

EFFECT OF DIFFERENT ROOT CANAL IRRIGATING SOLUTIONS ON THE APICAL SEALING ABILITY OF BIOCERAMIC AND RESIN-BASED SEALERS: AN IN VITRO STUDY

Radwa Nessem¹, Hayam Yousif Hassan², Dalia Mukhtar Fayyad³

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KEYWORDS

Apical Microleakage, Bioceramic BC Sealer, Citric Acid, EDTA, Ozonated Water, Resin-Based Sealer.

• E-mail address:

- radwa_nassem@dent.suez.edu.eg 1. Postgraduate student at End-
- odontics, Faculty of Dentistry, Suez Canal University. 2. Associate Professor of End-
- odontics, Faculty of Dentistry, Suez Canal University.
- Professor of Endodontics, Faculty of Dentistry, Suez Canal University.

ABSTRACT

Introduction: Ideal chemo-mechanical preparation with good obturation produces a monoblock and three-dimension hermetic root canal seal. The importance of sealing is the prevention of leakage, reinfection, periapical lesion, and the root fracture. Aim: The purpose of this in vitro study was to evaluate and compare the effect of different irrigating solutions on the apical sealing ability of two sealers (Bioceramic and Resin based sealers). Materials & Methods: One hundred fifty-five extracted human single-rooted mandibular premolars were prepared and classified into three main groups depending on their final irrigation regimens; group A: 17% Ethylene-diamine tetraacetic acid (EDTA), group B: 10% citric, and group C: 16 ppm ozonated water. Each group was subdivided into two subgroups based on the type of sealer applied, subgroup1: TotalFill® BC Sealer and subgroup 2: AH Plus® sealer. Each subgroup was then divided into two divisions based on the time of evaluation, T1: immediately after incubation and T2: after 30 days. The Apical Sealing Ability was measured for all samples using fluid infiltration method. Statistical analysis was performed using ANOVA (one-way and three-way) and t-test (paired and unpaired). Statistical significance was considered at P<0.05. Results: In relation to irrigation, the highest apical microleakage mean values were recorded in group B, and the least mean values were recorded in group A, followed by group C. In relation to sealers, the highest mean values were recorded in subgroup1, and the least mean values were recorded in subgroup2. In relation to time, the highest mean values were recorded in division 2 and the least mean values were recorded in division 1. Conclusion: AH plus sealer provided better apical sealing than Totalfill BC sealer. Sealers sealing performance is improved by 17% EDTA and 16 ppm Ozonated water. Apical sealing ability decreased over time, regardless of the irrigation and sealer types used.

INTRODUCTION

Root canal cleaning and shaping are performed to eliminate canal contents, particularly infective microorganism, and to prepare the root canal not only for disinfection but also to develop a shape that allows for the simplest and most effective 3D filling^(1,2).

Although advances in metal technology have improved root canal shaping, the actual cleaning of the canal still poses challenges due to anatomical factors, microbiological factors, and iatrogenic damage during preparation^(3,4). One of the challenges related to root canal instrumentation is the formation of smear layer⁽⁵⁾. This layer signifies the possibility of a gap between the fillings and the root canal walls by preventing obturation materials from fully adapting to the prepared root canal surfaces⁽⁶⁾. Several studies have described that this layer is eliminated by auxiliary chemicals for better intracanal medicament diffusion and proper sealer adaptation to the dentinal wall^(7–9).

Irrigation is the most effective strategy to reduce the smear layer as it washes loose, necrotic, and infected debris out of the root canal⁽¹⁰⁾. For its antimicrobial properties and potential to disintegrate dead tissue, vital pulp tissue, and the organic substances of dentin, sodium hypochlorite (NaOCl) is the most widely utilized irrigation^(11,12). However, it lacks the ability to eliminate the inorganic compounds from the instrumented root canal ^(13,14). Endodontic irrigation with Ethylenediamine Tetra-Acetic Acid (EDTA) is recommended because it can bind and eliminate the mineralized component of the smear layer. Its popularity as a chelator arises from its potential to sequester di-and tricationic metal ions⁽¹⁵⁾.

