	Negative control		
0.20±0.08^{B}	0.21±0.06^{B}	0.13±0.04^{BC}	0.47±0.11^{A}	0.09±0.07^{C}	**38.11**	**<0.001***

**=* *significant at p ≤ 0.05.*

Means with different superscript letters within the same horizontal row are significantly different.

There was a significant difference between different groups (p<0.001). The highest value was found in the positive control group (0.47±0.11), followed by group (B) (0.21±0.06), then group (A) (0.20±0.08), and group (C) (0.13±0.04), while the lowest value was found in the negative control group (0.09±0.07). Post hoc pairwise comparisons showed the positive control groups to have significantly higher values than other groups (p<0.001). In addition, they showed groups (A) and (B) to

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[1] R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

have significantly higher values than the negative control group ($p<0.001$).

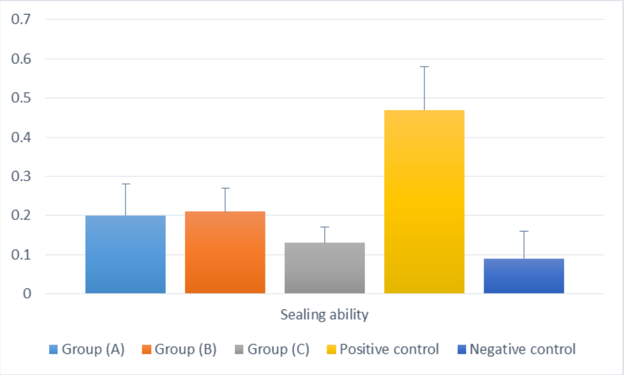

Figure (8): Bar chart showing average sealing ability for different groups.

DISCUSSION

Iatrogenic Perforation of the pulp chamber floor has been a concern for dentists for as long as endodontic procedures have been conducted. The most typical factors associated with perforation was the practitioner's expertise, the type of tooth, and the geometry of the tooth.[1] Perforations, the second most prevalent cause of failure correlated with endodontic treatment, are thought to account for up to 10% of root canal treatment failures.[2] If the perforation is not treated immediately and properly, the inflammatory reaction that ensues in the periodontium as a result of the perforation might result in tooth loss.[3] Several publications in the literature address perforation repair as well as a wide range of materials and methodology with varying degrees of effectiveness.[4,11,12,14] That leads to the aim of the present study of evaluating new perforation repair material. As MTA had been approved as gold standard repairing material, it has been used in this study to compare the evaluated materials.[5,6] Nanotechnology has been brought to the dental industry and introduced new nano-sized materials improving the physicochemical and biological properties.[9-11] Therefore, in this study Nano-MTA and Nano-hydroxyapatite have been chosen as perforation repair materials. The evaluation of perforation repair materials regarding their physical and biological properties of sealing ability, adaptability, radio-opacity, biocompatibility, and antibacterial action is crucial.[15,16] Sealing ability is considered an important property to be evaluated, whereas the success or failure of furcal perforation correction is achieved by isolating the communication between coronal and periradicular environments. The sealing ability property resists the micro-leakage of fluids and penetration of bacteria into the material itself and to the surrounding tissues.[12] To evaluate the micro-leakage of a material several methods used such as dye penetration[17,18] fluid filtration[19,20] bacterial leakage[21,22] and dye extraction. In the current study, the sealing ability of the material will be evaluated by dye extraction using UV Spectrophotometer.[13,14] In literature, the sealing ability of conventional MTA has been assessed.[14,24] However, nano-dental materials have been examined in previous works;[11,26-28] but under-examined for sealing ability this has been the main motive for their evaluation in the current study. Our contribution to this study is to introduce an accurate quantitative method of dye extraction with a Spectrophotometer UV) in evaluating two nano-sized particles of bioactive repair materials, (hydroxyapatite and MTA) against conventional MTA.

According to some research, the size of the tooth concerning the size of the perforation has a direct impact on the prognosis, whilst others found no correlation between the two variables.[29] In the current study, bur size #4 was used to induce a perforation with a defect of 2mm diameters into selected teeth. This was similar to Hashem et al[13] which allowed enough testing material to be examined. Another study (Hamad HA et al.),[30] on the other hand, used smaller-sized burs to create a small defect (1-1.5 mm) which creates small perforation with a small amount of material that should be tested.

The negative control group had low dye absorbance (0.09_+ 0.07) near the blank (nitric acid) which was 0.053. This little distinction is due to yellowish teeth tint while the blank is colorless. The positive control group with unrepaired perforation showed the maximum dye absorption of all samples indicating the technique's validity.

ProRoot MTA (conventional MTA) showed the lowest dye absorbance in comparison with Nano-MTA and Nano-Hydroxyapatite and the positive control group. The reason for this result is that it is hydrophilic and adapts easily to the dentine surface. This result is similar to Hashem A. et al.,[13] Janani B. et al.[23] and Shah S. et al.[24]

Nano-MTA results in comparison with Nano-hydroxyapatite showed better sealing ability and lower dye absorbance than Nano hydroxyapatite. This could be due to the granular mixing of nanohydroxyapatite and its difficulty in a placement to dentin walls.

CONCLUSION

With the limitation of this study, it can be concluded that:

- ProRoot MTA (conventional MTA) remains the material of choice as furcation perforation repair material
- Nano-MTA showed promising results in comparison with ProRoot MTA (conventional MTA) indicates to be used as a perforation repair material.

- Nano-Hydroxyapatite cannot be an alternative to conventional MTA as a perforation repair material.

Further investigations are required for Nano-materials.

CLINICAL RELEVANCE

The improvement of the sealing ability and adaptability of the material enhances the prognosis and the longevity of endodontically repaired molars with accidental furcal perforation. Work in this scope increases the life expectancy of the tooth function.

FUNDING RESOURCES

Non-funded research.

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

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