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THE EFFECT OF NANOPARTICLE ADDITION TO A BIOCERAMIC SEALER ON ITS INTRA-TUBULAR PENETRATION

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ABSTRACT

Introduction: The aim of this study was to evaluate the effect of titanium dioxide nanoparticles (TiO2NP) addition on Endoseal MTA intra-tubular penetration after cleaning and shaping of root canal with different irrigation protocols using the confocal laser scanning microscopy (CLSM).

Methods: 56 human mandibular premolars were used. The access cavities were opened. All teeth were instrumented with a crown-down technique, using a set of ProTaper rotary instruments to achieve a size #40 apical preparation. After biomechanical preparation, teeth were divided equally into 2 groups (n=28) G1 (Endoseal MTA without TiO2NP) and G2 (Endoseal MTA with 1 wt% of TiO2NP). Each group was further subdivided equally into 2 sub-groups (n=14): sub-group N (5.25% NaOCL) and sub-group T (Green tea). Each irrigant was further subdivided equally into 2 sub-groups (n=7): sub-group CI (conventional needle irrigation) and sub-group PUI (Passive ultrasonic irrigation). Sealers were mixed with the Rhodamine B fluorescent dye for analysis of sealer penetration under the CLSM at 10x magnification and obturation was carried out in lateral condensation technique. From each root 3 (1 mm) slices were obtained representing the coronal, middle and apical thirds. Results were tabulated and statistically analyzed.

Results: The results showed that NaOCl groups had a significantly higher value of sealer intra-tubular penetration than green tea groups in both types of sealer. PUI groups had also a significantly higher value of sealer intra-tubular penetration than CI groups in both types of sealer. The results also showed that Endoseal MTA sealer without TiO2NP had a higher value of intra-tubular penetration than Endoseal MTA sealer with TiO2NP yet the difference was not statistically significant.

Conclusion: Both of NaOCl and PUI permit a significantly greater sealer intra-tubular penetration compared to green tea and CI. Endoseal MTA exhibited greater intra-tubular penetration compared to Endoseal MTA with TiO2NP.

Key Words: Sealer intra-tubular penetration, Endoseal MTA, Titanium dioxide nanoparticles, Sodium hypochlorite, Green tea, Passive ultrasonic irrigation, conventional needle irrigation, confocal laser scanning microscopy.

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INTRODUCTION

The major aim of root canal therapy is to provide a three-dimensional obturation of the root canal system. A secure seal prohibits apical periodontitis, minimizes coronal leakage and bacterial infection, and entombs the residual irritants in the root canal. Different endodontic materials have been developed for finish and impermeable fillings. Root canal sealers are needful to fill the space between the obturating material and the root dentin wall. Sealers should fill voids and irregularities, and also plug the root canal apically and laterally. The capability of the sealer to permeate into the dentinal tubules is substantial, as this inhibit penetration by microorganisms and toxins and assists the sealer provide a fluid-tight seal (1).

Chemomechanical preparation of the root canal system is a main procedure for endodontic success. The bacteria, related irritants, pulp tissue, dentin debris, and the smear layer should be removed from the root canal system during the step of root canal therapy. The existence of the smear layer acts as a barrier that may impede the penetration of root canal medications, irrigantions, and/or sealers into the dentinal tubules. Numerous irrigant delivery techniques and agitation methods have been suggested to solve this problem and hence, preserve effective cleaning and disinfection during final irrigation. Agitation of irrigants may also improve the sealing properties of root canal filling, providing a better seal interface between root filling and canal walls. Also the efficiency of final irrigant has been a certain phenomenon for elimination of smear layer after the cleaning and shaping of the root canal system (2,3).

Bioceramic sealers have only been available for use in endodontics for the last 30 years, they are containing calcium phosphate, with a chemical composing and a crystalline construction analogous to tooth apatite materials and bone. Besides to their mechanical and physical characteristics and superb sealing capability, their biocompatibility to the surrounding tissue. A new systematic review stated that the permeation of bioceramic sealer is basically more considerable than that of epoxy resin-based sealers (AH Plus, for example), even with activated irrigation techniques and chelating agents (4,5).

