





A Comparative *ex-vivo* Study of Effects of Different Irrigation Protocols with/without Laser Activation on the Root Dentine's Micro-Hardness

 Morgana QUTEIFANI,  Ahmad A MADARATI,  Kinda LAYOUS,
 Mouhammad AL TAYYAN

ABSTRACT

Objective: To compare the effects of different irrigation protocols, with/without laser activation, on the radicular dentine's micro-hardness.

Methods: Eighty-two human extracted premolars were decoronated and divided into 7 groups. Roots were longitudinally split into two halves. The micro-hardness was measured for one half before and after irrigation protocols. The groups were; G1: MTAD without laser-activation, G2: MTAD with laser-activation, G3: sodium-hypochlorite (SH) with laser-activation, G4: SH then EDTA with laser-activation, G5: SH then MTAD with laser-activation, G6: SH without laser-activation. G7: distilled water (control). In the two-irrigants groups G4 and G5, samples were irrigated first with SH then with MTAD or EDTA irrigants, which were activated by the laser. The difference between the before- and after-irrigation micro-hardness was calculated to obtain the micro-hardness difference. Data were analyzed using the Paired Sample-t and Two-ways ANOVA tests at $P=0.05$.

Results: Overall, the mean dentine's micro-hardness after-irrigation (103.1) was lower than before-irrigation (116.1) ($P<0.001$); except for the distilled-water group, (116.6 and 112.9, respectively) ($P=0.075$). The micro-hardness reduction of SH without laser-activation group (32.5) was the greatest ($P<0.001$). The single-irrigant or laser-activation irrigation protocols caused significantly less micro-hardness reduction compared to the two-irrigants or no laser-activation protocols. The mean micro-hardness reduction of SH and MTAD groups (both with laser-activation) (5.8 and 9.3, respectively) were significantly lower than other groups, but not from that of the control group (3.7).

Conclusion: Using irrigants significantly reduced the root-dentine's micro-hardness. Although irrigants agitation by an Er: Yag laser significantly minimized micro-hardness reduction, it did not suppress the adverse effects on dentine micro-hardness when two-irrigants were used.

Keywords: Agitation, EDTA, irrigation, laser, micro-hardness, MTAD, sodium hypochlorite

Please cite this article as: Quteifan MQ, Madarati AA, Layous K, AL Tayyan M. A Comparative *ex-vivo* Study of Effects of Different Irrigation Protocols with/without Laser Activation on the Root Dentine's Micro-Hardness. *Eur Endod J* 2019; 3: 127-32

From the Department of Endodontic and Operative Dentistry (M.Q.Q., K.L., M.A.T.), College of Dentistry, Damascus University, Syria; Department of Retorative Dental Sciences (A.A.M. ✉ahmad.madarati@hotmail.co.uk) College of Dentistry, Taibah University, Saudi Arabia; Department of Endodontic and Operative Dentistry, College of Dentistry, Aleppo University, Syria

Received 10 July 2019,
Accepted 18 September 2019

Published online: 03 December 2019
DOI 10.14744/ej.2019.49369

This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.



HIGHLIGHTS

- This study showed that laser activation of irrigants can minimize their adverse effects on dentine microhardness
- An irrigation protocol that includes single irrigant is better than multiple irrigants in terms of dentine microhardness reduction.

INTRODUCTION

Continues irrigation of the root-canal system is as important as the mechanical action of instruments during cleaning and shaping. In that, removal of microorganisms, their by-products and the smear layer created during instrumentation is a major objective towards successful RCTs. Many irrigation solutions have been used for this

goal. Sodium hypochlorite (SH) (NaOCl) is the most common solution and has been the gold standard to which other irrigants are compared. It has antimicrobial effects and works as a dissolvent against vital and necrotic pulp tissues (1). However, it is highly scrubble to periapical tissues, especially at high concentrations (1). Hence, it is better used at the lowest concentrations and should not be extruded beyond the root-canal foramen (1). Also, it cannot remove the smear layer effectively, as it can only remove the organic component of the smear layer (2).