Citric acid is an organic acid that has been demonstrated to be effective in eliminating the smear layer at various concentration^(16,17). It is a chelating compound that combines with metals to create a nonionic soluble chelate⁽¹⁸⁾. One of the latest generations of disinfection agents is ozone, a strong oxidizing irrigation used to remove microorganisms from root canals⁽¹⁹⁾. The generation of vaporcontaining bubbles inside a fluid is one of the cavitation properties of ozone. This subsequently leads to the creation of pressure waves/shockwaves, which are associated with significant amplitude fluctuations in pressure⁽²⁰⁾. In the root canal system, such shockwaves might possibly break bacterial biofilms, burst bacterial cell walls, and eliminate the smear layer⁽²¹⁾.

Three-dimensional obturation must be achieved after adequate chemo-mechanical preparation. Because the goal of obturation is to close all "portals of exit," the microorganisms are entombed and re-infection is prevented by the distribution of microbial toxins⁽²²⁾. A root canal filling is composed of two key components: a solid core material and a sealer.

To avoid leakage, root canal sealers should ideally be able to produce an efficient bond between the core substance and the root canal dentin.⁽²³⁾.

AH Plus[®] (resin based-sealer) provides its bonding capabilities by penetrating dentinal tubules and forming resin tags, leading to micromechanical interlocking. Also develops an intimate hybrid layer when it is in close contact with the surrounding collagen fibers⁽²⁴⁾.

Totalfill[®] BC sealer is one of the most recent Bioceramic sealers; it derives its bonding properties from monobasic calcium phosphates. Monobasic calcium phosphates were added to the sealer to facilitate reaction with calcium hydroxide, which resulted in the production of water and hydroxyapatite via sealer activation by water⁽²⁵⁾. Hydroxyapatite co-precipitates with calcium silicate hydrate phase to form a composite-like structure that reinforces the set cement^(26,27).

Up to our knowledge, there is a lack of dental research that concerned with the effect of ozonated water on the smear layer elimination, sealer penetration and sealing ability of bioceramic and resin based sealers. Therefore, this study was conducted to evaluate and compare the effect of 17%EDTA, 10%Citric acid, and 16ppm Ozonated water on the apical sealing ability of TotalFill BC and AH Plus sealers at two time intervals. The null hypothesis stated that there are no differences in apical sealing ability between Totalfill BC and AH Plus sealers at different time intervals when using

different irrigation solutions (EDTA, Citric acid & Ozonated water).

MATERIALS AND METHODS

A. Study design

This study was carried out with the permission of the Faculty of Dentistry, Suez Canal University Research Ethics Committee ; Number 77/2018.

A total of 155 extracted single-rooted human mandibular premolars were calculated using G*power software version 3.1.9.6 with an effect size of 0.19, a power of 0.95 at a significance level of p<0.05, and partial eta-squared of $0.035^{(28)}$.

B. Teeth collection and preparation:

Single-rooted mandibular premolars were gathered from the Oral and Maxillofacial Surgery outpatient clinic at the Faculty of Dentistry of Suez Canal University. Teeth were extracted for orthodontic reasons. Each tooth was radiographed to ensure that it had a single canal, a mature root apex, no resorptive defects (if any), and no prior root canal filling.

All teeth were decoronated, adjusted, and standardized to 15 mm from the apex. The working length was determined by subtracting 0.5 mm from the estimated length. All roots were cleaned and shaped with a Revo-S® rotary system (Micro-Mega, Besançon, France) to an apical size of 40/0.06. Following each file, all root canals were irrigated with 5mL of freshly made 2.5% sodium hypochlorite (CalixE, DHARMA, USA).

C. Grouping of samples:

One hundred fifty-five prepared teeth were randomly divided into three main groups (n=50)

based on the final irrigation regimens: group A: 17% EDTA (CalixE, DHARMA, USA), group B: 10% citric acid was prepared by diluting 100 gm citric acid salt in 1000 mL distilled water using a mechanical stirrer (Freshly prepared at the Faculty of Science, Suez Canal University, Egypt), and group C: 16 ppm ozonated water was prepared by bubbling O3 through sterile distilled water at 16 mg/L using the O3 generator digitally (Ozone Department, National Research Centre Community, Egypt).

Each irrigating solution was irrigated for one minute with a capacity of 5 mL. Then, sterile absorbent paper points (40/.06) (Dentsply maillefer, Tulsa, USA) were used to dry the root canals.

