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Sealing Ability and Dislodgement Resistance of Three Materials for the Repair of Furcation Perforation (*In-vitro* Study)

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ABSTRACT

Background: Root canal treatment is considered one of the most important and challenging procedures in the field of dentistry and the modern daily dental practice. Procedural Errors during root canal treatment occurs often due to lack of knowledge of the root canal system. One of these common challenging errors is tooth or root perforation. Sealing of such defects is a must to eliminate and inhibit any bacterial infection. ***Objectives:*** To assess the sealing ability by dye penetration method and dislodgement resistance of three different perforation repair materials namely MTA, nano-hydroxyapatite and hydroxyapatite. ***Materials and Methods:*** Sixty extracted human molars were selected for the study. All teeth had a standard endodontic access cavity preparation to expose the pulp chamber. Using a carbide rose head bur size # 2, a furcation perforation was created. All Teeth were divided into three experimental groups (n=20) according to type of perforation repair material used. Repaired perforation sites were evaluated for sealing ability and push-out bond strength of the repair materials by using Stereomicroscope and universal testing machine respectively. ***Results:*** MTA showed the least amount of dye penetration and its resistance to dislodgment was the best ***Conclusion:*** MTA can be considered the material of choice for the treatment of furcation perforation as compared to Hydroxyapatite and Nanohydroxyapatite.

Keywords: MTA, Hydroxyapatite, Nanohydroxyapatite, Perforation.

INTRODUCTION

Furcation perforation is a serious error that may occur during root canal treatment, tooth extraction may even result if perforation is not properly treated. Different techniques and materials have been suggested and described over the years. Repair materials should have the ability of Osseo-induction and cementum formation,

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ease of manipulation and application, bacteriostatic, radiopaque, un affected by moisture contamination, non-resorbable, non-toxic and provide a good bacterial and fluid tight seal.[1]

In order to ensure the accurate treatment of a furcation perforation, immediate action should be taken as the defect should be proper isolated, debrided, and sealed. According to studies repair materials or underlying matrix material such as amalgam, Cavit, calcium hydroxide, glass ionomers, hydroxyapatite, tricalcium phosphate, and demineralized freeze-dried bone were not able to produce consistent outcomes. However Current research on novel materials like mineral trioxide aggregate, on the other hand, may greatly advance furcation repair treatment techniques.[2]

Mineral trioxide aggregate had the ideal properties of sealing material being superior to amalgam, glass ionomer, super EBA and intermediate restorative material due its biocompatibility to the surrounding tissue, ability to set in moisture either saliva or blood, ease of manipulation, ease of application, Osseo inductive ability, high adaptability and cementum formation also having perfect seal as it shows a minimal dye leakage during their test.[3]

Skucaite and Busauskas[4] compared sealing ability in an in-vitro study of materials (synthetic bone substance, amalgam, and mineral trioxide aggregate) used to treat furcation perforation through the use of methylene blue dye. MTA has been shown to be superior to amalgam and synthetic bone in terms of sealing ability.

Different furcation perforation was repaired in vitro with variable diameters was evaluated by Zou *et al.*[5] The repair materials were calcium sulphate, composite resin and calcium sulphate under composite resin. They reported that in large perforations, composite resin alone exhibited substantially more leakage. Using calcium sulphate significantly decreased leakage of smaller perforations. Leakage in smaller perforations was significantly lower in groups repaired with calcium sulphate under composite resin than in larger ones.

Chordiya *et al.*[6] compared the sealing ability for furcation perforation repair using 3 different repair materials; MTA, calcium phosphate cement and bone cement. The authors reported that MTA-repaired furcation perforations had the least amount of dye leakage, calcium phosphate cement had the most, and bone cement had intermediate dye leakage.

Singla *et al.*[7] evaluated the push-out bond strength of glass ionomer cement,

hydroxyapatite, mineral trioxide aggregate, and biodentine when used to repair furcation perforations with and without blood contamination in permanent molars. They revealed that the biodentine contaminated with blood had the maximum pushout bond strength, while hydroxyapatite contaminated with blood had the least.