Nanotechnology is regarded an upcoming technology. In dentistry it aids in diagnosis, curative and preservation. Nanotechnology involves the usage of nanoparticles less than 100 nm. The technology assists in modifying the present restoration in terms of chemical, physical and biological aspects to progress their quality. Since the size of nanoparticles can permeate the dentinal tubules to ensure that all the spaces have been locked efficiently, the development of a sealer based on nanotechnology may be a remarkable step to obtain a preferable sealer in endodontics. It is worth mentioning that TiO2NP have drawn attention in the dental materials scope (6,7,8).

This in vitro study evaluated the effect of TiO2NP addition on Endoseal MTA intra-tubular penetration after cleaning and shaping of root canal with different irrigation protocols using the CLSM.

MATERIALS AND METHODS

56 human mandibular premolars that were freshly extracted for orthodontic reasons. Teeth were thoroughly cleaned from any hard and/or soft tissue deposits, then they examined for the following inclusion criteria: sound crowns and roots, complete formation of root apex, no visible crown or root caries, no cracks and no signs of internal or external resorption or calcification.

Sample classification and preparation: Access cavity was opened, and canal patency was verified using a #10 K file (Mani, Tochigi, Japan), then root canals were instrumented with a crown-down technique using ProTaper universal rotary system (Dentsply, USA) till f4 following the manufacture guides. Irrigation was performed using 3 ml of 2.6% NaOCL (Alexandria for detergents and chemicals, Alexandria, Egypt) after every change of instrument. After biomechanical preparation, teeth were distributed as shown in the below diagram:

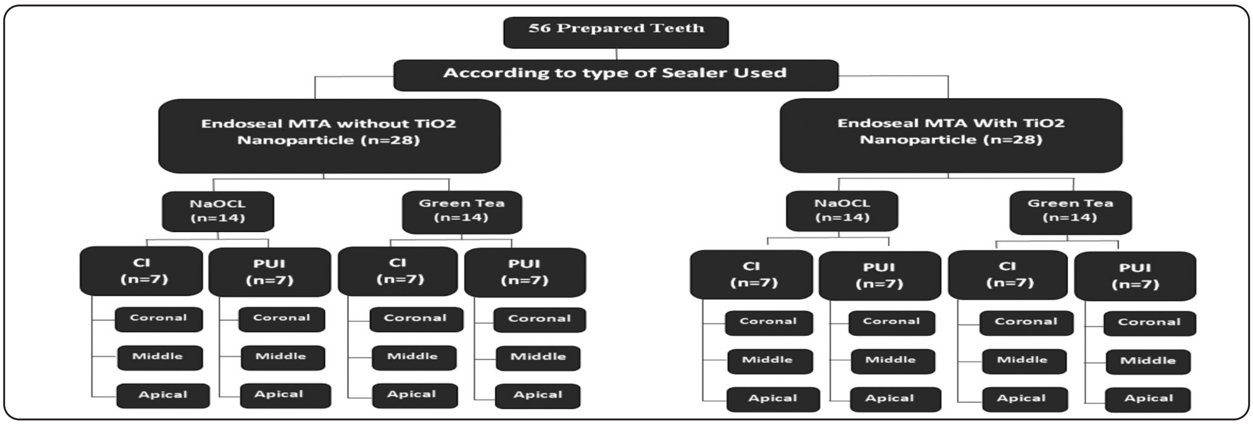


Diagram (1): Sample distribution for the sealer penetration test.

NaOCL with CI sub-groups: Irrigation with (5 ml) 5.25% NaOCl over 60 seconds was applied as a final flush with CI by a 27 G side vented needle mounted on a handle held syringe(Ameco, Egypt). The irrigation needle was placed 1 mm short of the working length and the needle was constantly pulsed 1-2 mm in the apical to coronal direction during irrigation.

Green tea with CI sub-groups: Irrigation of the samples was done as the same manner as NaOCL with CI sub-groups but (5 ml) Green tea (Twinning s of London) was used instead of NaOCL.

