

# **RESEARCH ARTICLE**

#### SEALING ABILITY ASSESSMENT OF A NEW COMBINATION OF MTA AND HYALURONIC ACID USED AS A RETROGRADE FILLING MATERIAL.

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Manuscript Info	Abstract			
Manuscript History	Introduction: Resection of the root end during periradicular surgery			
Received: 16 May 2018 Final Accepted: 18 June 2018 Published: July 2018	results in an exposed apical dentin surface bounded by cementum with a root canal at its center. After ultrasonic root-end preparation, a root- end filling material is usually placed to seal the root-end cavity preparation. In order to enhance the complete regeneration of hard			
<i>Keywords:-</i> Hyaluronan, MTA, Sealing ability.	tissues after periradicular surgery. A new mix of MTA and Hyaluronan			
	Material and Methods: Sealing ability evaluation was done using the fluid filtration method at four evaluation testing periods (24hours, 1week, 1month and 3months) comparing MTA and MTA/Hyaluronic acid mix (MHM) when used as a retrograde filling material. Data was analyzed statistically.			
	<b>Results:</b> No statistical significant difference was found between MTA and MTA/Hyaluronic acid mix regarding sealing ability at the four evaluation testing periods.			
	<b>Conclusion:</b> MTA/Hyaluronic acid mix satisfied the sealing ability requirments to be used as a retrograde filling material in comparison with the golden standard of MTA as a comparative group.			
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#### **Introduction:-**

Complete regeneration of hard tissues after periradicular surgery consists of a renewel of the apical attachment apparatus and osseous repair of medullary and cortical bone. It is thought that undifferentiated mesenchymal cells arise from the periodontal ligament and bone and participate in the healing process.

Resection of the root end during periradicular surgery results in an exposed apical dentin surface bounded by cementum with a root canal at its center. After ultrasonic root-end preparation, a root- end filling material is usually placed to seal the root-end cavity preparation.

**Corresponding Author:- Raief HM.** Address:- Researcher Assistant, Restorative and Dental Materials Research Department, National Research Center, Egypt. The ideal root-end filling material should be biocompatible, bactericidal, or at least bacteriostatic; should be neutral to neighboring tissues; and should provide excellent sealing. Furthermore, it should promote regeneration of the original tissues.

A new prospection towards the accelaration of the healing process of the periapical lesions and fastening the new bone formation. For this sake new materials and formulations was used. The use of Hyaluronic acid in the treatment of inflammatory process is established in many medical areas. In the field of dentistry, hyaluronate has shown anti-inflammatory, antiedematous and anti-bacterial effects for the treatment of gingivitis and periodontitis.

The purpose of this study to evaluate the sealing ability of the new combinations of MTA together with hyaluronic acid when used as a retrograde filling material using the fluid filtration method.