The bond strength of different tricalcium silicate cements to retrograde cavity using a push out test was evaluated by Stefaneli Marques *et al.*[8] The tested materials were MTA Angelus, ProRoot MTA and Biodentine. The results revealed that all tricalcium silicate-based root-end filling materials showed similar bond strength.

Other study by Bhagya *et al.*[9] assessed in the presence of blood contamination, the push-out bond strength of four endodontic root perforation repair materials. Biodentine has a higher push-out bond strength than ProRoot MTA, GIC, and Cavit, according to their study.

Hydroxyapatite, chemically, is similar to the mineral components of bone and the other hard tissue such as teeth and cartilage.[10] It's considered one of the most widely explored biomaterial for its various applications in the medical field. Under different physiological conditions, the higher biocompatibility and stability of Hydroxyapatite leads to its wide application in medical and dental field as bone substitution and remodeling, bone grafting, regeneration, implant coating and in periodontal lesions filling.

Hosseinzade *et al.*[11] explored the physiochemical properties of hydroxyapatite, MTA, calcium enriched mixture and nanohydroxyapatite chitosan cement in endodontics. The tested materials shared the bioactivity and biocompatibility in a manner leading to bone differentiation and stimulation of mineralization in the pulpal system serving also a good antibacterial property.

Armi *et al.*[12] reviewed the recently developed nanotechnology and its beneficial usage in the field of dentistry producing a promising and imminent applications with the materials. Nanohydroxyapatite represents crystals ranging from 50-1000nm has a strong ability to bond with proteins and bacterial fragments when being contained in toothpastes. This feature is due to the nanoparticle sizes that lead to increase of the surface area to which it can bind with proteins, it can also act as a filler as it can close the small gaps and holes. Nanocrystalline form of hydroxyapatite showed a great osteointegrative properties as it can bind and stimulates bone healing encouraging the osteoblastic activity.

Kantharia *et al.*[13] illustrated the hydroxyapatite nanoparticles combined with restorative materials such as glass ionomer cement and composite resins. These nano-sized particles have been shown to improve biocompatibility and mechanical strength. Nano-hydroxyapatite has been used for various applications like pulp capping agent, root canal sealer, filler for bleaching agents and toothpastes, osseo-conductive bone graft. Nano-hydroxyapatite has been obtained using natural bovine bone, carbon template technique, hydroxyapatite chitosan template technique, wet precipitation technique, and plasma spraying technique.

MATERIALS AND METHODS

Sixty extracted sound or minimally restored human permanent mandibular molar teeth free from any cracks or fracture lines were collected from Misr International University's tooth bank. Patients from which the teeth had been extracted were given a clinical consent for the use of their teeth in a scientific research after extraction (IRB 800010118).

Teeth were autoclaved for 15 minutes at 121°C under 15 psi pressure, and immersed in distilled water which was daily renewed to prevent their dehydration until being tested.

All of the sixty samples according to the perforation repair materials were allocated randomly, Group **(1):** repaired by MTA (N=20), **Group (2):** repaired by Hydroxyapatite (N=20**)**, **Group (3):** repaired by Nano-Hydroxyapatite (N=20). Each group was further subdivided into two subgroups **(A, B);** 10 samples each, according to the evaluation method utilized, **Subgroup A:** measuring sealing ability using dye penetration, **Subgroup B:** measuring dislodgement resistance (Push-out bond strength test) using direct compressive force.

Teeth preparation:

Each tooth had a standard endodontic access cavity prepared with rose head carbide bur (size #2). After removing the pulp chamber, the cavities were irrigated using Sodium Hypochlorite (NaOCL) 2.5 %. An Endo -Z bur was used for finishing of cavity walls after extension of the access cavity before doing the perforation. Intentional perforation defects were created in the bifurcation area perpendicular to the long axis in the middle of the pulp chamber floor using a round bur (size 2#) mounted on a high-speed handpiece (**Figure 1).** The perforation width was corresponding to the diameter of the bur (2.33 mm), and perforation length was determined by the dentin and cementum thickness of each tooth separately.

