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The Effect of Final Irrigation Protocols on the Apical Sealing Ability of Epoxy Resin-based and Bioceramic-based Root Canal Sealers

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Abstract

AIM: This study was designed to investigate the effect of different final irrigation protocols on the apical sealing ability of bioceramic and epoxy resin-based sealers.

MATERIALS AND METHODS: Thirty human single-rooted mandibular premolars were instrumented using ProTaper Next rotary files. Teeth were randomly divided into three groups according to the final irrigation regimen; Group I: 5 ml 0.2% chitosan nanoparticles (CNPs)/3 min, Group II: 2.5 ml 0.2% CNPs/1.5 min followed by 2.5 ml 17% EDTA/1.5 min, and Group III: 5 ml 17% EDTA/3min. All groups were subdivided into two subgroups based on the obturation material; Subgroup A: Gutta-percha/Sure-Seal Root BC Sealer; and Subgroup B: Gutta-percha/AH Plus. All canals were obturated using single cone obturation technique. The apical sealing ability was assessed using modified silver staining technique with ammoniacal silver nitrate tracer solution. Samples were sectioned longitudinally and examined using scanning electron microscope.

RESULTS: Sure-Seal Root BC sealer showed significantly lower nanoleakage compared to AH Plus ($p < 0.001$). No significant difference was recorded in the nanoleakage of Sure-Seal Root BC sealer among the three groups ($p = 0.284$), while AH Plus showed a significantly higher nanoleakage in the EDTA group ($p = 0.002$). The depth of silver nitrate penetration into the dentinal tubules was significantly higher in AH Plus subgroup with the three different irrigation protocols ($p < 0.001$). For both sealers, the highest penetration depth for silver nitrate tracer solution was recorded in the EDTA group ($p < 0.001$).

CONCLUSIONS: The apical sealing ability of bioceramic sealers is better than that of epoxy resin based sealers. The type of the final irrigating solution seems to affect the post-obturation seal of both AH Plus and Sure-Seal Root BC sealer.

Introduction

Successful endodontic treatment depends on thorough chemo-mechanical debridement of the root canal system, and three-dimensional obturation of the canal space with an inert filling material. Three-dimensional obturation aims to completely seal the spaces previously occupied by the pulp tissues and provide a fluid tight seal between the canal walls and root canal filling materials [1].

The smear layer is a thin layer of organic and inorganic remnants formed on the instrumented surfaces of the root canal walls. It acts as a barrier hindering the optimum penetration of disinfecting agents, intra-canal medicaments, and root canal sealers into the dentinal tubules [2]. The presence of the smear layer may compromise the integrity of the apical and coronal seal which negatively affects the treatment outcome [3]. Apical leakage is considered to be the most common cause for endodontic failure which is influenced mainly by the presence or absence of the smear layer as well as chemical and physical properties of root canal filling materials [4].

Many chelating agents have been proposed for removal of the smear layer including EDTA, citric acid, MTAD and Qmix. 17% EDTA solution is the most commonly used chelating agent, however prolonged exposure to EDTA adversely affects dentin structure resulting in significant reduction of dentin microhardness as well as dentin erosion [5].

Chitosan is a natural biopolymer obtained by deacetylation of chitin, which is derived from crustacean exoskeletons as crabs and shrimps [6]. In the last few years, chitosan has been used as a final irrigant for removal of the smear layer because of its high chelating capacity. In addition, it has a broad spectrum antimicrobial activity against a wide range of Gram-negative and Gram-positive bacteria as well as fungi [7], [8]. Contrary to EDTA, chitosan can remove the smear layer effectively with less dentin erosion [9], [10]. Furthermore, chitosan treatment improves collagen resistance to enzymatic degradation by collagenase [11].

Nanoscale materials have enhanced and unique physiochemical properties compared to their bulk counterparts in terms of ultra-small structure, large surface area/mass ratio, and increased chemical

reactivity [12], [13]. The previous *in vitro* studies demonstrated that chitosan nanoparticles (CNPs) can serve as a useful alternative to EDTA because of its ability to act as a chelating and an antibiofilm agent [14], [15], [16].

The aim of the present study was to assess and compare the effect of different final irrigation protocols; EDTA, CNPs, and CNPS/EDTA on the apical sealing of bioceramic and epoxy resin-based sealers. The null hypothesis tested was that these final irrigation protocols had no effect on the apical sealing ability of the tested sealers.