Ethylene diamine tetra acetic acid solution (EDTA) is recommended as part of irrigation protocols, as it is effective in elimination of the inorganic component of the smear layer (3). Other chelating solutions have been studied, such as peracetic and citric acids because they decrease calcium

ions concentrations and clear away the smear layer of the root-canal dentine (4). A mixture of a tetracycline isomer, an acid, and a detergent (MTAD) irrigant (Dentsply, Tulsa, OK) was developed by Torabinejad and Johnson (5). It is an aqueous solution of 3% doxycycline, 4.25% citric acid, a demineralizing agent; and 0.5% polysorbate 80 detergent (Tween 80). The doxycycline increases the water solubility and gives continuance antibacterial effects. It can eliminate microbes, especially *Enterococcus facialis* that are resistant to conventional endodontic irrigants (5, 6). It is also effective in elimination of the smear layer when is used as a final irrigant with minimal erosive changes on the surface dentine (1).

The light amplification by stimulated emission of radiation(s) (lasers), have been used widely in a range of biomedical and dental applications (7). In the field of the endodontic, lasers, such as erbium: yttrium-aluminum-garnet (Er: YAG–2940-nm wavelength) and neodymium: yttrium-aluminum-garnet (ND: YAG–1064-nm wavelength), have been used during cleaning and shaping of the root canal system; either for root-canals preparation or for irrigants agitation (8, 9) An alternative high-energy and cost-effective laser is the 980-nm diode laser (10). The laser's energy crosses through thin flexible fibers that are suitable for the use in slightly curved root-canals (10, 11). Diode lasers' wavelengths have good penetration ability and low reaction with water and hydroxyapatite (11). The penetration ability of diode lasers (810–980 nm) is 10.000 times greater than that of the Er: YAG, laser and may reduce the number of microorganisms up to 500 μm in depth within the dentinal tubules (10).

Irrigants may affect the radicular dentine and adversely change its chemical and physical properties such as the micro-hardness, which, in turn, may affect the sealing ability of endodontic sealers to root dentine's walls (1). There is lack of information regarding the effects of irrigants when are activated by lasers on root dentine's micro-hardness. Therefore, the aim of this study was to compare the effects of different irrigation protocols, with or without laser's activation, on the micro-hardness of the radicular dentine. The two null hypotheses were:

1. There would be no significant difference between radicular dentine's micro-hardness before-irrigation and that of after-irrigation.
2. There would no significant difference in radicular dentine's micro-hardness between groups of irrigants with laser activation and those without laser activation.

MATERIALS AND METHODS

Collection and preparation of teeth

This study was approved by the Research and Ethics Committee at College of Dentistry, Damascus University (No: 1134, Date 22/6/2016). Eighty-two human, sound freshly extracted (not for the purpose of the study) and single-canal premolars (confirmed by mesio-distal and bucco-lingual periapical radiographs) were stored in 0.1% thymol solution until they were used. Teeth with caries, fractures, restorations, internal or external resorptions, canal's curvature greater than 20° or with mul-

ti-roots were excluded. Each tooth was mounted in a limpid acrylic to stabilize it during decoronation; which was performed at the cemento-enamel junction using a diamond impregnated disc under sufficient water coolant, leaving 14.7±0.8 mm long roots. These were randomly divided into 7 groups (as will be defined in the irrigation protocols sub-section). Using a diamond disc, two grooves; one on the buccal and one on the lingual external roots' surfaces were created under water-cooling without penetration into the root-canals. Each root was then split longitudinally into two halves by a chisel (12, 13).

Microhardness tests

One half of each root was mounted on the stage of the Vicker's micro-hardness tester (MST-10, DMLM from Leica, Germany) and indentations were marked with a Vicker's diamond indenter at 100 g load and dwell time of 20 seconds for measuring a baseline micro-hardness. The micro-hardness was measured at three different points in the middle third of the apico-coronal direction and in the middle second third of the bucco-lingual direction of each sample. The mean micro-hardness number (VHN) (of the three measurements) was calculated and recorded for each sample. Six samples (from different groups) with irregular surfaces, following decoronation, were excluded as they were unstable during the micro-hardness test.