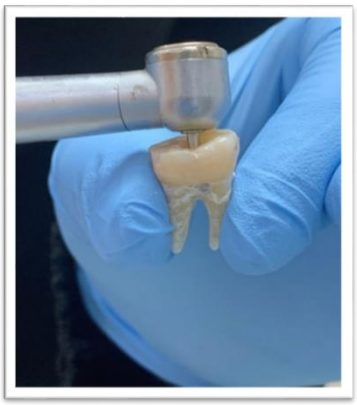

Figure 1: showing a standard endodontic access cavity in a mandibular molar using a round bur size #2.

The pulp chamber was filled with sodium hypochlorite (NaOCL) 2.5% for few minutes before being dried. Before repair processes, excess moisture was removed from the perforation site with paper points and small cotton pellets.

Perforations were repaired and sealed using three different materials in powder liquid form.

Application of repair materials:

The following steps were followed during the application of the three repair materials, on a glass slab, powder was mixed with distilled water. In a 3:1 powder/water ratio; after 30 seconds of mixing the mixture exhibited putty-like consistency for the MTA. The mix was positioned in the perforation site using an MTA carrier. After mixing of 4:2 powder/liquid ratio for hydroxyapatite and Nanohydroxyapatite a granular-like mixture occurred after 1 minute for both materials immediately after that it was placed into the perforation site using an MTA carrier. Endodontic plugger size 110/130 with tip size 1.1 followed by a wet cotton pellet were used to condense and apply the materials over the perforation site. In the pulp chamber, a moistened cotton pellet was placed. The excess material was removed gently by a sharp small excavator and sharp endodontic probe.

Evaluation Methods:

a. Evaluation of Sealing Ability:

Thirty samples prepared and filled with the three tested repair materials representing samples of subgroup A (groups 1, 2, 3) were used for this part of study. The sealing ability was evaluated utilizing dye penetration method as described by Shetty *et al.*[14] As follows:

Composite resin material was placed to seal the access cavities. Using a microbrush, double coats of universal bond were applied. Air thinning for five seconds then light-cured for 20 seconds**.** To avoid dye penetration through other areas, all experimental teeth were coated homogeneously with double coats of nail varnish except for 1 to 2 mm around the perforation defect. Samples were allowed to air dry for 24hrs then were immersed in the methylene blue dye (2% buffered) and left for another 48 hours for

assessment of dye leakage. The root trunk area was placed under running tap water for 30 minutes to remove any excess residue of methylene blue dye and then the varnish was removed with a Parker blade #15. All samples were sectioned using a diamond disc on a low-speed in a buccolingual direction. Sectioned samples were scanned under stereomicroscope at 25x.

Linear measurement of dye penetration was done using Image J software which is a java-based image processing program (LOCI, university of Wisconsin).

b.Evaluation of Dislodgment Resistance (Push-out Test):

Thirty Samples representing subgroup B (groups 1, 2, 3) were used for this part of the study. The Prepared Samples were placed in acrylic resin cylindrical blocks and then left to be fully set. Each sample was cut into slices Using an IsoMet 5000 linear sawing under water coolant. The coronal portion of each sample was removed at the level of the cervical line. One millimeter below the apical end of the furcation area, another horizontal cut was done to separate the roots. **(Figure 2).**

The test was done as follows: Each sample segment was mounted on a metal holder upside down where the apical surface of the repaired furcation defect was facing