Materials and Methods

CNPs preparation

The CNPs were prepared using the ionotropic gelation method based on a previously published protocol [17]. The chitosan powder (Acros Organics, Belgium) was dissolved in 1% acetic acid (Sigma Aldrich, USA) under magnetic stirring at room temperature. Afterwards, 0.7 mg/ml sodium tripoly-phosphate (TPP) solution (Sigma-Aldrich, USA) was added into the chitosan solution. The preparations were mixed at 800 rpm with drop-wise addition of TPP solution to form the nanoparticles, thus achieving a final CNPs concentration of 2 mg/ml (0.2%).

Sample size

Sample size calculation was performed using G*Power software version 3.1.9.7. By adopting a 5% significance level, power 80% and based on the results of Al-Zaka *et al*. [18]; the calculated sample size was a total of 30 samples.

Sample selection

The study was approved by the Ethics Committee of Cairo University, Faculty of Dentistry, Cairo, Egypt. Thirty single-rooted human mandibular premolars with straight roots and mature apices were collected from the Oral Surgery Department. Teeth were radiographically examined in both mesiodistal and buccolingual directions to confirm the presence of a single patent canal and rule out teeth with calcified canals, root resorption, or any other anomalies. The external teeth surfaces were thoroughly cleaned with flowing water and disinfected in 5.25% sodium hypochlorite (NaOCl) solution for 15 minutes. An ultrasonic scaler was used to eliminate any soft-tissue remnants or hard deposits.

Teeth were accessed using round and tapered diamond burs mounted in a high-speed contra angle handpiece under water cooling. A size 15 K-file (Mani Inc., Tochigi, Japan) was used to verify the canal patency and determine the working length. The study included mandibular premolars with approximately similar apical diameters (size #20) and similar root length of (17 ± 1 mm) to ensure maximum standardization of the experimental groups. After working lengths were determined, the root canals were prepared using ProTaper Next rotary NiTi files (Dentsply Maillefer, Ballaigues, Switzerland) according to the manufacturer's instructions up to size X4 (#40/.06). The canals were irrigated between each file with 3 ml of 2.5% NaOCl.

Samples were randomly assigned into three groups (n = 10) according to the type of the final irrigation regimen: Group I, 5 ml of 0.2% CNPs; Group II, 2.5 ml of 0.2% CNPs followed by 2.5 ml of 17% EDTA (Meta biomed, Cheongju-si, Chungbuk, Korea); Group III, 5ml of 17% EDTA. In Groups I and III, irrigants were allowed to remain in the canal for 3 min, but in Group II, each irrigant was used for 1.5 min. Five milliliters sterile saline were used between the irrigants in Group II.

All canals were rinsed with 5 mL sterile saline and dried with paper points (Dentsply Maillefer, Ballaigues, Switzerland). The samples in each group were allocated into two subgroups (n = 5) based on the root-filling material: A, bioceramic root canal sealer (Sure-Seal Root bioceramic sealer; Sure-Dent Co., Seongnam-si, Gyeonggi-do, Korea); B, epoxy resin root canal sealer (AH Plus; Dentsply DeTrey GmbH, Konstanz, Germany). In subgroup A, the root canals were obturated with a size 40/.06 gutta-percha master cone (Dentsply Maillefer, Ballaigues, Switzerland) in combination with Sure-Seal Root BC Sealer according to the manufacturer's instructions, while in subgroup B, obturation was performed with a size 40/.06 gutta-percha master cone coated with AH Plus sealer. AH Plus was mixed according to the manufacturer's instructions and introduced into the canal in a pumping motion using the master gutta-percha cone. All canals were obturated using single-cone obturation technique.

Teeth were radiographed at different angulations to verify the presence or absence of voids in the filling mass and confirm the quality of obturation. Specimens were placed in 100% humidity at 37° C for 1 week to ensure complete setting of the sealers.

Nanoleakage evaluation

Evaluation of apical leakage was done using silver nitrate tracer penetration test. A modified silver staining technique was used with 50 wt% ammoniacal silver nitrate tracer solution. The solution was freshly prepared according to the protocol first described by Tay *et al*. [19].