Irrigation's procedures

The two halves of each root were placed together in the acrylic block to facilitate performing the irrigation protocol procedures according to each group. In single-irrigant solution's groups, 5ml of the irrigant was used for 5 mins; 1ml for every 1 min (5). In the laser-activation groups, the irrigant was activated by the Er:Yag laser (Fotona d.o.o. Ljubljana, Slovenia) for 1 minute. The Er: Yag laser was used at a 2 w power, 15 Hz frequency and without coolants by inserting the laser's fiber (200 μm) into the root-canal, 5 mm shorter of the apex, then moving it coronally by half circle (14). The groups were; G1: MTAD without laser-activation (n=12 samples), G2: MTAD with laser-activation (n=11 samples), G3: SH with laser-activation (n=11), G4: SH then EDTA activated by the laser (n=10), G5: SH then MTAD activated by the laser (n=11), G6: SH without laser-activation (n=11) and G7: distilled-water as a control group (10 samples). In the SH+MTAD and SH+EDTA groups: samples were irrigated first with 5ml of SH for 4min, rinsed with distilled water and then they were irrigated for 1 mine with 5ml of the supplementary irrigant (MTAD or EDTA, corresponding to each group), then the latter irrigant was activated by the laser as described above. A final rinse with 5 ml distilled water was performed to remove any residue of the chemical solutions. A second micro-hardness measurement was obtained as previously described.

Statistical analysis

The difference between the first and second microhardness values was calculated to obtain the micro-hardness's reduction for each sample. Data were tabulated for statistical analysis using the SPSS software (SPSS 19 for windows, SPSS Inc, IL, USA). They were a little skewed and kurtotic, but they did not significantly differ from normality ($P>0.05$ for all groups). Therefore, the Paired Sample-t and the Two-ways analysis of variance (ANOVA) tests at 0.05 significant level were used.

RESULTS

The mean and standard deviation (SD) values of the radicular dentine's micro-hardness before- and after-irrigation for each group are presented in Table 1. There were no significant differences in the mean dentine micro-hardness values among groups before-irrigation (P=0.310). Overall, the mean dentine's micro-hardness after-irrigation (103.1 HV) was significantly lower than that before-irrigation (116.1 HV) (P<0.001); except for the distilled-water group, (116.6 and 112.9 HV, respectively) (P=0.075) (Fig. 1). The mean micro-hardness's reduction of the SH without Laser-activation group (32.5 HV) was significantly the greatest among all groups (P<0.001). Irrigation protocols including a single-irrigant or laser-activation significantly resulted in less reduction of micro-hardness when compared to two-irrigants or no-laser-activation protocols, respectively (Figs. 2 and 3). The mean micro-hardness reduction of the SH

and MTAD groups (both with laser-activation) (5.8 and 9.3 HV, respectively) were significantly lower than other groups and were not different from that of the control one (3.7 HV).

DISCUSSION

The long-term success of RCTs depends on the quality of the root-canal preparation, irrigation, and the tight seal of the root-canal system (15). Irrigation solutions used during cleaning and shaping may affect the physical and chemical properties of radicular dentine, including hardness (1, 15). The reduction of dentine's micro-hardness may decrease the sealing of sealers to the radicular dentine's walls (1). This study evaluated the impact of different irrigation protocols, with or without laser-activation, on the micro-hardness of root-canals' dentine. The micro-hardness's test is a simple and widely used method to investigate fine scale changes in the hardness (4). Vicker's