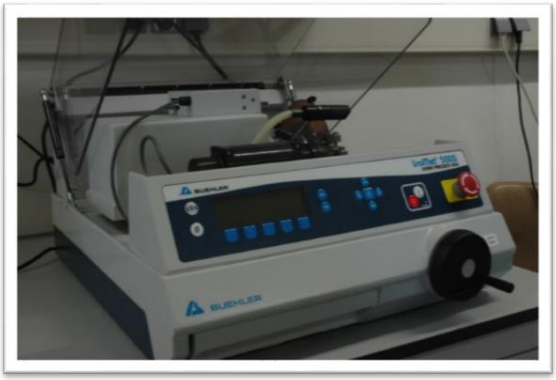

Figure 2: IsoMet 5000 linear saw (Buehler).

upward. Metal stylus of different diameters (0.8, 1, 1.5 ,2) mm equivalent to the diameters of the repair materials was mounted on a universal testing machine. The samples were positioned on a metal slap with a central hole to allow the plunger with a diameter of 1.2 mm to move freely, at a constant vertical downward pressure at a speed of 1 mm/min. The plunger tip was positioned to contact the test material touching only the filling material without stressing the surrounding dentin, in a coronal-apical direction. The test was completed until total bond failure. The highest force applied to materials at the time of dislodgement was recorded in megapascal (MPa), as described by Tomer.*et al.*[15] **(Figure 3,4**). Bond strength was calculated from the following equation as illustrated:[16] **Bond strength= F/πdh,** (**F)** is the compressive force applied to the specimen, (**d**) is the diameter of the plunger, **(h)** is the thickness of the specimen, **(π)** is constant (3.14). All Samples were examined

under dental operating microscope to determine the type of bond failure.

Figure 3: Universal testing machine (Instron).

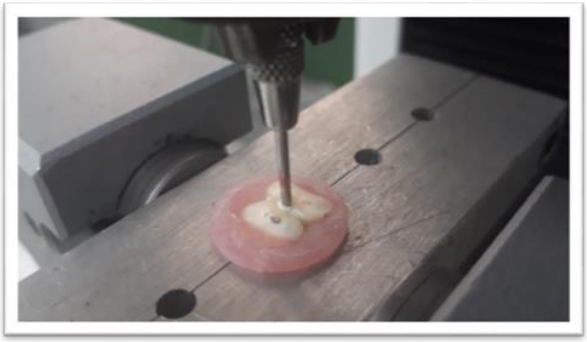

Figure 4: a sample tested for push out bond strength under universal testing machine.

Statistical Analysis:

The collected data were tabulated and statistically analyzed using IBM® and SPSS® Statistics version 20 for windows. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to evaluate the parametric and non-parametric distribution of the collected data. One-way ANOVA followed by Tukey post hoc test was used to compare between more than two groups in non-related samples.

RESULTS

<u>Sealing ability (Dye penetration):</u> (Table 1, figs 5-8)

Table 1: The Mean and Standard Deviation (SD) Values of Sealing Ability (Dye Penetration) of Different Groups.

Variables	Sealing ability (Dye penetration)	
	Mean	SD
MTA	0.26 mm	0.09 mm
Hydroxyapatite	1.50 mm	0.34 mm
Nano-Hydroxyapatite	2.01 mm	0.31 mm
p-value	<0.001*	

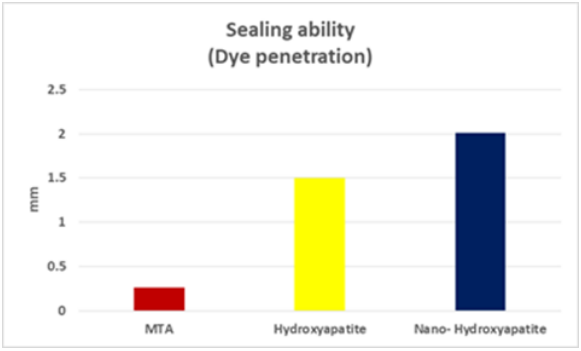

Figure 5: Bar Chart Representing Sealing Ability for Different Groups.

Figure 6: sample of MTA group showing minimal dye penetration under 25X.

