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The effectiveness of different irrigation strategies with laser activation on the bond strength of MTA as root end filling material: in vitro study

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Abstract

Objective: To compare the push out bond strength of MTA apical plug in a simulated immature root, after using different irrigation protocols; NaOCl + 20% Etidronic acid (HEBP) and NaOCl + 17% Ethylenediaminetetraacetic acid (EDTA) with and without diode laser activation.

Methods: Twenty-eight extracted single straight rooted permanent human teeth were selected. The root lengths were standardized (15 mm) by decontamination of all samples. Instrumentation was done using the ProTaper Next system (Dentsply Sirona), the canals were flushed with 1 mL NaOCl after each instrument. Then, the apical 3 mm was cut perpendicular to the root's long axis and the canals were prepared till file 90 (master file) using the balanced force technique to simulate immature teeth. Samples were divided into four experimental groups ($n = 7$) in accordance with the irrigation protocol; G1 (NaOCl + HEBP), G2 (NaOCl + EDTA) both G1 and G2 were activated with diode laser, while G3 (NaOCl + HEBP) and G4 (NaOCl + EDTA) were activated with manual agitation. Canals were filled with a 5-mm MTA (ProRoot MTA, Dentsply Tulsa Dental) apical plug. Each root was transversely sectioned perpendicular to their long axis to have a 3 mm \pm 0.1 sections in thickness from the root apical portion. A push-out test was performed, and the failure pattern was assessed. Push out bond strength values were analyzed with student t test for compared pairs. Two-way ANOVA was used to detect the effect of each variable (chelating agent with/without laser activation).

Result: Effect of diode laser activation in G1 and G2 showed no significant differences ($p > 0.05$) on the push out bond strength values of MTA, while in manual activated groups (G3 and G4), there was a statistically significant difference ($p < 0.05$) where G4 recorded higher push out bond strength mean values than G3.

Conclusions: The diode laser activation when used with NaOCl and HEBP increased the effect of HEBP as chelating agent and improved the push out strength values of MTA, rendering it an alternative mild chelating agent in comparison with the EDTA.

Keywords: Immature permanent teeth, Mineral trioxide aggregate, Bisphosphonates, Diode laser, Intracanal irrigation activation, NaOCl, EDTA

Background

The development and apical closure of permanent teeth are completed following three or four years after the process of eruption (Hargreaves et al. 2013). Deep caries or traumatic injuries within the developmental period usually result in irreversible pulpitis or periapical

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periodontitis or even necrotic pulp (Hargreaves et al. 2013). Risks accompanied to traditional root canal treatment to undeveloped permanent teeth may occur. It can be due to fractures of thin dentinal walls or inadequate apical seal as apices are widely opened (Kalaskar and Kalaskar 2013; Shabahang 2013). Apexification is used for immature teeth with necrotic pulp, where the induction of apical calcified barrier by using certain materials to avoid the extrusion of root canal filling to the periapical area (Apexification 2005).

Therefore, adequately done root canal debridement supports and stimulates the reparative processes in apical periodontal tissues. Endodontic treatment is based on proper biomechanical preparation (Hargreaves et al. 2013). Furthermore, to the apical hermetic sealing, a biologic material that could prevent overfilling should be used (Kalaskar and Kalaskar 2013; Shabahang 2013). A biocompatible material is required to be used. Also, with adhesive and physio-chemical stability to accomplish obturation (Apexification 2005; Sa-maria et al. 2019). Mineral trioxide aggregate (MTA) material accomplishes many of required characteristics (Sa-maria et al. 2019).

MTA is a calcium silicate material incorporated Portland cement with bismuth oxide as radio-opaque (Coomaraswamy et al. 2007). MTA increased patient compliance, minimizing the number of treatment visits, improved rate of healing (Songtrakul et al. 2020) and decreasing the risk of subsequent fracture. This may be referred to advantage features including biocompatibility, dimensional stability, high alkaline pH rendering it bacteriostatic, and its ability of hard tissue formation which aids in induction of apical barrier in necrotic immature teeth (Erdem and Sepet 2008; Torabinejad et al. 2018).