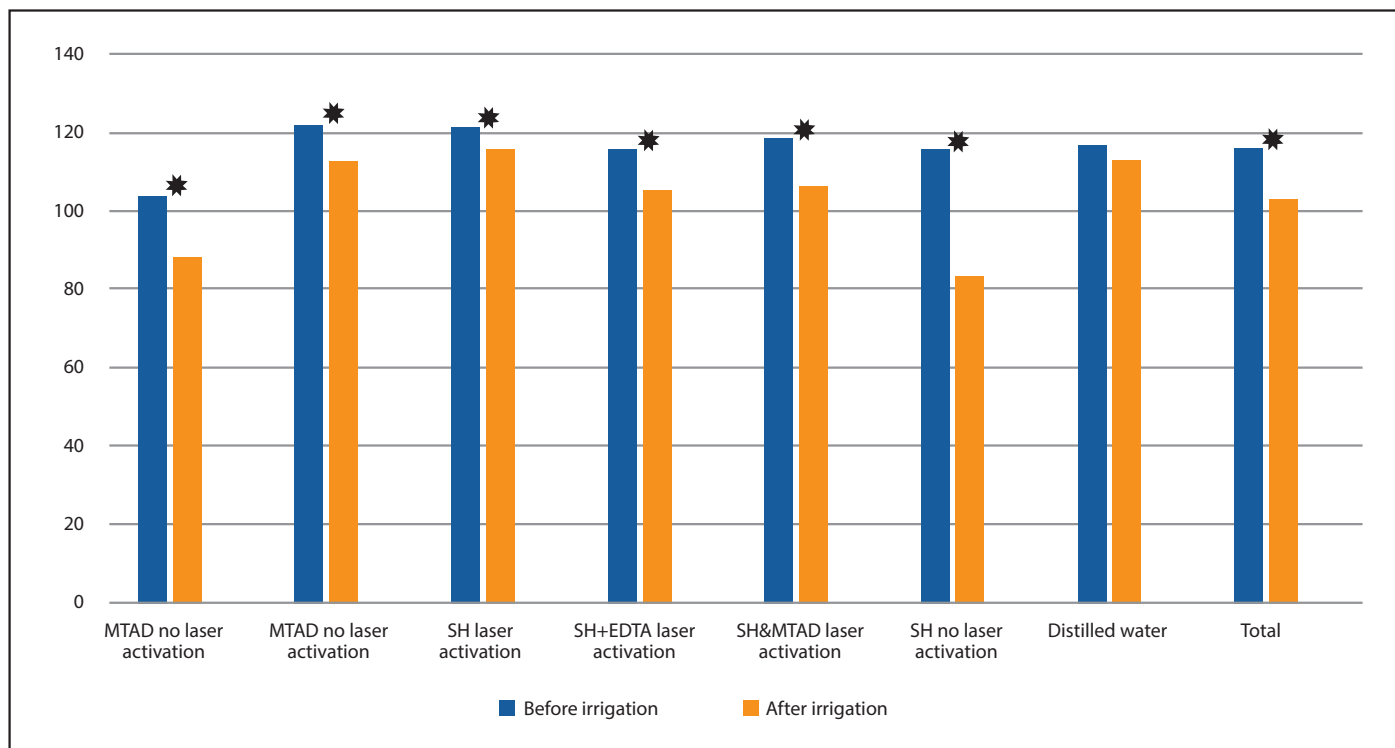


Figure 1. The differences between micro-hardness of radicular dentine between before- and after-irrigation's protocols in study groups. * Indicate significant statistical differences between before and after-irrigation

TABLE 1. The mean and Standard deviation micro-hardness of radicular dentine in study groups

Groups (n)	Root-dentine micro-hardness (HV) (Mean±SD)		
	Before irrigation	After irrigation	Before-after difference
Distilled Water (10)	116.6±16.6	112.9±14.7	3.7±2.2 ^a
MTAD no laser-activation (12)	103.9±25.5	88.1±22.9	15.8±10.1 ^{a,b}
MTAD laser-activation (11)	121.9±10.8	112.7±10.2	9.3±2.7 ^c
SH laser-activation (11)	121.4±13.5	115.7±13.5	5.8±2.3 ^{b,d}
SH+EDTA laser-activation (10)	115.6±21.3	105.2±15.2	10.4±8.8 ^e
SH&MTAD laser-activation (11)	118.5±20.3	106.3±22.7	12.1±4.9 ^{a,f}
SH no laser-activation (11)	115.6±17.8	83.1±11.9	32.5±8.3 ^{a,b,c,d,e,f}
Total (76)	116.1±18.8	103.1±20.1	13.0±10.9
Sig (Two-ways ANOVA)	P=0.310	P<0.001	P<0.001

Symmetrical letters indicate a significant different between paired groups (P<0.001)

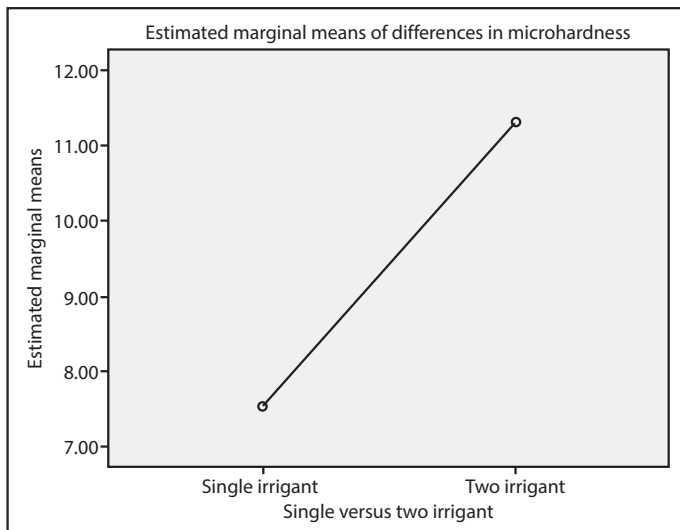


Figure 2. The effect of single- or two-irrigants irrigation protocols on dentine' micro-hardness reduction