# Materials and Methods:-

After the determination of the powder/liquid ratio and the assessment of the physical properties of the new mix of MTA/Hyaluronic acid (MHM) according to ADA/ANSI specification no. 57.Testing the leakage and the sealing ability of the new root end filling material was conducted. Thirty four extracted human single rooted teeth were used in the experimental part of the study. These teeth had no root caries, fractures or cracks, signs of internal or external resorption or calcification with completely formed apicies. Following extraction, the teeth were soaked in 5.25% NaOCl for 30 minutes. The teeth were stored in 0.9% isotonic saline at room temperature until being used. A total of 34 teeth were used and divided according to the following grouping system, Mineral trioxide aggregate (MTA) n=15, mineral trioxide aggregate/Hyaluronic acid mix n=15, positive control group n=2 and negative control group n=2. The teeth were decapitated with a diamond disk to create teeth with a standardized length of 16mm at the cementoenamel junction (2, 3, 4, 5, 6, 7). K-file#15 was inserted until the file tip became visible at the apical foramen and then subtracting 1mm. K-file#10 passed after the apical foramen to ensure apical patency. Canals were prepared in a crown-down technique using Protaper universal rotary nickel-titanium for all specimens and final apical preparation will be completed up to Protaper F4 as a master apical file to facilitate the delivery of endodontic irrigants of NaOCl (5.25%) using a plastic syringe with a 27gauge needle and EDTA for 5minutes to remove the smear layer during instrumentation. The canals were dried with paper points and obturated with laterally condensed gutta percha (META) without sealer to act as a stop for placement of the root end fillings. The sealer was not used to ensure that the apical leakage only was a function of the apical filling material alone. Each root filled tooth was restored with a zinc phosphate cement. The teeth were stored in 100% humidity for 48hrs to prevent fracture during the cutting process. Root-end resection was made 90 degrees to the longitudinal axis of each tooth, 3mm away from the root apex, using a water coolant at a high speed diamond stone (Komet, Germany). Root end cavities of 3mm were prepared using an ultrasonic tip (NSK, E32D). The root end cavities were filled according to the grouping system except for the positive control group without any retrofills. (MTA) grey<sup>1</sup> was mixed according to the manufacturer instructions. The new formulation hyaluronic acid liquid (Hyalgan)<sup>2</sup> and MTA was mixed according to the proposed result of powder: liquid ratio got from the tested physical properties, with no retrofilling in the positive control group. The filling materials were applied using MTA carrier (Angelus) and condensed with a small plugger into the retrocavities. Two layers of nail polish were applied to the whole surface of each root in addition to a layer of adhesive cyanoacrylate except for the apex. On the other hand, teeth in the negative control group had the whole surfaces protected by the nail polish (including the apex). After the preparation of the specimen, radiographs were taken to ensure that the obturation was adequate throughout the canal length and width, and to ensure the proper condensation of the retrofill material. The teeth were tested for the following evaluation periods (24hours, 1 week, 1 month and 3 months) Fig (1).

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Fig 1:-(a) showing teeth after the nail polish coating, (b) showing x-rays of the prepared teeth.

The sealing ability was evaluated (2, 3, 4, 7, 8) using the fluid filtration method described by Derkson et al and Wu et al (9, 10). The fluid filtration apparatus consists of nitrogen pressure tank with a pressure regulator of 2 gauges, one gauge indicates the pressure inside the tank, the other gauge adjust the pressure of the out flowing nitrogen of 20Psi Fig(2). Pressurized water reservoir cylinder which is a stainless- steel cylinder of metal thickness 5mm which is partially filled with water, such that an empty space exists on the top of the water surface. The cover of the reservoir has 2 holes through which 2 tubes are inserted, through one hole a rubber polyethylene tube connects the water reservoir with the nitrogen tank and the other hole connects the reservoir to the glass micropipette. The first tube is fitted to a short metal tube reaching above water surface to deliver nitrogen gas at 20 Psi. Through the other hole, another long metal tube is immersed in water to reach a distance 1cm from the bottom of the cylinder. All the junctions were properly sealed with an adhesive cyanoacrylate resin. Glass micropipette is connected to the pressure reservoir via polyethylene tube, its length of 75mm length, and internal diameter of 1.1-1.2mm by calculating the volume of the cylinder through the following  $v=h\pi r^2$  where h is the length of the tube and r is radius of the tube (cylinder)  $v = 75x\pi x(0.6)^2 = 85 \text{ mm}^3$ . The micropipette is fitted onto a graph ruler to measure the distance moved by the bubble. It contains a bubble of 3mm which moves according to the pressure changes. Microsyringe assembly contains a plastic syringe which is partially filled with a water, with a totally withdrawn Plunger. its plastic hub is connected to the T-shaped junction via a polyethylene tube. A screw piston is fixed to the end of the syringe plunger to allow gradual pushing of the plunger. The syringe is fixed in an aluminium holder that allowed movement of the pistol in a screwing manner to deliver an air bubble to the capillary tube. T-Junction with the first horizontal opening connected to the micropipette via polyethylene tube. The second horizontal opening is connected to the tooth assembly unit via another polyethylene tube, the third vertical opening is connected to the microsyringe via polyethylene tube. Tooth assembly unit with a standardized length of obturated root sample with root end filling material of about 13mm was connected to the micropipette via polyethylene tube. The coronal part of the root assembly was properly sealed with an epoxy putty material (Epobond) in order to provide a leak proof closed system. After snugly fitting the tubes, every point of tubing connections in the assembled apparatus is sealed with cyanoacrylate adhesive resin to provide a leak proof closed system. Measurement is done where the tooth assembly unit is first disconnected, the nitrogen tank was opened and the flow of the nitrogen was adjusted at 20Psi by pressure gauges. The nitrogen flows to the pressure reservoir which leads to forcing of nitrogen through the system to displace air before connecting the root sample. The system was allowed to equilibrate for 15 minutes to allow free flow of water without any problem, the nitrogen tank was closed and the root sample was cemented to the polyethylene with an adhesive cyanoacrylate epoxy resin. The nitrogen gas was allowed to flow under a pressure of 1.2bar and forced the distilled water to move and displace the air out of the system. The system was allowed to equilibrate for 5minutes before any measurement. A small air bubble of 3mm was introduced into the system by the microsyringe till reaching the center of the micropipette. The linear movement of the bubble was measured in millimeters along 10 minutes at 2minutes interval. The reading was averaged and converted from mm/min to  $\mu$ /min. To evaluate the efficiency of the fluid filtration device as a tool for measuring leakage, both positive and negative control groups were used. The positive control (without retrofill) should allow the free movement of the air