NaOCL with PUI sub-groups: (5 ml) 5.25% NaOCl was delivered inside the root canal using conventional syringe and 27G side vented needle placed 1 mm from the working length. PUI was performed using an ultrasonic scaler and an endo ultrasonic tip (Woodpecker, China) in 6th power setting. The tip was inserted into the canal 1 mm short of the working length, and the irrigant was ultrasonically activated for 60 s. The tip was kept as centered as possible to decrease contact with the canal walls, as any contact with the canal wall could inhibit the oscillatory movement of the file.

Green tea with PUI sub-groups: Irrigation of the samples was done as the same manner as NaOCL with PUI sub-groups but (5 ml) Green tea was used instead of NaOCL.

Manufacturing method of TiO2NP (Nanogate company, Cairo, Egypt): Anatase particles were prepared by precipitation from homogeneous solution using titanium (IV) isopropoxide as precursor in aqueous solution acidified with nitric acid to pH 2 using a water-to-titanium mole ratio of about 200 [9]. Morphology and size were measured by using TEM, JEOL JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 kV, respectively.

After mechanical preparation and the final irrigation procedures, the root canals were irrigated with 5 ml saline solution and dried using paper points. Then:

G1 (Endoseal MTA without TiO2NP): The sealer was mixed with the Rhodamine B fluorescent dye (Sigma–Aldrich,St. Louis, MO, USA) for further analysis of the sealer penetration under CLSM (Carl Zeiss Microscopy, Thornwood, NY, USA). 1gm of the Endoseal MTA sealer (Maruchi, Wonju, Korea) was mixed with 0.001gm of the 0.1% Rhodamine B dye [10]. Obturation of the samples was carried out in lateral condensation technique [11] using the fluorescent labeled Endoseal MTA and gutta-percha in low light conditions as the Rhodamine B dye is sensitive to light) and heat. After obturation the orifices of all teeth were sealed with temporary filling material Cavit (3M, ESPE, and St. Paul,USA) and teeth were coated with 2 layers of nail varnish (Yolo,France) and stored in an incubator at 37 c and 100 % humidity till complete setting of the sealer.

G2 (Endoseal MTA with TiO2NP): 1gm of the Endoseal MTA sealer was mixed with 0.01gm of the TiO2NP (1% weight ratio) [8,12] and 0.001gm of the 0.1% Rhodamine B dye. Then Obturation of the samples was completed as the same manner as G1.

Evaluation of sealer penetration: Then the roots were loaded onto resin stubs and then were transversely cut using microtome (BUEHLER, Illinois, USA). From each root 3 slices were obtained representing the coronal, middle and apical portions (3, 6 and 9 mm from the root apex) making a 1 mm thickness slices. The slices were then thoroughly investigated under CLSM. The images were analyzed by using Ziess Microsystems software (LAS-AF). Each x10 sample was evaluated for a consistent fluorescent ring around the canal wall, indicating the sealer-dye distribution. The area of deepest penetration was recorded and viewed at x40. The sealer intra-tubular penetration depth was illustrated by the fluorescence, which was traced from the sealer-dentin junction until the maximum depth. The measurements were recorded by using the digital measuring ruler, a feature present in the CLSM image recorder software. The canal wall served as the starting point, and sealer penetration was measured to the outer limit of the visible field in the microscope. Measurements of the tubular dentin sealer penetration were carried out. The following formula [11] was used to calculate the tubular dentin sealer penetration: Dentin area = Total area - root canal area and Percentage of tubular dentin sealer penetration =

$$\frac{\text{Area filled by sealer} - \text{root canal area}}{\text{dentin area}} \chi 100$$

RESULTS

Data were presented in table1 and figure1. The results showed that NaOCl groups had a significantly higher value of sealer intra-tubular penetration than green tea groups in both types of sealer (p=0.043). PUI groups had a significantly higher value of sealer intra-tubular penetration than CI groups in both types of sealer (p<0.001). Also the results showed that Endoseal MTA sealer without TiO2NP had a higher value of intra-tubular penetration than Endoseal MTA sealer with TiO2NP yet the difference was not statistically significant (p=0.061). The maximum sealer penetration was achieved in coronal third, followed by the middle third and least in the apical third area.