Each group was subdivided into two subgroups based on the type of sealer applied (n=25), subgroup 1: TotalFill® BC Sealer (FKG Dentaire, La-Chauxde-Fonds, Switzerland) and subgroup 2: AH Plus® sealer (Dentsply, DeTrey, Konstanz, Germany). The cold lateral compaction technique was used to obturate the root canals in each subgroup. Total fill sealer was provided with a syringe and an intracanal tip. Following the manufacturer's instruction, the provided syringe tip was inserted into the canal at the coronal one-third, and a small amount (2 reference markings) of sealer was gently dispensed into the canals. AH plus sealer was mixed according to the manufacturer's instructions. An appropriate amount of base and catalyst (1:1 wt. ratio) was squeezed onto a mixing plate. They were mixed with the spatula for 15-20 s or until the creamy and homogeneous mix was obtained. Then spreader #25 (Micro-Mega, Besançon, France) was used to lightly coat the canal walls with the mixing sealer. A gutta-percha master cone #40 taper .06 (Dentsply maillefer, Tulsa, USA) was chosen to fit at the apical portion of the root canal. In all subgroups, the master cone was coated with a layer of the sealer and fitted inside the canal and compacted using a spreader size #25 (Micro-Mega, Besançon, France) that can reach 1mm shorter of the determined working length. The auxiliary cones #25 (Dentsply maillefer, Tulsa, USA) were added and compacted inside the canal using the spreader, and the procedure was repeated until the spreader no longer introduced more than 2 mm. The excess gutta-percha protruding from the coronal end of each root canal was removed using a warm instrument and then the hole was filled with glass ionomer (GC Corporation, Tokyo, Japan). To allow for the full set of sealers, all roots were kept for one week in an incubator at 37°C and 100 humidity. Except for the apical 3 mm of the root apex, all experimental group specimens were coated with a double coat of nail varnish.

One hundred and fifty five samples were divided into positive control(n=5) and experimental samples (n=150), 150 samples were divided into 3 main groups (n=50) based on irrigation type. Each group was divided into (n=25) based on type of sealer used, and each subgroup was divided into (n=10) based on time of evaluation and remaining 5 samples from each subgroup were used as negative control.

Positive and negative control samples were used to ensure that the fluid filtration apparatus was working properly, they were used at both time intervals. Positive samples were obturated with gutta-percha without a sealer, allowing the bubble to move and confirming that all system paths were open. They received the same coating as such experimental group. Each subgroup was then separated into two divisions(n=10) based on the period of evaluation, T1: roots were examined immediately after incubation, T2: roots were preserved in saline and tested after 30 days.

To ensure no bubble movement, negative control: Five obturated roots from each subgroup

were completely covered including the apex with nail varnish.

Evaluation of the apical sealing ability:

Using a fluid filtration apparatus (Figure 1&2), all samples were tested for apical sealing ability. First, positive control samples were connected to the system to check that all system paths were open and unobstructed. Then, negative control samples were then connected to verify there was no leakage. After passing these two tests, the system was ready for usage.

Observing the movement of the air bubble allowed us to calculate the volume of fluid transport. The air bubble linear movement was recorded in millimeters in 4 successive readings at two-minute intervals. The average of these readings was calculated and the millimeter per minute linear records were converted into microliters per minute by using this equation: $(V = \pi r^2 l/ pt)^{(29,30)}$.



Fig. (1) A photograph showing assembled fluid filtration apparatus

Statistical analysis

Statistical analysis was carried out using IBM® SPSS® Statistics Version 20 for Windows at significant level of P≤0.05 (Statistical Package



Fig. (2) A photograph showing parts of fluid filtration apparatus. (A): oxygen tank with a pressure gauge (B) Glass bottle containing distilled water (C) Glass bottle cap (D) micropipette (E) T-junction (F) Sample (G) Microsyringe.

for Social Science, Armonk, NY: IBM Corp). Kolmogorov-Smirnov and Shapiro-Wilk tests revealed that fluid filtration data had a parametric distribution. A one-way ANOVA followed by a Tukey post hoc test was performed for comparing multiple groups in unrelated samples. An independent sample t-test was performed to evaluate two groups in unrelated samples. A paired sample t-test was performed to evaluate two groups in related samples. Interactions between different variables were investigated using three-way ANOVA tests.