Proper conditioning and debridement of root canal walls is crucial step that facilitates the removal of bacteria, debris, necrotic tissue and allow proper adaptation and bond of MTA.

Smear layer existence has a role in the diffusion of bacteria, molecules and ions into the filling material interface and root canal walls as it shelters and nutritive to it. It is considered as a barrier between the filling material and canal walls, obliterating dentinal tubules thus, preventing the penetration of disinfecting agents. (Cobankara et al. 2011; Lui et al. 2007).

Proper mechanical bonding between root end filling materials and root canal walls is achieved across the penetration of the cements to the root canal substrate to result in mechanical interlocking. Obtaining proper adhesive bond, the root canal walls must be thoroughly disinfected, through the elimination of the smear layer, and creation of a rough surface (Asnaashari and Safavi 2013).

The gold standard irrigation protocol assigns the association of sodium hypochlorite (NaOCl) and ethylenediaminetetraacetic acid (EDTA) in association with dynamic agitation/activation of the irrigating solutions to enhance a 3D cleaning of the complex canals' anatomy. (Van der Sluis et al. 2007a).

Nevertheless, using EDTA during root canal disinfection has several pitfalls. The integrated implementation of NaOCl and EDTA reported a reduction in the volume of free chlorine in the mixture that is important for the antibacterial action and dissolving abilities of the NaOCl (Grawehr et al. 2003). Moreover, it was revealed that the high surface tension of EDTA decreased its wetting capacity at the apical thirds of the root canals, thus limiting smear layer removal (Zivanovic et al. 2019). Other possible causes for the limited decalcification effect of EDTA are the reduced non-collagenous organic matrix in the apical third of root canals, as the chelation process includes withdrawal of the water-soluble phosphoproteins, non-collagenous proteins, beside the calcium removal. (Yilmaz et al. 2011).

Etidronic acid or etidronate or 1-hydroxyethylidene-1,1-bisphosphonate (HEBP) is a suggested substitute chelating agent to EDTA, which is commonly used in a concentration of 20%. In the pharmaceutical industry, it is used as an osteoporotic agent, while in the metal industry, it is used as an anticorrosive agent and in the soap industry to prevent oxidation and rancidification of fatty acids. (Yadav et al. 2015).

In consideration of increasing the irrigants effectiveness and safety delivery to the peripheral portion of the RC, activation of the endodontic irrigants turned to be very essential. It can be performed using ultrasonic, laser and even manual agitation. (Van der Sluis et al. 2007b).

Laser application in endodontics, like carbon dioxide (CO₂) lasers and Neodymium: Yttrium–Aluminum–Garnet (Nd:YAG), had proved their ability in disinfecting and cleaning the root canal and lateral dentinal tubules (Kimura et al. 2000). The diode laser has acquired expanding significance due to its density, low cost, and efficiency. The diode laser is advocated for root canal treatment due to its wavelength is within the infrared range. Also, its intracanal tips are thin and flexible fibers are easily used inside the canal. Previous studies illustrated the bactericidal effect of 810-nm wavelength, and 980-nm wavelength diode lasers (Moritz et al. 1997; Gutknecht et al. 2004).

The design of the current study is comparing the push out bond strength of MTA apical plug in a simulated immature root, after alternating use of 20% bisphosphonates (HEBP) and 17% EDTA in combination with 5.25% NaOCl with and without diode laser activation.

Methods

Sample collection & preparation

A total of 28 freshly extracted human single straight rooted permanent teeth were stored from the surgery clinic at the National Research Centre, Egypt.

Inclusion and exclusion criteria

Teeth were examined under magnification $\times 10$ to exclude teeth with caries, cracks and fractures. They were also radiographed buccolingual and mesiodistal to exclude those with any resorptive defects. Teeth were washed under running water and scaled to remove any calcified tissues. They were then immersed for 10 min in 5.25% NaOCl to dissolve any tissues on the root interfaces and then immersed in distilled water till time of use.