After setting of the sealers, teeth were sealed coronally with Fuji II glass ionomer restoration. The external root surfaces were coated with two layers of

nail polish except for the apical 2 mm. After that, teeth were immersed into the freshly-prepared ammoniacal silver nitrate solution for 24 h in total darkness. The silver-impregnated samples were rinsed with distilled water for 5 min, and then placed in a photodeveloping solution under fluorescent light for 8 h to reduce the diamine silver ions into metallic silver grains within the voids along the sealer-dentin interface. After removal from the developing solution, samples were placed under running distilled water for 5 min.

Each sample was sectioned longitudinally in a bucco-lingual direction using a low-speed diamond disc under water cooling. The cut surfaces were then lightly sanded using a sequence of 600, 800, and 1000-grit abrasive papers followed by light polishing with a diamond paste. One half from each specimen was randomly selected for analysis of nanoleakage using a field emission scanning electron microscope (FESEM) operated in the backscattered-electron mode.

Nanoleakage was expressed in terms of silver nitrate penetration along the bonding interface. First, a group of images with 200x magnification was taken for assessment of linear nanoleakage from the root apex along the sealer-dentin interface on both sides. The measurements of silver nitrate penetration were calculated using Image J software. For statistical analysis, the longest line was considered. Another group of images with 400× magnification were used the extent of silver nitrate penetration into the dentinal tubules.

Statistical analysis

One-way analysis of variance with a *post hoc* Tukey test was performed to assess the impact of different irrigation protocols on the apical leakage of both sealers. Independent *t* test was used for intragroup comparisons between the tested sealers. The significance level was set at $p < 0.05$ for all statistical analyses.

Results

The results of the silver nitrate tracer penetration test are presented in Tables 1 and 2. SureSeal Root BC sealer showed significantly lower linear nanoleakage compared to AH Plus ($p < 0.001$), except for the CNPs group where there was no significant difference between both sealers ($p = 0.083$). For Sure-Seal Root BC sealer, no statistically significant difference was recorded among the three groups ($p = 0.284$). On the other hand, AH Plus displayed a significantly higher nanoleakage in the EDTA group ($p = 0.002$). Figure 1 presents SEM images of linear nanoleakage for AH Plus and Sure-Seal Root BC sealer in the EDTA group.

Table 1: Linear nanoleakage values (Mean ± SD) for both sealers in the different experimental groups

Linear nanoleakage (mm) (Mean ± SD)			
Group	Gutta-percha/ SureSeal BC sealer	Gutta-percha/AH Plus	p-value
Group I (CNPs)	1.15 ± 0.30	1.58 ± 0.38[b]	0.083
Group II (CNPs/EDTA)	1.17 ± 0.07	1.88 ± 0.25[ab]	<0.001*
Group III (EDTA)	1.40 ± 0.33	2.37 ± 0.15[a]	<0.001*
p-value	0.284	0.002*	

*Significant at $p < 0.05$, Different superscript letters in the same column indicate statistically significant values.

Table 2: The depth of silver nitrate tracer solution into the dentinal tubules (Mean ± SD) for both sealers in the different experimental groups

Depth of silver nitrate tracer solution into the dentinal tubules (μm) (Mean ± SD)			
Group	Gutta-percha/ SureSeal BC sealer	Gutta-percha/AH Plus	p-value
Group I (CNPs)	517.19 ± 30.81[b]	625.17 ± 23.80[b]	<0.001*
Group II (CNPs/EDTA)	436.83 ± 19.48[c]	646.54 ± 28.53[b]	<0.001*
Group III (EDTA)	565.53 ± 15.03[a]	717.12 ± 10.29[a]	<0.001*
p-value	<0.001*	<0.001*	

*Significant at $p < 0.05$, Different superscript letters in the same column indicate statistically significant values.