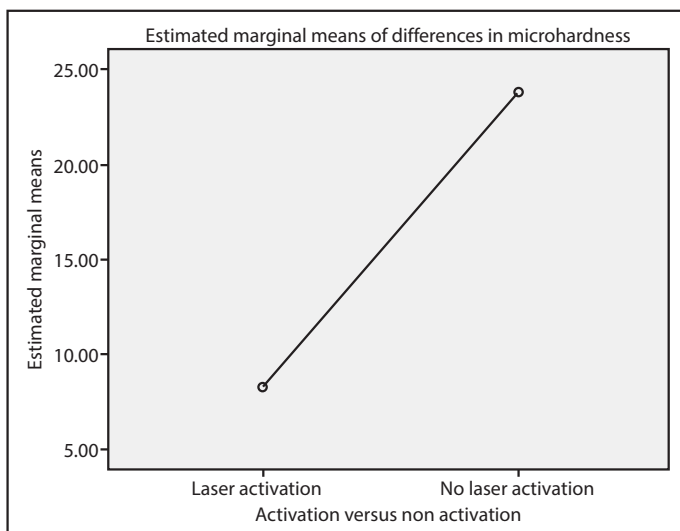


Figure 3. The effect of Laser-activation of irrigants on dentine' micro-hardness reduction

hardness test has been considered a suitable and a practical one, to estimate the changes in the surface and as well as deeper hard tissues' structures (16). In this study, three points in each sample were selected for measuring the micro-hardness, in order to minimize structural variations (13). More importantly, as the micro-hardness reduction may vary among teeth, it was measured at the baseline for each sample following sampling of roots-samples into different groups. There were no significant differences in micro-hardness among the groups at the baselines, which confirmed the randomization and that any post-irrigation significant differences would be attributed to the irrigation protocol(s) only. In addition, efforts were made to perform the post-irrigation indentations for each sample almost at the same areas of the pre-irrigation ones. Nevertheless, like other studies, the current one has few limitations. There was no negative control group (with no irrigation procedures). Also, unlike the recommended irrigation time (20-30 minutes), the irrigation in the current study was limited to 5-6 minutes. However, this enabled better compar-

isons with previous studies, which adopted the same irrigation time.

The dentinal tubules number increases from the cervical to the apical third of the radicular dentine (17). The dentine layer consists of organic and inorganic elements. Any alteration in the ratio of the calcium element can significantly change the original rate of these elements, which in turn affects the dentine characteristics. Structure's changes can also result from the changes of the number of mineral components of the radicular dentine and the depth of penetration of irrigants used (3). Dentine has an intense collagen network coated by hydroxyapatite (18, 19).

The first null hypothesis of the current study was: there would be no significant difference between dentine's micro-hardness before-irrigation and that of after-irrigation. The results showed that the mean dentine micro-hardness after-irrigation was significantly lower than that of before-irrigation, except for the distilled water group. Hence the first null hypothesis was rejected. This result can be attributed to the fact that distilled water was unable to chemically change dentine's structure (20). By contrast, SH solution is a non-specific oxidant and an effective solvent of organic tissues, which causes dissolution of collagen. The latter includes the breakdown of the bonds between carbon atoms, the disorganization of the protein base structure and the change in the magnesium and phosphate ions; magnesium ions are removed by SH (3, 21-23). The dentine's apatite crystals do not fully protect the collagen layer from chemical oxidation (3). Consequently, the dentine micro-hardness is adversely affected (21). These explain the results of a previous study (17) and those obtained in our study, which showed the significantly worst micro-hardness reduction when SH was used without laser-activation. Nevertheless, the increased negative effects when higher concentrations of SH are used, suggests that removal of the organic phase of the mineralized dentine occurs by diffusion with a relation of time and concentration dependence (3).