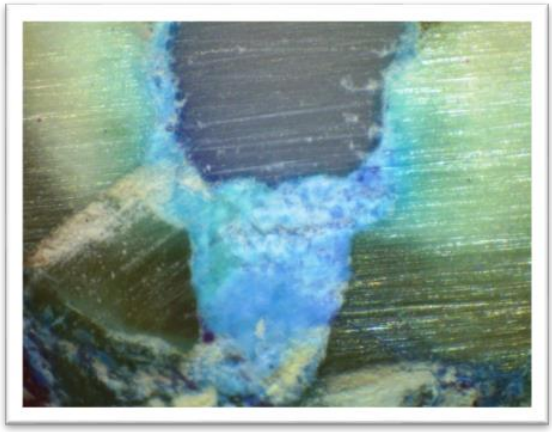

Figure 7: sample of Hydroxyapatite group showing moderate dye penetration under 25X.

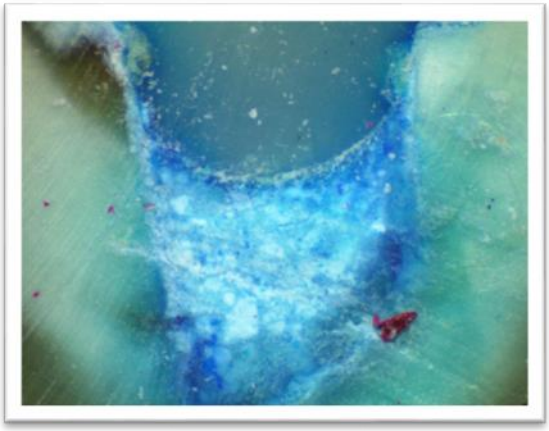

Figure 8: sample of Nanohydroxyapatite group showing severe dye penetration under 25X.

Comparing the dye penetration values for the three experimental groups showed that the least dye penetration was seen within the samples repaired by MTA (0.26 mm ± 0.09 mm). The highest dye penetration was recorded with the samples repaired by nanohydroxyapatite (2.01 mm ± 0.3 mm). Samples repaired by hydroxyapatite showed intermediate dye penetration value (1.50 mm ± 0.34mm). Statistically, the dye penetration values for the three tested materials were significantly different at P<0.001.

<u>Push-out Bond Strength:</u> (Table 2, figs 9-12)

Table 2: The Mean and Standard Deviation (SD) Values of Push-out Bond Strength of Different Groups.

Variables	Push out bond strength	
	Mean	SD
MTA	20.46 MPa	4.37 MPa
Hydroxyapatite	2.54 MPa	1.01 MPa
Nano- Hydroxyapatite	2.81 MPa	0.65 MPa
p-value	<0.001*	

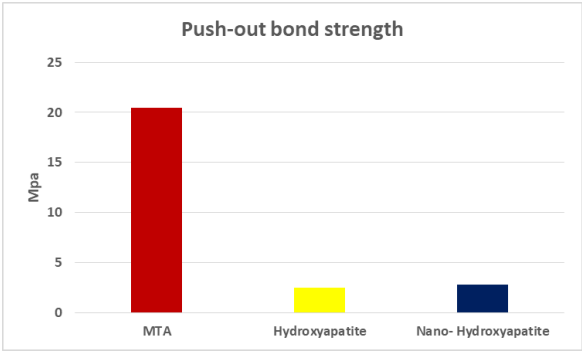

Figure 9: Bar Chart Representing Push-out Bond Strength for Different Group.

Figure 10: Push-out sample of MTA showing the cohesive failure.