RESULTS

Effect of time (Table 1):

• Group A (17% EDTA):

Subgroup A1 (Total Fill BC sealer): The highest statistically significant apical microleakage

mean value was found after 30 days (0.56 ± 0.07), while the least mean value was found after 7 days (0.13 ± 0.04).

Subgroup A2 (AH Plus sealer): The difference between the two time intervals was not statistically significant. The highest apical microleakage mean value was found after 30 days (0.25 ± 0.06), while the least mean value was found after 7 days (0.19 ± 0.09).

• Group B (10% Citric acid):

Subgroup B1 (Total Fill BC sealer): The highest statistically significant apical microleakage mean value was found after 30 days (1.06 ± 0.29), while the least mean value was found after 7 days (0.62 ± 0.05).

Subgroup B2 (AH Plus sealer): The difference between the two time intervals was not statistically significant. The highest apical microleakage mean value was found after 30 days (0.50 ± 0.05), while the least mean value was found after 7 days (0.46 ± 0.05).

Variables -	Irrigants							
	Group A (EDTA)		Group B (Citric acid)		Group C (Ozonated water)			
Sealers _ Time	TotalFill	AH Plus	Totalfill	AH Plus	TotalFill	AH Plus		
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
7 days	0.13 ± 0.04	0.19 ± 0.09	0.62 ± 0.05	0.46 ± 0.06	0.17 ± 0.03	0.19 ± 0.10		
30 days	0.56 ± 0.07	0.25 ± 0.06	1.06 ± 0.29	0.50 ± 0.05	0.61 ± 0.05	0.21 ± 0.06		
p-value	<0.001*	0.199ns	0.002*	0.106ns	<0.001*	0.503ns		

Table (1) Comparison of the mean and standard deviation (SD) of apical microleakage values on two tested sealers irrigated with different irrigation solutions over time.

Subgroup C1 (TotalFill BC sealer): The highest statistically significant apical microleakage mean value was found after 30 days (0.61 ± 0.05), while the least mean value was found after 7 days (0.17 ± 0.03).

Subgroup C2 (AH Plus sealer): The difference between the two time intervals was not statistically significant. The highest apical microleakage mean value was found after 30 days (0.21 ± 0.06), while the least mean value was found after 7 days (0.19 ± 0.10).

Effect of sealer type (Table 2):

• Group A (17% EDTA):

After 7 days: The highest statistically significant apical microleakage mean value was found with the AH Plus sealer (0.19 ± 0.09) subgroup, while the least mean value was found with TotallFill BC sealer (0.13 ± 0.04) subgroup.

After 30 days: The highest statistically significant apical microleakage mean value was found with the TotalFill BC sealer (0.56 ± 0.07) subgroup, while the least mean value was found with AH Plus (0.25 ± 0.06) subgroup.

• Group B (10%Citric Acid):

After 7 days: The highest statistically significant apical microleakage mean value was found with the TotalFill BC sealer (0.62 ± 0.05) subgroup, while the least mean value was found with AH Plus sealer (0.46 ± 0.06) subgroup.

After 30 days: The highest statistically significant apical microleakage mean value was found with the TotalFill BC sealer (1.06 ± 0.29) subgroup, while the least mean value was found with AH Plus sealer (0.50 ± 0.05) subgroup.

• Group C (16ppm ozonated water):

After 7 days: There was no statistically significant difference between both sealers where (p=0.446). The highest apical microleakage mean value was found with the AH Plus sealer (0.19 ± 0.10) subgroup, while the least mean value was found with TotalFill BC sealer (0.17 ± 0.03) subgroup.

After 30 days: The highest statistically significant apical microleakage mean value was found with the TotalFill BC sealer (0.61 ± 0.05) subgroup, while the least mean value was found with AH Plus sealer (0.21 ± 0.06) subgroup.

Variables	Irrigants							
	Groups A (EDTA)		Group B (Citric acid)		Group C (Ozonated water)			
Sealers	7 days	30 days	7 days	30 days	7 days	30 days		
Time	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD		
TotalFill	0.13 ± 0.04	0.56 ± 0.07	0.62 ± 0.05	1.06 ± 0.29	0.17 ± 0.03	0.61 ± 0.05		
AH Plus	0.19 ± 0.09	0.25 ± 0.06	0.46 ±0.06	0.50 ± 0.05	0.19 ± 0.10	$\textbf{0.21} \pm 0.06$		
p-value	0.045*	<0.001*	<0.001*	<0.001*	0.446ns	<0.001*		

Table (2) Comparison of the mean, standard deviation (SD) values showing effect of sealer type on apical microleakage at different time intervals.