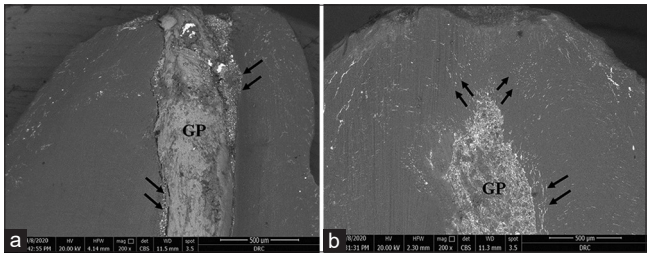

Figure 1: SEM images showing linear nanoleakage in (a) EDTA/AH Plus subgroup, (b) EDTA/Sure-Seal subgroup. (GP): Gutta-percha, the black arrows refer to the silver nitrate stain

The depth of silver nitrate penetration into the dentinal tubules was significantly higher in AH Plus subgroup compared to Sure-Seal subgroup within the three experimental groups ($p < 0.001$). For both sealers, the highest penetration depth for silver nitrate tracer solution was recorded in the EDTA group ($p < 0.001$). The penetration of silver nitrate stain into the dentinal tubules for both sealers is presented in Figure 2.

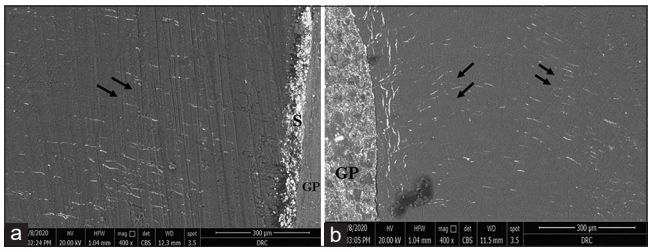

Figure 2: SEM images showing silver nitrate penetration into the dentinal tubules in (a) EDTA/AH Plus subgroup, (b) EDTA/Sure-Seal subgroup. (GP): Gutta-percha, (S): Sealer, the black arrows refer to the silver nitrate stain

Discussion

Three-dimensional obturation of the root canal system is one of the most important factors for long

term success of root canal treatment. Its main objective is to obtain a hermetic seal between root canal filling materials and radicular dentin. Endodontic sealers are used mainly to improve the seal between core filling materials and radicular dentin since most of the endodontic failures are due to leakage at the filling-dentin interface [20].

No current gutta-percha/sealer combination prevents leakage entirely, however, tubular penetration of root canal sealers increase the interface between filling materials and dentinal structure which may decrease the possibility of leakage. Therefore, maximum penetration of sealers into the dentinal tubules is essential to achieve a satisfactory seal and prevent microleakage [21]. The smear layer acts as a physical barrier preventing penetration of root canal sealers into the dentinal tubules, which increase the risk of bacterial infection and microleakage [2,22].

The sealing ability of root canal filling materials has been evaluated by various methods and techniques such as colored dye penetration test, bacterial penetration, radioisotopes, fluid filtration method, and electrochemical method. However, there is no universally accepted method for evaluation of leakage [23]. The dye penetration test is commonly used for assessment of the apical seal since it's easy to perform and does not require sophisticated materials [24].

Microleakage test was traditionally used to evaluate the sealing ability of root canal filling materials. In modern endodontics, nanoleakage test was introduced instead of microleakage for evaluating the sealing ability of root canal sealers. The term "nanoleakage" was introduced to describe the submicrometer-sized spaces located within the hybrid layer in the absence of gap formation, that allow for fluid infiltration reducing the bond efficacy with time [25].

The use of chitosan at 0.2% concentration was justified in previous studies that demonstrated its efficiency in removal of the smear layer at this low concentration with minimal dentin erosion [10], [26]. Nanoparticles have shown advanced physical and chemical properties in comparison to their parent materials in terms of ultra-small size, larger available surface area, and increased chemical reactivity [12], [13]. The concept of using CNPs in combination with EDTA (1:1) was elaborated to achieve the ultimate chelating effect as proposed in a previous study [27].

AH Plus is an epoxy resin-based sealer that chemically bonds to root dentin, as a result of the covalent bonding between the epoxy rings and collagen amine groups [28], [29]. On the other hand, Sure-Seal Root BC sealer is a premixed calcium alumino silicate paste developed for permanent obturation of the root canal. Bioceramic sealers composed mainly of zirconium oxide, calcium silicates, calcium phosphate monobasic, calcium hydroxide, filler, and thickening agent [30]. They are dimensionally stable with excellent physical properties since they utilize the moisture naturally present in the dentinal tubules to initiate and complete the setting reaction [31], [32]. Bioceramic sealers bond mechanically to dentin by tubular diffusion of the sealer particles forming mechanical interlocking bonds, and chemically by infiltration of the sealer mineral content into the intertubular dentin establishing a mineral infiltration zone. In addition, calcium silicate hydrogel and calcium hydroxide formed from the hydration reaction of calcium silicate tend to react with phosphate ions forming a hydroxyapatite layer along the mineral infiltration zone [33].