Table (1): Mean, Standard deviation (SD) values of percentage of intra-tubular dentin sealer penetration for different types of sealers

Irrigation material	Irrigation mode	Root section	Percentage of intra-tubular dentin sealer penetration (mean±SD)		p-value
			Without TiO2	With TiO2	
NaOCl	Conventional needle irrigation	Coronal	68.46±24.36	56.40±6.34	**0.247ns**
		Middle	48.99±2.32	33.70±10.64	**0.008***
		Apical	54.11±21.96	54.06±7.08	**0.995ns**
	PUA	Coronal	70.85±11.92	52.98±3.31	**0.007***
		Middle	62.10±15.10	72.12±12.89	**0.207ns**
		Apical	35.18±14.84	72.78±6.52	**<0.001***
Green tea	Conventional needle irrigation	Coronal	45.85±9.92	47.81±19.04	**0.815ns**
		Middle	50.71±5.20	54.85±10.92	**0.390ns**
		Apical	39.22±17.98	30.34±8.14	**0.266ns**
	PUA	Coronal	83.15±11.69	56.28±16.60	**0.005***
		Middle	71.39±6.67	43.53±5.12	**<0.001***
		Apical	49.22±12.85	42.25±11.58	**0.307ns**

; significant (p ≤ 0.05) ns; non-significant (p>0.05)

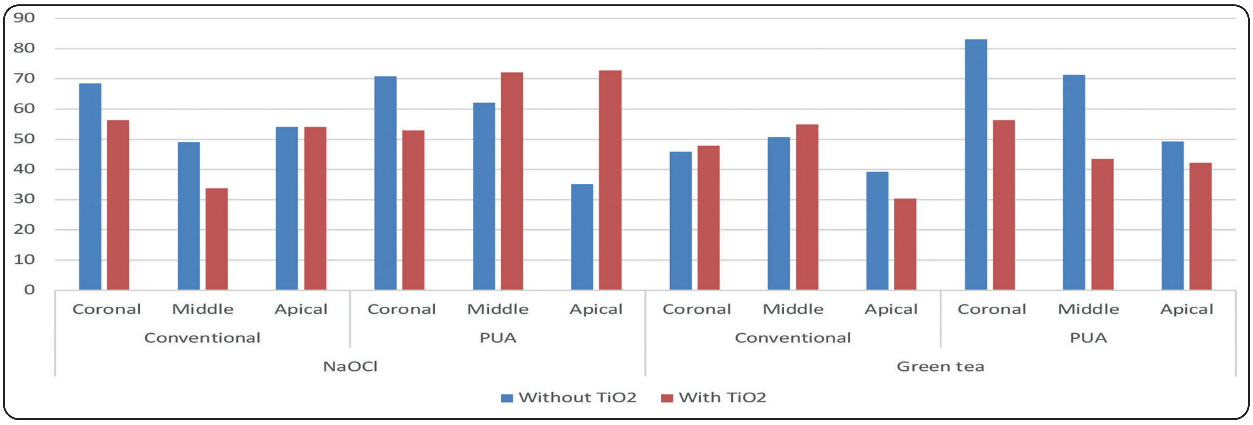
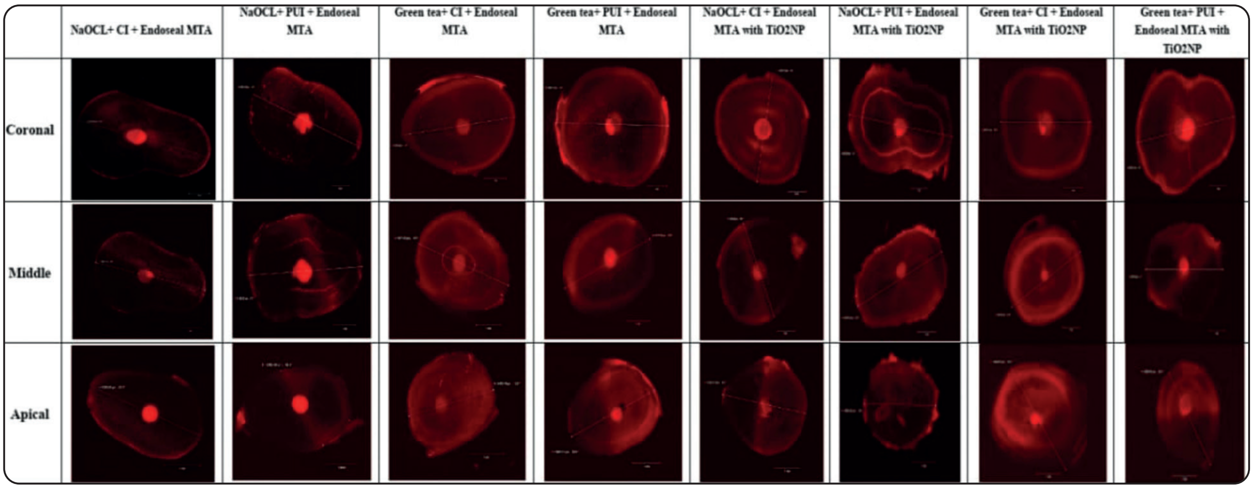