Effect of irrigants (Table 3):

• TotalFill BC sealer (subgroup 1):

A statistically significant difference (p <0.001) was found between the 17 % EDTA (Group A), 10% Citric Acid (Group B), and 16ppm Ozonated water (Group C) groups at both intervals (7 and 30 days). A statistically significant difference was found between group B and each of group A and C (p <0.001). At 7 and 30 days. No statistically significant difference was found between groups A and C (p=0.078, p=0.769, respectively). The maximum apical microleakage mean value was found with 10% Citric Acid (0.62 ± 0.05), (1.06 ± 0.29) at 7&30 days, followed by the 16ppm Ozonated water group (0.17 ± 0.03), (0.61 ±0.05) at 7&30 days, and the lowest mean value was reported in the 17% EDTA group (0.13 ± 0.04), (0.56 ± 0.07) at 7&30 days.

• AH Plus sealer (subgroup 2) :

A statistically significant difference (p < 0.001) was found between the 17% EDTA (Group A), 10%Citric Acid (Group B), and 16ppm Ozonated water (Group C) groups at both intervals (7 and 30 days). There was a statistically significant difference between group B and each of group A and C (p<0.001). At 7 and 30 days, no statistically significant difference between groups A and C (p=0.999, p=0.451). The greatest apical microleakage mean value was obtained in the 10% Citric Acid group (0.46 ± 0.06) , (0.50 ± 0.05) at 7&30 days, followed by 17% EDTA and 16ppm ozonated water (0.19 ± 0.09) , (0.19 ± 0.10) at 7 days. Then, after 30 days, 17 percent EDTA (0.25 0.06) was added, and the lowest mean value was discovered in 16ppm ozonated water group (0.21 ± 0.06) at 30 days.

Table (3) Comparison of the mean and standard deviation (SD) values showing the effect of irrigation on the apical microleakage of two tested sealers at different time intervals.

Variablas	Sealers					
variables	Totalf	ill sealer	AH Plus sealer			
Time	7 days	30 days	7 days	30 days		
Irrigants	Mean \pm SD	$Mean \pm SD$	Mean \pm SD	$Mean \pm SD$		
Group A (EDTA)	0.13 ± 0.04	0.56 ± 0.07	0.19 ± 0.09	0.25 ± 0.06		
Group B (Citric acid)	0.62 ± 0.05	1.06 ± 0.29	0.46 ± 0.06	0.50 ± 0.05		
Group C (Ozonated water)	0.17 ± 0.03	0.61 ± 0.05	0.19 ± 0.10	0.21 ± 0.06		
p-value	<0.001*	< 0.001*	< 0.001*	<0.001*		

DISCUSSION

The sealer basic function is to aggregate the root filling material and maintain it as a compact mass free of gaps that adheres to the root canal dentin and produces a single block structure that hermetically seals the canal system and its irregularities⁽³¹⁾.

Totalfill BC sealer has good physical properties that allow well flow of the sealer into root canal irregularities⁽³²⁾. Besides, their good biochemical properties lead to the formation of a hydroxyapatite layer on hydration that initiates chemical bonding between dentin and the sealer⁽³³⁾. AH Plus is considered as the gold standard sealer due to its excellent properties such as dimensional stability, very low shrinkage, and adhesion to dentin providing good sealing^(34,35). In the current study, the AH Plus sealer was used to compare its apical sealing ability with bioceramic-based sealers (TotalFill BC) using different irrigation solutions.

The formation of a smear layer during root canal instrumentation is one of the factors influencing adherence of sealer to the root canal dentin ⁽³⁶⁾. It behaves as a barrier between the obturating materials and the canal walls, impairing its establishment of even a suitable seal and maybe resulting in microfiltration⁽³⁷⁾. One of the most significant chelating agents, EDTA, solubilizes the inorganic component of the smear layer by establishing a chelate with the calcium within the dentin tissue⁽¹⁰⁾. In this study, the concentration of 17% was used due to the high effect in a short time preventing dentin destruction ⁽³⁸⁾.