In the present study, nanolakage was evaluated using ammoniacal silver nitrate tracer solution as it provides good results as described in previous studies [34], [35]. This tracer solution can penetrate dentin because of the small silver ion size (0.059 nm in diameter). In addition, silver ions have a very small diameter compared to the size of some bacteria (0.5–1.0 mm) which would provide clinically significant information in evaluation of nanoleakage [36]. Consequently, if the sealer prevents silver nitrate penetration *in vitro*, it would probably also prevent bacterial leakage *in vivo*. The teeth were sealed coronally with glass ionomer restoration and the root surfaces were coated with two layers of nail varnish except for the apical 2 mm, so the tracer solution was allowed to work only from the apical foramen. The specimens were sectioned longitudinally to evaluate the extent of apical leakage along the root filling-dentin interface [34], [35], [37].

The teeth were immersed in 50 wt% ammoniacal silver nitrate solution for 24 h in total darkness to allow the silver nitrate to diffuse along the bonding interface. The silver impregnated samples were rinsed thoroughly with distilled water for 5 min, and then placed in a photodeveloping solution for 8 h under fluorescent light to reduce the silver ions into metallic silver grains within voids along the sealer-dentin interface [19].

Silver uptake was evaluated using FESEM operating in the backscattered-electron mode that has been widely used to evaluate interfacial nanoleakage expression at the adhesive-dentin interfaces [37], [38]. The backscattered-electron mode of FESEM can produce high contrast images because of its element atomic number dependent characteristics. Silver (Ag) used in the present study has a high atomic number, so it can reflect a larger number of electrons and produce a brighter image than the background [38].

In the present study, the results obtained with SEM examination showed that AH Plus had a significantly higher nanoleakage compared to Sure-Seal Root BC sealer in the EDTA and CNPs/EDTA groups. The extensive nanoleakage observed with epoxy resin sealers is due to incomplete resin infiltration of the thick demineralized collagen matrices created after EDTA irrigation [39], [40]. These demineralized collagen matrices are susceptible to collapse after drying the canals with paper points hindering complete resin infiltration [39], [40]. Additionally, epoxy

resin sealers tend to shrink during setting which might result in poor sealer adaptation and cause de-bonding from root canal wall [41]. On the other hand, Sure-Seal Root BC sealer showed less nanoleakage as it does not shrink on setting, and the apatite-like interfacial deposits formed during the setting reaction tend to seal gaps at the sealer-dentin interface [42], [43]. In the CNPs group, no significant difference was found between AH Plus and Sure-Seal Root BC sealer as chitosan causes less dentin demineralization compared to EDTA [9]. This could be also the reason behind the significant difference in AH Plus nanoleakage between CNPs and EDTA groups.

The results of the current study showed that silver nitrate penetration within the dentinal tubules was significantly higher with AH Plus compared to Sure-Seal Root BC sealer in all groups. This may be explained by the higher flowability and smaller particle size of Sure-Seal Root BC sealer which enhances sealer penetration into the dentinal tubules, especially the smaller-sized tubules at the apical root area. While AH Plus sealer contains larger particles which might not easily penetrate the smaller tubules at the apical root part [41], [44]. These results are in agreement with some recent studies that showed better adaptation of bioceramic sealers to the root canal wall and higher penetration of these sealers into the dentinal tubules compared to AH Plus, especially in the apical third of root canals [45], [46]. For both sealers, the highest penetration depth of silver nitrate tracer solution in the dentinal tubules was observed in the EDTA group, as EDTA causes more alterations in dentin surface structure and Ca/P ratio [9].

Conclusions

Based on the findings of this study, it can be concluded that the apical sealing ability of bioceramic sealers is better than that of epoxy resin-based sealers. Moreover, the type of the final irrigating solution seems to affect the post-obturation seal of both AH Plus and Sure-Seal Root BC sealer. Compared to EDTA, CNPs had less adverse effects on the apical sealing ability of both sealers. Further studies are required to confirm the benefits of CNPs as a chelating and antibacterial agent, and to assess its effect on the bacterial biofilm and smear layer removal in combination with different irrigation activation techniques.

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