bubble. The negative control group prevented any movement of the air bubble Fig (3, 4). In order to calculate the amount of leakage in  $\mu$ l/mm, by knowing the volume and the length of the micropipette, the linear movement of the air bubble was converted and expressed in  $\mu$ l/min20Psi so, the volume in a length of 1mm can be calculated. By recording the length traveled by the bubble, its corresponded water volume can be calculated as follows:

Volume of the distance travelled by the bubble= length in mm x volume in a length of 1mm and the time the air bubble to travel.

Thus, the value expressed in  $\mu$ /min.20psi.= Volume travelled by the bubble Time for the air bubble to travel

The data of the sealing leakage tests were collected and subjected to ANOVA and T-test to test the statistical difference of each group and subgroup.



Fig 2:-Digrammatic illustration of the fluid filtration system





Fig3:-showing the whole fluid filtration system

Fig4:-showing the Pressurizied water cylinder

#### **Results:-**

Fig (5), Table (1)

#### Effect of time variable in each group:-

where regarding MTA, the highest mean value of leakage was found in (24 hours) (5.33  $\pm$  1.56) followed by (One week)  $(4.08 \pm 1.67)$  and (One month)  $(1.56 \pm 0.50)$ , while the least mean value of leakage was found in (Three months)  $(0.64 \pm 0.36)$ . There was a statistically significant difference between (24 hours), (One week), (One month) and (Three months) where (p<0.001). A statistically significant difference between (24 hours) and each of (One week), (One month) and (Three months) where (p < 0.001). Also, a statistically significant difference was found between (One week) and each of (One month) and (Three months) where (p<0.001). A statistically significant difference was found between (One month) and (Three months) where (p<0.001). On the other hand, regarding **MTA/Hyaluronic acid mix (MHM):** The highest mean value was found in (24 hours) (5.57  $\pm$  2.86) followed by (One week)  $(3.76 \pm 2.61)$  and (One month)  $(1.37 \pm 0.46)$ , while the least mean value was found in (Three months)  $(0.41 \pm 0.26)$ . There was a statistically significant difference between (24 hours), (One week), (One month) and (Three months) where (p<0.001). A statistically significant difference between (24 hours) and each of (One week), (One month) and (Three months) where (p<0.001). Also, a statistically significant difference was found between (One week) and each of (One month) and (Three months) where (p=0.004) and (p=0.001) respectively. A statistically significant difference was found between (One month) and (Three months) where (p<0.001). On comparing the 2 materials in each time period, at 24 hours, the highest mean value was found in (MTA/Hyaluronic acid mix) ( $5.57 \pm 2.86$ ), while the least mean value was found in (MTA) ( $5.33 \pm 1.56$ ). There was no statistically significant difference between (MTA) and (MTA/Hyaluronic acid mix) where (p=0.808). While at one week, the highest mean value was found in (MTA) (4.08  $\pm$  1.67), while the least mean value was found in (MTA/Hyaluronic acid mix)  $(3.76 \pm 2.61)$ . There was no statistically significant difference between (MTA) and (MTA/Hyaluronic acid mix) where (p=0.731). In addition to that, the highest mean value was found in (MTA) (1.56  $\pm$  0.50), while the least mean value was found in (MTA/Hyaluronic acid mix) (1.37  $\pm$  0.46). There was no statistically significant difference between (MTA) and (MTA/Hyaluronic acid mix) where (p=0.370). At three months evaluation period, the highest mean value was found in (MTA) ( $0.64 \pm 0.36$ ), while the least mean value was found in (MTA/Hyaluronic acid mix) (0.41  $\pm$  0.26). There was no statistically significant difference between (MTA) and (MTA/Hyaluronic acid mix) where (p=0.091).