Citric acid, an organic acid that serves as a chelator, enables removing the inorganic component in the smear layer via dentin decalcification⁽³⁹⁾. Because lower concentrations of citric acid with its original pH were proven to be almost as effective as greater concentrations in removing the inorganic

component in the smear layer, 10% citric acid was utilized in this study⁽⁴⁰⁾.

Ozonated water is one of the novel endodontic irrigations. The basic principle of ozone therapy is the high oxidant potential of the ozone, which is the triatomic form of oxygen that grants an important antimicrobial action and stimulates the cellular metabolism of the healthy cells, thereby favoring tissue repair⁽⁴¹⁾. There has been little investigation on the effect of ozonated water on smear layer removal. There are scientific researches that showed the effect of ozone on removing natural organic matter (NOM) and insoluble particles during purifying water ⁽⁴²⁾. As a result, the influence of 16ppm ozonated water on the sealing ability of different root canal sealers was investigated in this study⁽⁴³⁾.

Derkson *et al*⁽⁴⁴⁾ and **Wu** *et al*⁽⁴⁵⁾ devised a fluid filtering technique that has been frequently utilized to assess microleakage. This technique has various advantages over frequently used methods, including the fact that the samples are not destroyed, it allows for the measurement of microleakage over time, and most importantly the results are accurate since very small volume is recorded ⁽⁴⁶⁾.

The current study found that root canals treated with 17% EDTA and 16ppm Ozonated water irrigant had statistically significant higher sealer adaptability and less apical microleakage than root canals treated with 10% Citric acid, with different sealer types. There was no significant difference between 17 % EDTA and 16ppm Ozonated water; both had reduced apical microleakage values. The EDTA result might be attributable to the fact that the EDTA irrigant is more able to eradicate the smear layer due its chelation action ⁽⁴⁷⁾. EDTA is a complex molecule with a claw-like structure, which binds and seizes divalent and trivalent metal ions such as Ca2+ and Fe3+. EDTA removes bacterial surface proteins by combining with metal ions from the cell envelope leading to bacterial death . EDTA forms a stable complex with calcium⁽¹⁵⁾.

Sealer penetration was not observed through dentinal tubules in totally coated canal by a smear layer, according to **De-Deus et al**⁽⁴⁸⁾. Despite the fact that the major goal of this research is to examine the effect of various irrigation solutions on sealing ability, our findings are consistent with those that investigated smear layer removal ⁽⁴⁹⁻⁵²⁾.

These 17 % EDTA results agreed with those of Jardine *et* al⁽⁵³⁾, who observed that 17 % EDTA increased AH Plus sealer apical penetration over BioPure MTAD and saline. This might be due to EDTA, a weak acid that may promote protein denaturation, increasing dentinal permeability to intracanal medicine and making the link between dentin and endodontic cement simpler ⁽⁵⁴⁾.

This conclusion was also consistent with the findings of **Fouda et al**⁽⁵⁵⁾. According to their findings, the method of final irrigation impacts the attachment of Total Fill Bio-ceramic and AH plus sealers to root canal walls. When 17 % EDTA was employed as final irrigation, both sealers demonstrated good push-out bond strength. It might be ascribed to increased adhesion caused by the use of chelating solutions, which increase the surface contact between both the sealer and the root canal dentin. This is a positive adaptation because it increases the number of opened dentinal tubules and reduces the smear layer. ⁽³¹⁾.

However, this study findings contradicted with **Carvalho** et al., ⁽⁵⁶⁾ that discovered no significant variation in bond strength between calcium silicatebased sealers (MTA Fillapex, Totalfill BC) and epoxy resin-based sealers (AH Plus) to dentin when different irrigation solutions were used (17%EDTA, 10 % Citric acid, 2.25%PA). They determined that the type of sealer had a considerable impact on the bond strength. This discrepancy might be related to different sample preparation and assessment methods.

Ozonated water (16ppm) showed a good result with different types of sealers. The results of ozonated water agreed with **Ibrahim** et al.⁽⁵⁷⁾, **Garcia** et al.⁽⁵⁸⁾, but not with **Ibraheem** et al.⁽⁵⁹⁾, who indicated that ozonated water increased bond strength. These findings might be explained by the fact that Ozone is a potent oxidant, however ozonated water becomes very unstable and rapidly breaks down due to a sophisticated chain reaction, therefore diminishing the oxidative impact ⁽⁶⁰⁾. Other ozone processes include organic biodegradation and some possibility for smear layer removal⁽⁵⁹⁾.