Preparation of specimens

A sectioning disk was mounted on a low-speed hand-piece, and the coronal segments of all samples were sectioned, to standardize the tooth length at 15 mm.

Instrumentation was done using the ProTaper Next system (files X1-X3) (Dentsply Sirona). Using a 27 gauge needle, the root canals were flushed with 2 mL 5.25% NaOCl solution, followed by 2 ml of distilled water.

Root-end resection samples and root-end filling

A sectioning disk was employed to remove 3 mm from the apical end of the root in a right-angle direction. The apical portion of all samples was enlarged with K-files till size 90 using balanced force technique.

The prepared samples were randomly divided into four ($n=7$ /group) experimental groups in accordance to the irrigation protocol. Four groups were formed as follows:

Group 1 (G1), irrigation using 5 ml 5.25% NaOCl followed by 5 ml HEBP activated with the diode laser (wavelength: 980 nm) with 2.0 W output power in continuous mode (Doctor Smile, Italy). The optical fiber (200 μ m) was positioned 1 mm short of the length of the root canals. The irradiation was sustained three times with ten seconds interval.

Group 2 (G2), the root canals were irrigated in the same way like group 1, but using 5 ml 17% EDTA. The irradiation was repeated three times with 10 s interval.

Group 3 (G3), irrigation using 5 ml 5.25% NaOCl followed by 5 ml HEBP activated with manual agitation for 5 min.

Group 4 (G4), irrigation using 5 ml 5.25% NaOCl followed by 5 ml EDTA activated with manual agitation for 5 min.

Slight dryness of the root canals was done using paper points. A 5-mm apical plug was prepared as stated to the respective of manufacturer's recommendations using white MTA (ProRoot MTA, Dentsply Tulsa Dental). It was incrementally placed in ortho-grade direction.

Push-out test procedure

A transverse section for each specimen at a right angle direction of the roots long axis using a water-cooled precision disc to have a 3 mm \pm 0.1 sections out the root apical third. Specimens were measured using digital caliper (Pachymeter, Electronic Digital Instruments, China). Each section was labeled and photographed by a stereomicroscope -magnification of 65x (SZ-PT; Olympus, Tokyo, Japan) from coronal and apical planes. Calibration was done in this study by comparing a ruler of a known length, using the "Set Scale" tool obtained by Image J analysis software (NIH, Bethesda, MD). The cement diameter was measured, and accordingly, the radius was calculated.

Custom-made loading fixture was used to mount the samples, then samples were exposed to a crosshead speed of 1 mm/min compressive load through a computer controlled materials testing machine (Model 3345; Instron Industrial Products, Norwood, MA, USA).

A plunger of 0.75 mm diameter was used to apply the load on tested cement filling samples, where the tip was positioned touching the filling only, away from the circumferential dentin, from the narrow dimension of the specimens to push the filling toward the wider coronal direction, therefore, preventing any constraint of the cement displacement. This guarantees that the dentin was adequately supported in the loading course.

The maximum load failure was calculated in MPa. The bond strength was calculated as the maximum load divided by the computed surface area by the following formula (Nagas et al. 2007)

$$\left[A = \left(3.14 * H * \left(r^1 + r^2 \right) \right) \right],$$

r^1 = apical radius, r^2 = coronal one, H = sample thickness in millimeters.

Failure was demonstrated through the displacement of cement, confirmed alongside the sudden decrease the load-deflection curve, that was recorded by Blue-hill computer software. The push-out bond strength was calculated for each specimen.