Variables	Sealing abili						
	МТА		MTA/Hyaluronic acid mix				
	Mean	SD	Mean	SD	p-value		
24 hours	5.33 <sup>aA</sup>	1.56	5.57 <sup>aA</sup>	2.86	0.808ns		
One week	$4.08^{bA}$	1.67	3.76 <sup>bA</sup>	2.61	0.731ns		
One month	1.56 <sup>cA</sup>	0.50	1.37 <sup>cA</sup>	0.46	0.370ns		
Three months	$0.64^{dA}$	0.36	0.41 <sup>dA</sup>	0.26	0.091ns		
P-value	<0.001*		<0.001*				

 Table 1:-The mean, standard deviation (SD) values of leakage (Sealing ability) of all experimental groups in different time periods

Means with different small letters in the same column indicate statistically significance difference, means with different capital letters in the same row indicate statistically significance difference. \*; significant (p<0.05) ns; non-significant (p>0.05)





Figure 5:-Bar chart representing means of sealing ability in all experimental groups in different time periods

# **Discussion:-**

The ultimate success of the periapical surgery depends on the regeneration of a functional periodontal attachment apparatus. Regeneration has been defined as the replacement of tissue components in their appropriate location in the correct amounts and the correct relationship to each other (20).

Among various types of endodontic surgery, retrograde filling aims to seal the root canal system using a material with adequate physical, chemical, and biological properties, preventing egression of infectious content of root canal toward the surrounding tissues (13). The ideal root-end filling material must present certain characteristics, i.e, biocompatibility, adequate marginal sealing ability, dimensionally stable, has osteogenic potential, antibacterial effect, radiopaque, nontoxic, noncorrosive, noncarcinogenic and ease of placement and manipulation. (11, 12)

Endodontic surgery is performed in case of a persistant chronic periapical infection, which still symptomatic after orthograde retreatment (13).

The technique and type of root-end filling materials used are of great importance in this treatment; using an optimum root-end filling material has a critical effect on the success of endodontic surgery (13).

Mineral trioxide aggregate (MTA) was available in the late 1990's. It was reported as a biocompatible material that induces osteogenesis and odontogensis, when MTA directly contacts fibroblasts, cementoblasts and osteoblasts cells of the periodontal ligament, making it ideal material for root-end filling for endodontic microsurgery (12). The main constituents of this material are tricalcium silicate (CaSiO<sub>4</sub>), Bismuth oxide (Bi<sub>2</sub>O<sub>3</sub>), dicalcium silicate (2CaOSiO<sub>4</sub>), calcium sulphate (CaSO<sub>4</sub>), tricalcium aluminate (3CaOAl<sub>2</sub>O<sub>3</sub>), tetracalcium aluminoferrate (4CaOAl<sub>2</sub>O<sub>3</sub>FeO<sub>3</sub>) and amorphous structure consisting of 33% Calcium, 49% Phosphate, 2% carbon, 3% Chloride and 6% Silica.

Hyaluronic acid (HA) was chosen due to its beneficial effect in previous periodontal studies. HA is a key element in the soft periodontal tissues, gingiva, and periodontal ligament, and in the hard tissue, such as alveolar bone and cementum. It has many structural and physiological functions within these tissues (14, 15). It can play a regulatory role in inflammatory response, the hydrophilic nature of hyaluronan creates an environment for cellular migration, in addition to promoting many blood cells' functions especially in the inflammatory response, e.g., phagocytosis and chemotaxis (15).