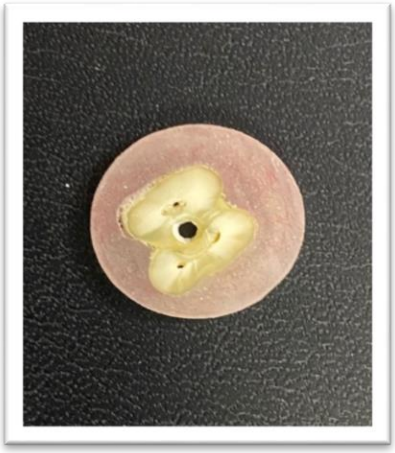

Figure 11: Push-out sample of Hydroxyapatite showing the adhesive Failure

Figure 12: Push-out sample of Nanohydroxyapatite showing the adhesive failure.

Comparing the values of push-out bond strength for the three experimental groups showed that the least push-out bond strength was seen within the samples repaired by hydroxyapatite (2.54 MPa ± 1.01 MPa). The highest push-out bond strength was recorded with samples repaired by MTA (20.46 MPa ± 4.37 MPa). Samples repaired by nanohydroxyapatite showed intermediate push-out bond strength (2.81 MPa ± 0.65 MPa). Statistically, the bond strength values of samples repaired by MTA was significantly higher than the samples repaired by hydroxyapatite and nanohydroxyapatite. On the other hand, the bond strength values for the samples repaired by hydroxyapatite and nanohydroxyapatite were not significantly different at $P<0.001$.

Mode of Failure among the experimental groups: Table (3)

All samples were examined after the push-out bond strength test under dental operating microscope for detection of mode of failure. The results showed that there were two types of failure either Cohesive or adhesive.

Table 3: Type of failure for MTA, Hydroxyapatite and Nanohydroxyapatite.

Mode of Failure	Cohesive	Adhesive
MTA	90%	10%
Hydroxyapatite	30%	70%
Nanohydroxyapatite	40%	60%

-In MTA group, 9 samples out of 10 showed a cohesive failure.

-In Hydroxyapatite group 7 samples out of 10 showed adhesive failure.

-In Nanohydroxyapatite group 6 samples out of 10 showed adhesive failure.

DISCUSSION

Furcation perforation is considered a serious error which may occur during root canal treatment while searching for canal orifices. Perforation in Bi or tri-Furcation area is considered one of the hardest endodontic mishaps due to difficulty in locating the original canals. Furthermore, for the repair of perforation defects various materials have been suggested and tested, however, more of these materials satisfied all the criteria for the ideal repair materials. So, it was the purpose of the present study to search for a new perforation repair material. MTA was chosen to be one of the test materials being considered the gold standard of all perforation repair materials as a result of biocompatibility and its high sealing ability property. Hydroxyapatite thought to be one of the materials which could be used in repair as it resembles one of tooth structures component also its wide variety usage in field of dentistry. Utilizing the nanotechnology in changing the materials properties to a better form and structure thought to show a promising result. Thus, hydroxyapatite in a Nano form was selected to be the third test material in the present study aiming to have a repair material with enhanced properties.

The effect of the size of the furcation perforation on the efficacy of restorative material is still unknown. Some studies stated that the size of the tooth in proportion to the size of the perforation has a direct impact on the prognosis, while others found no link between the two variables.[17]

Different methods for induction of perforation have been suggested regarding the size of the perforation and the tool used. In the present study a rose head bur size #2 was used. This was similar to the study of Grover *et al*.[29] and Yahya *et al.*[30] This size was selected so that the size of the induced defect would be wide enough to accept a good amount of repair material. Other studies used larger sizes of burs #4 [42] which can be considered too large and might jeopardize the integrity of the furcation area. Other studies used smaller burs which results in narrower perforation defects (1-1.5 mm).[4,6,18,19,26] These narrower defects might not allow proper placement of the repair material.

In the present study, the materials of repair were tested for their sealing ability and resistance to dislodgment. Several studies suggested different methods of evaluation of microleakage to determine the materials ability to seal furcation perforations. These methods included fluid infiltration, dye penetration, bacterial leakage models, dye extraction, air pressure method, radioisotope

method, an electrochemical method, metal solution tracers, and reverse diffusion as.[20,21,22,23,24]

In this study, dye penetration method for evaluation of sealing ability was used for its easiness in comparison to other tests.[19,25,26]

On one hand, dye penetration test has some drawbacks including its small molecular size of the dye compared to bacteria. However, on the other hand Torabinejad *et al.*[27] stated that the material that can prevent small dye molecules from penetrating should also be able to prevent larger substances like bacteria and their byproducts from penetration.