Results

The results were analyzed using Graph Pad InStat software (Graph Pad, Inc.). The $P \leq 0.05$ value was reviewed statistically significant. Continuous variables were revealed as standard deviation and the mean values. Student t test was used for compared pairs after similarity of variance and normal dispensation of errors had been settled. Two-way ANOVA was used to distinguish the effect of every variable (chelating agent -laser activation). Sample size ($n=7$) was enough to detect large consequence sizes for the outcomes and pair-wise comparisons, with the adequate level of power set at 80% and a 95% confidence level.

Push out bond strength

The standard deviation and mean values of Push out bond strength (MPa) as function of chelating agent group and laser activation are summarized in Table 1 and graphically drawn in Fig. 1.

For HEBP chelating agent group, it was proved that G1 (laser activated) recorded higher push out bond strength mean values than G3 (manual agitation). This was statistically non-significant ($p > 0.05$) as revealed by paired t test—Table 1 and Fig. 1

For EDTA chelating agent group, it was demonstrated that G2 (laser activated) group recorded higher push out bond strength mean values than G4 (manual agitation). This was statistically significant ($p < 0.05$) as demonstrated by paired t test—Table 1 and Fig. 1

Either without or with laser activation; it was found that EDTA chelating agent group recorded higher push out bond strength mean values than HEBP chelating agent group mean value, that was statistically significant ($p < 0.05$) in manual agitation groups while non-significant ($p > 0.05$) in laser activated groups as verified by unpaired t test—Table 1 and Fig. 1

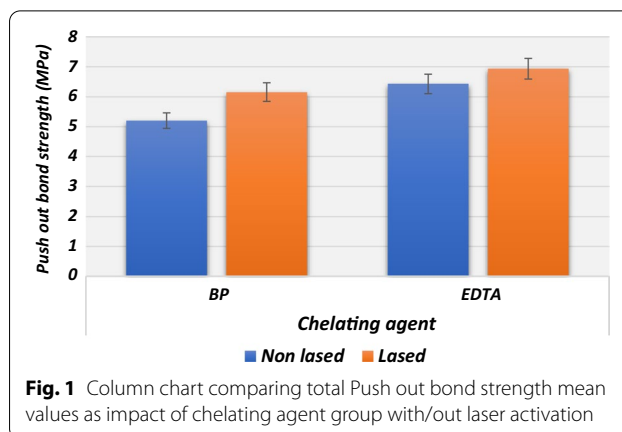


Fig. 1 Column chart comparing total Push out bond strength mean values as impact of chelating agent group with/out laser activation

Total effect of chelating agent type on push out bond strength

Regardless to laser activation, totally, it was found that EDTA chelating agent groups recorded higher push out bond strength mean values than HEBP chelating groups mean value. This was statistically significant ($p < 0.05$) as demonstrated by two-way ANOVA—Table 1 and Fig. 1

Total impact of laser activation on push out bond strength

Irrespective to chelating agent type, totally, it was found that the push out bond strength in the groups after laser activation recorded higher mean value than the manual agitation, but it was non-significant ($p > 0.05$) as demonstrated by two-way ANOVA,—Table 1 and Fig. 1

Discussion

Successful outcome of MTA apexification has been reported in necrotic immature permanent teeth (Holden et al. 2008; Wakiaa et al. 2013). The core of this treatment technique lies in the convenient debridement of the root canal system of immature teeth, along with placement of an apical plug by a cement that is compliant to the healing of apical tissue and its regeneration (Matt et al. 2004; Steinig et al. 2003). The MTA sealing ability of may

Table 1 Push out bond strength results (Mean values ± SDs) as function of chelating agent group type and laser activation

Variable	Laser activation	Statistics						
		Without		With		P value		
		Mean ± SD	95% CI	Mean ± SD	95% CI	Low	High	
Chelating agent	HEBP	5.203 ± 0.456	4.781	5.625	6.155 ± 1.771	4.517	7.792	0.1312 ns
	EDTA	6.429 ± 0.002	6.427	6.430	6.932 ± 0.429	6.635	7.327	0.021*
Statistics	P value	0.0004*			0.2809 ns			

ns: non-significant ($p > 0.05$)

* Significant ($p < 0.05$)

emerge from physicochemical reactions between dentin surface and MTA (Sarkar et al. 2005).