Fig. (1): Bar chart showing average percentage of intra-tubular dentin sealer penetration for different types of sealers.



DISCUSSION:

It is known that sealer intra-tubular penetration is valuable for an appropriate therapy result. Penetration of sealer would increase the interface between the material and dentin, thus would enhance sealer's mechanical retention, this will in turn decrease micro leakage. It has also been observed that the farther a sealer intra-tubular penetration, the more it could do its antibacterial impact, if present. There are numerous factors which would affect the depth of sealer permeation such as type of irrigating technique, sealer application, final irrigating solution used, obturation technique, physical and chemical properties of a sealer such as viscosity, solubility, particle size, surface tension and film thickness. Flow is a significant physical property of a sealer as it would allow sealer penetration deeper into the accessory canals and irregularities of the root canal system [13].

In the present study, preparation of canal was done using the Protaper Universal system up to a master apical file size-F4, in accordance with the studies carried out by ***Agrawal et al.*** [14]; ***Machado et al.*** [15]; ***Mumcu et al.*** [16]. Endodontic instrumentation creates a smear layer, many reports suggest that the smear layer itself can be infected and might prevent the root canal medication and irrigant intra-tubular penetration. This is why removal of the smear layer prior root canal obturation is generally recommended, in spite of the argument around its elimination [17]. The efficiency of final irrigant has been a certain phenomenon for elimination of smear layer after the cleaning and shaping of the root canal system [18]. Subsequently one of the most important factors affecting sealer intra-tubular penetration is the type of final irrigating solution used. NaOCl has been long used as the irrigation solutions in clinical dental practice. Its use in demineralizing surface dentin to expose the contents of the dentinal tubules has been already reported. A previous study showed that the usage of NaOCl with 5.25 % concentration showed larger zones of inhibition of *E.faecalis* biofilms compared to other NaOCl concentration [13]. Hence in our study we have used 5.25% conc. of NaOCl for our study. Also numerous plant types have been tested to define their capabilities to disinfect the root canal system in root canal therapy. The main advantages of the use of herbal alternatives in root canal treatment are that the products are easy to acquire and inexpensive, have long shelf lives and low toxicity, and cause no microbial resistance [19]. Green tea was used in this study as it was shown to contain epigallocatechin gallate (EGCG) [20]. Latterly, ***Lee et al.*** [21] showed that EGCG is an efficient antimicrobial agent versus both the planktonic and biofilm forms of E. faecalis and inhibiting bacterial growth. Green tea extract also found to be a good chelating agent [22].

To enhance the elimination technique of the smear layer, the final irrigant used in this study were associated with PUI. Its active tips make mechanical-vibrating impacts on the dentin walls, providing more efficient cleaning and sealing of the dentinal tubules [23]. Also in our study we used CI by using a 27 G side vented needle mounted on a handle held syringe. This technique is still vastly agreeable by both endodontists and general practitioners. One of the advantages of CI is that it relatvely permits easy control of the irrigant quantity that is flushed through the canal and needle penetration depth within the canal [24].