For better analysis of dye penetration most of the studies used stereomicroscope but with different magnification power as Nikoloudaki *et al.*[30] used 20 X and Mohan *et al.*[19] used 10 X. In the present study and in agreement with Yahya *et al.*[29] 25 X was used for better and accurate analysis in a bigger scale with a clear image to detect the leakage and the gaps between the material and the dentin walls.

The bond strength of materials used to repair perforation is a necessary attribute because it represents the material's ability to withstand dislodging forces such as mechanical stresses caused by tooth function or operational procedures.[31]

The adhesive capabilities of dental materials to the surrounding dentin have been evaluated using a variety of approaches. Tensile, shear, and push-out bond strength tests are among these approaches. The push-out bond strength test, which was utilized in this investigation, was shown to be efficient, practical, and accurate.[33]

The results of the present study were presented in the form of evaluating the sealing ability and the dislodgement resistance of the tested materials used to seal the perforation sites.

The Push-out bond strength results in this study showed that the samples repaired with MTA exhibited the highest strength. These results came in agreement with Shokouhinejad *et al.*[33], Akublut *et al.*[16] while it came in disagreement with Majeed *et al.*[34], Vanderweele *et al.*[35] The latter two studies showed different results due to difference in particle sizes of the material tested that affected the penetration to dentinal tubules. This could be related to difference in bond strength and the mode of bond failure.

Analyzing the mode of failure, it was found that 90% of samples of MTA showed a Cohesive bond failure. Only 10% (one sample) showed adhesive bond failure. This result reflects the perfect sealing properties of this material. This came in agreement with

Shokouhinejad *et al.*[33], Akbulut *et al.*[16] However, it came in disagreement with Shokouhinejad *et al.*[32], Vanderweele *et al.*[35] These two studies reported that the type of failure was adhesive. The adhesive failure can be regarded as an inherent property of the sealing ability of the tested material. This difference between our study and these studies could be attributed to difference in the experimental model employed in different studies

Samples of Hydroxyapatite and Nanohydroxyapatite showed lower bond strength. This low resistance to dislodgment reflects bad sealing qualities. These results were further explained by the mode of failure, where 60-70 % of the samples repaired showed adhesive failure. Those bond failures of those two materials revealed that their compressive strength is considerably low to withstand any force applied.

It is thought that the main reason for this type of failure is the material property and chemistry which is related to their setting time and poor adaptability.

The sealing ability results in this study showed that the samples of the MTA group have the best values showing a very good outcome of such material in sealing the perforation site with least amount of dye leakage.

These results were in agreement with several studies [4,6,7,18,36,37,38]**,** however**,** when MTA was compared to biodentine it ranked the second, where the latter showed superior sealing properties.[20,38,39,40,41]

The samples repaired with hydroxyapatite showed maximum dye penetration reflecting very poor sealing properties. This was in agreement with Singla *et al.*[7], Taneja and Kumari[42] Converting hydroxyapatite into a Nano form was thought to improve the physical and mechanical properties of the original material.[43] However, in the present study, this was not clearly seen as the sealing ability of the nanohydroxyapatite was significantly inferior to MTA. The overall results of the present study didn't succeed in adding a new perforation repair material but in fact it confirmed the previous concepts of considering the MTA to be the golden standard for sealing of root defects.

CONCLUSION

Based on the findings of this investigation, it could be concluded that:

(1)MTA continue to be the material of choice for repair of perforation defects.

(2)Characterization of hydroxyapatite into a Nano form did not improve the physical properties required for a standard perforation repair material.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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