Ozonated water efficacy might be attributed to cavitation property. Freshly dissolved O3 created vapors including bubbles within a fluid, leading to the pressure production on surfaces. Disintegration on the surface is caused by the forceful collapse of bubbles⁽⁶¹⁾.

Ozonated water performed well with many types of sealers, particularly AH Plus. This might be due to its potential to promote collagen type-1 synthesis, and decreased pro-inflammatory cytokine release leads to collagen augmentation⁽⁶²⁾. The capacity of AH Plus sealer to create covalent connections between exposed amino groups in the collagen network and epoxide rings contributes to its high adhesion ability⁽⁶³⁾.

Although Totalfill BC sealer demonstrated superior apical sealing over AH Plus after 7 days. At 30 days, the AH Plus sealer outperformed the Bioceramic sealer (Totalfill BC). The extended setting time of the bioceramic sealer drawing moisture from dentinal tubules and generating hydroxide gel for the initial setting may be attributed to the Totalfill BC sealer result at 7 days. AH Plus sealer, on the other hand, has lower solubility, micro retention to the root dentin, and a firm apical seal. The strong cross-links in epoxy resin-based materials are responsible for AH Plus poor solubility ⁽⁶⁴⁾.

This result of AH plus sealer came in accordance with many studies ⁽⁶⁵⁻⁶⁹⁾. They indicated that the higher apical sealing of AH Plus could be attributed to its excellent penetration into micro imperfections due to its creep capacity and extended setting time, which improves mechanical interlocking between sealer and root dentin. ^(70,71). Furthermore, AH Plus sealer can generate covalent links between exposed amino groups in the dentin collagen network and epoxide rings ⁽⁷²⁾. This gives it excellent adhesion properties. Also, as AH Plus is somewhat acidic, when it comes into contact with dentin, it may cause self-etching, which improves interfacial adhesion⁽⁷³⁾.

This study findings were in accordance with those of **Donnermeyer** et al ⁽⁷⁴⁾, who reported that AH Plus sealer had greater bond strength than Totalfill BC sealer over time. It might be related to the AH Plus sealer low solubility.

However, the findings of this study contradicted those of **Zhang** et al ⁽⁷⁵⁾, **Salem** et al ⁽⁷⁶⁾, who claimed that in terms of apical sealing performance, iRoot SP and Total Fill BC (Bioceramic sealer) were equal to AH Plus sealer. Such disparity might be attributed to differences in sample size (smaller), obturation technique (using a single cone, continuous wave), and various assessment periods employed in their investigations.

Furthermore, the findings of this investigation contradicted those of **Yap** et al⁽⁷⁷⁾ and **Al-Hiyasat** et al.⁽⁷⁸⁾, who reported that Totallfill had stronger binding strength than AH Plus sealer and that it grew with time. This discrepancy might be attributed to

variances in evaluation methodologies as well as various evaluation times.

Furthermore, the findings of this investigation contradicted the findings of **Asawaworarit** et al⁽⁷⁹⁾, who reported that EndoSequence BC Sealer outperformed AH Plus at all tested times. This discrepancy might be attributed to changes in root canal preparation (using a Protaper rotary file till F5) and irrigation regimen throughout preparation (2ml of 17% EDTA acid followed by 5 ml of 2.5% NaOCl). When NaOCl is administered after EDTA, it works directly on collagen, causing fast collagen degradation in the superficial dentin⁽⁸⁰⁾.

Within the limitation of the present study, Sealers sealing performance is improved by 17% EDTA and 16 ppm Ozonated water, so the null hypothesis was rejected. Further studies are recommended to investigate the effect of ozonated water on the smear layer elimination and sealer penetration.

CONCLUSION

Apparently final irrigating solution had an impact on the apical sealing ability of root canal sealers. The AH Plus sealer provides better apical sealing than bioceramic-based sealers (TotalFill BC). Sealers sealing performance is improved by 17% EDTA and 16 ppm Ozonated water. Apical sealing ability decreased over time, regardless of the irrigation and sealer types used.

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