Regarding the materials, Endoseal MTA was choosen for our study. This sealer as claimed by the manufacturer has the advantages of hardening even at the most complex and wet canal. Endoseal MTA is made of fine particles. It has calcium silicate that increases its flow characteristics and makes it perfect to be used as a sealer [13]. TiO2NP were also selected for our study. They were added to Endoseal MTA sealer. They have anatase crystalline structure. They are powder, spherical like shape, and with average size less than 15 nm. In this study, TiO2NP were used in combination with Endoseal MTA at 1 wt%, based on the results of a studies by ***Khataee et al.*** [25]; ***Samiei et al.*** [8,12].

Laboratory studies have concluded that sealer distribution inside the canal walls is not influenced by the sealer application method in the canal [13]. Hence in our study obturation of all samples was done with lateral condensation technique [11]. CLSM has particular advantages over SEM and other techniques that evaluate sealer adaptation and permeation. The labelling with Rhodamine B dye is necessary for observation the sealer penetration and adaptation amount. The fluorescence imparted by the Rhodamine dye aids in the visualization of the penetration depth as sealers adaptation in the horizontal section, even at very low magnifications. In the present study, we used a magnification of 10x, for easy visualization of tubule permeation and canal walls [13].

According to our study, sealer penetration was deeper and more equally diffused at the 9mm compared to that at the 6 and 3 mm. The findings are in line with a preceding studies with ***Akcay et al.*** [26]; ***Mamootil et al.*** [27]. This reduced penetration in the apical portion could be explained by small diameter of dentinal tubules, decreased tubules number in the apical portion, inefficient irrigant delivery in this portion and greater tubule sclerosis in the area. Besides, during obturation higher compressive forces may have been applied that could also ease deeper permeation into the coronal and middle parts of the canal wall [13].

The results showed that NaOCl groups had a significantly higher value of sealer intra-tubular penetration than green tea groups in both types of sealer. This might be due to that NaOCl was able to eliminate smear layer more than green tea which might be due to acid metabolites absence -in case of green tea - which is needed for the elimination. ***Sebatni et al.*** [22]. Agreed with the present results, but on contrast ***Kartik et al.*** [28]. The results also showed that PUI groups had a significantly higher value of sealer intra-tubular penetration than CI groups in both types of sealer. Ultrasonic activation probably enhanced the smear layer / debris elimination by causing shear stress in the inorganic particles of the smear layer by acoustic streaming, facilitating its elimination. However, the irrigation delivered by traditional needle permeates only from 0 to 1.1 mm deeper than the tip of the needle, and gas particles are produced and restricted in the apical part, creating a vapor lock and preventing the irrigation debridement efficacy. On the other hand, PUI permits the removal of vapor lock impact, enhancing the effectiveness of the irrigant. The safety of CI has been also questioned as the positive pressure used to deliver the irrigant into the canal may extrude it to the periapex, causing harm of tissue and pain postoperative [29]. These reasons could prove our results and hence confirm that PUI as a final irrigation protocol is superior over CI concerning increase sealer intra-tubular penetration. The present results are in agreement with ***Machado et al.*** [15]; ***Akcay et al.*** [26]; ***De Souza et al.*** [29]; ***Gu et al.*** [30]; ***Aksel et al.*** [31]; ***Zand et al.*** [32], but on contrast ***Yilmaz et al.*** [33] as curved root canals were used which is a challenging case to deliver the irrigation to the apical portion regardless of the irrigation technique.

Also the results showed that Endoseal MTA sealer without TiO2NP had a higher value of intra-tubular penetration than Endoseal MTA sealer with TiO2NP yet the difference was not statistically significant. This might be attributed to that the flow of the sealer was decreased by the addition of TiO2NP, which may explain the difference in penetration inside dentinal tubules. This also could be due to that the surface energy of the sealer was decreased by the addition of TiO2NP as the surface energy of Endoseal MTA is higher than the surface energy of TiO2NP.

CONCLUSION

Both of NaOCl and PUI permit a significantly greater sealer intra-tubular penetration compared to green tea and CI. Endoseal MTA exhibited greater intra-tubular penetration compared to Endoseal MTA with TiO2NP, yet the difference was not statistically significant.

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