On the other hand, it has been revealed that the physical properties of endodontic cements change after using intracanal irrigants (Elnaghy 2014). Also, the smear layer removal causes an improved adaptability and closer contact of root canal filling materials, which contributes to the disinfection of root canal space. (Violich and Chandler 2010).

The current study showed that the effect of diode laser activation had no significant difference in the push out bond strength values on NaOCl+EDTA and NaOCl+HEBP groups.

The same results were shown within the groups of HEBP with laser activation and manual agitation, and there is no significant difference between the two groups. Alternatively, the NaOCl+EDTA laser activated group recorded higher bond strength values than the manual agitated NaOCl+EDTA group. Generally, the combination of NaOCl+EDTA showed higher bond strength followed by the NaOCl+HEBP group, the possible explanation may refer to the adequate removal of smear layer.

The elimination of the smear layer allows a better adaptation and penetration of MTA through the dentinal tubules which improves its bond to dentin surface, as well as an enhanced seal. (Eldeniz et al. 2005; Behrend et al. 1996).

Also, it is likely that the depth of demineralized zone, created by the NaOCl+EDTA either with laser activation or manual agitation, was deeper than the one created with the NaOCl+HEBP group. Previous studies illustrated that EDTA resulted in 2-4 μm demineralized dentin zone deep (García-Godoy et al. 2005). While the HEBP being a softer chelating agent, creates a less demineralized dentin zone, which explains the lower push out strength values of that group.

However, the activation of irrigants by laser was applied aiming in smear layer eradication with the cavitation process (George et al. 2008; Moor et al. 2010). The emergence of bubbles collapse and their vapor, acoustic streaming, and cavitation action, all results in irrigant activation. (Tomita and Shima 1986) This is affected by the laser wavelength and its absorption by the irrigants. Wavelengths ranging from 940–2940 nm were advocated to be effectively actuate intra canal irrigants (Hmud et al. 2010; Marissen et al. 2007). In the current study, 980 nm diode laser was used with NaOCl+EDTA, and NaOCl+HEBP for 30 s. of laser agitation. According to the present study, both chelating agents had no significant differences in the bond strength of MTA, this indicates that the laser activation increased the effectiveness of HEBP. The values of bond strength are indirectly

convenient in recognizing the effect of many irrigation protocols on altering the mineral content of dentin (García-Godoy et al. 2005). Insignificant difference in bond strength of MTA when the NaOCl+EDTA and NaOCl+HEBP were laser activated may refer to the exposure time which was 30 s. Also, it may be attributed to the laser activation mode, using continuous activation mode for 10 s that was repeated on three-time intervals for each tested irrigation protocols chelating. Hence, it can be considered as an advantage as the use of HEBP as a softer chelating agent with less erosive effect on dentine walls compared to EDTA. (Kandaswamy et al. 2011).

Conclusions

In conclusion, with the limitations of this study, both chelating agents, especially the diode laser NaOCl+HEBP group, showed satisfactory push out bond strength results with the MTA. The activation of NaOCl+HEBP could be a good protocol to enhance the success rate of root canal treatment and reduce the deleterious impact of EDTA on root canal dentine. Other activation protocols or periods of application of HEBP may be further investigated.

Abbreviations

HEBP: Etidronic acid; NaOCl: Sodium hypochlorite; EDTA: Ethylenediaminetetraacetic acid; MTA: Mineral trioxide aggregate; CO₂laser: Carbon dioxide laser; NdYAG laser: Neodymium–Yttrium–Aluminum–Garnet laser; G: Group.

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Author contributions

NO and NK planned the work and did the practical part. MA did the tests and the statistics. NO, NK, and MA contributed in writing the paper. All authors read and approved the final manuscript.

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Consent for publication

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Competing interests

The authors declare that they have no competing interests.

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