The aim of the present study was to evaluate the sealing ability of the new mix of MTA/Hyaluronic acid mix when used as a retrograde filling material. MTA was developed to seal communication between the tooth and the external surfaces (19, 20). MTA was chosen because of its superior sealing ability, osteoinductive ability and its potential for hard tissue formation (21, 22). Recently, MTA is considered a gold standard repair material for various clinical applications. It is a unique material that is not influenced by moisture or blood contamination; the presence or absence of blood appears not to influence the sealing capacity of the material (23, 24).

MTA has some known drawbacks such as a long setting time, high cost, poor handling properties due to its sandy consistency providing difficulty during its placement and potential of discoloration. Hydroxyapatite crystals form over MTA when it comes in contact with tissue synthetic fluid. This can act as a nidus for the formation of calcified structures after the use of this material in endodontic treatments (24).

Hyaluronan (HA) can play a regulatory role in inflammatory response: the high-molecular-weight HA synthesized by hyaluronan synthase enzymes in the periodontal tissues, undergoes extensive degradation to lower molecular weight molecules in chronically inflamed tissue, such as gingival tissue inflammation (26).

HA is one of the most hygroscopic molecules known in nature. When HA is incorporated into an aqueous solution, this feature allows HA to maintain conformational stiffness and to retain water. HA also presents important viscoelastic properties reducing the penetration of viruses and bacteria into the tissue, which also plays a role in the wound-healing process in both mineralized and non-mineralized tissues (26).

The new formulation of (MTA+Hyaluronic acid) was conducted and developed, this requires the characterization of the new mix in order to be eligible to be used as a root-end filling material.

Sealing ability testing have become some of the most popular, but at the same time most controversial laboratory tests for the clinical performance of root filling materials. Most endodontic failures occur as a result of leakage of irritants from pathologically involved root canals into the periradicular tissues (25).

The apical leakage of the root-end filling material has been measured by several methods: degree of penetration of dye (3, 5), radioisotope penetration, bacterial penetration, electrochemical means, scanning electron microscopy and fluid filtration method (1, 2, 5).

In this study, the apical leakage of the root-end filling material was measured by fluid filtration method that overcomes the disadvantages of previous methods. Samples are not destroyed, and it is possible to obtain measurements of microleakage at intervals over extended time periods. it's quantitative and nondestructive (1, 2). Nevertheless, the fluid transport model has some disadvantages, it is time-consuming, technique-sensitive, and if not automatized, the precision of the measurements is dependent on the precision of the human eye (2).

Under clinical conditions a root-end filling material is subject to pressure such as local bleeding and swelling caused by surgery. Pressure applied on a root-end filling material might be increased by tooth function, and for this reason a pressure of 20Psi was applied to simulate the clinical conditions (2).

Results showed that no significant statistical difference between MTA and the new MTA/ Hyaluronic acid mix over all the testing period with the observation that the new MTA/ Hyaluronic acid mix exhibited more sealing ability than MTA at the first week, one month and three months. On the other hand, there was a gradual decrease in the microleakage over time in both mixes improving the sealing ability by time.

The high sealing ability of MTA can be attributed to being a bioactive hydrophilic material form an apatite-like layer on its surface when it comes in contact with physiologic fluids in vivo or with simulated body fluids. in vitro. The hydroxyapatite crystals can release calcium and phosphorus continuously, improving the sealing ability of the MTA (27, 29).

Fluid filtration investigations showed the superiority of MTA when used as a retrograde filling material over amalgam (30, 32, 34, 35) and equal (37, 35) or superior to Super EBA (31, 34) and equal to intermediate restorative material (IRM) (35), this was consistent with our results. Shahi et al revealed no statistical significant difference regarding the sealing ability between white MTA, gray MTA, white Portland cement and gray Portland cement(4).

Another fluid filtration study revealed no significant leakage was observed when at least 3 mm of MTA remained after root-end resection. However, the authors reported significantly more leakage when 2 mm or less thickness of MTA remained after root-end resection (36).

Todate there is no available literature discussing the sealing ability of the new combination of MTA/hyaluronan mix.

Thus it could be concluded that MTA/Hyaluronic acid mix satisfied the requirements concerning the apical sealing ability to be used as a retrograde filling material.

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