Similarly, MTAD without laser-activation resulted in significant dentine micro-hardness reduction. MTAD is a chelating irrigant, especially due to the tetracycline's low pH, and can reduce the calcium and phosphor elements' concentrations in the radicular dentine (5, 24). The concentration of these two elements is essential and determines the dentine's mechanical properties (3, 25). The lower the concentrations of calcium and phosphor elements within the radicular dentine, the less the micro-hardness. Moreover, MTAD consists of 4.25% citric acid, which is capable of dissolving 67% of the dentine's mineral contents (26). Nevertheless, the current study results are consistent with those obtained by Kandil et al, who showed that MTAD decreased the micro-hardness of radicular dentine because it weakened the dentine matrix's structure (1). A previous study showed that the exposure of root dentine to calcium hydroxide dressing, mineral trioxide aggregate or sodium hypochlorite for 5 weeks resulted in weakening of the root dentine (27). However, it should be noted that the irrigation process in the current study was only for 5 min.

The study's second null hypothesis (there would no significant difference in dentine's micro-hardness between groups of irri-

gants with laser activation and those without laser activation) was also rejected. The results revealed that laser-activation of irrigants resulted in significantly less reduction of micro-hardness when compared to no-laser-activation. The mean micro-hardness reduction of SH and MTAD groups (both with laser-activation) were significantly lower than other experimental groups and were not different from that of the control group. Shetty et al found that the loss of the calcium element (34.15 ppm) was significantly less when Er:Ysgg laser was used (28). Also, Prabhakar et al found that irrigation solutions activated by lasers were better in disinfecting the root-canal system without affecting dentine's properties (2). They explained; the Er:Yag laser has a low penetration ability into the dental tissue, because of its high absorption grade in water and hydroxyapatite. This laser inactivates mainly with the superficial layers of the dentine and supports changes in micro-hardness up to 60µm (8). Our unpublished data showed that MTAD activated by laser did not significantly affect the mineral content of the radicular dentine (calcium, phosphor and magnesium) when compared to irrigation with MTAD without laser-activation. Nevertheless, Macedo et al found that EDTA agitation by Nd: YAG 1064 and 980 nm diode lasers caused greater reduction in micro-hardness and increased roughness of the radicular dentine when compared to irrigation with EDTA without agitation or with manual agitation (29). EDTA can make changes in dentine by adjusting the Ca/P ratio (23, 30), hence changes in micro hardness, and solubility characteristics (19, 2 3). One possible reason for such inconsistency with our results is the use of different lasers. Using different lasers (with different parameters), hence different abilities in penetrating tissues and different degree of changes in the chemical components. Therefore, this may result in different effects on the radicular dentine micro-hardness.

However, laser activation, in the current study, did not suppress the effects of using two irrigants in one irrigation protocol. The two-irrigants irrigation protocols significantly resulted in greater micro-hardness reduction when compared to single-irrigant protocols. This could be attributed to the additional effects of using two irrigants instead of one irrigant, as long as the irrigants have some chemical effects on the radicular dentine. Another possible reason is that using SH could maximize the chelating effects and ability of smear layer's removal of EDTA and MTAD. Arslan et al found that agitation of 15% EDTA with an 808nm diode laser for 20 sec was effective in removing the smear layer in the apical thirds of root canals dentine (31). Cruz-Filho et al also showed that chelating agents caused significant loss in root dentine's micro-hardness (25). Nevertheless, future research to investigate the effects of different lasers, with different parameters, for activation of different irrigants on root dentine's microhardness will be paramount.

CONCLUSION

Within the limitations of the current study it can be concluded that using endodontic irrigants significantly reduced the root-dentine's micro-hardness. Although agitation of irrigants by the Er: Yag laser significantly minimized reduction of the micro-hardness, it did not suppress the adverse effects on dentine micro-hardness when two-irrigants irrigation protocol was adopted.

Disclosures

Conflict of interest: None declared.

Ethics Committee Approval: This study was approved by the Research and Ethics Committee at College of Dentistry, Damascus University (No: 1134, Date 22/6/2016).

Peer-review: Externally peer-reviewed.

Financial Disclosure: None declared.

Authorship contributions: Concept – M.Q., A.A.M., K.L., M.A.T.; Design – M.Q., K.L., M.A.T.; Supervision – K.L., M.A.T.; Funding – M.Q.; Materials – None; Data collection &/or processing – M.Q., A.A.M.; Analysis and/or interpretation – M.Q., A.A.M.; Literature search – M.Q., K.L., M.A.T.; Writing – M.Q., A.A.M., K.L., M.A.T.; Critical Review – A.A.M.

REFERENCES

- Kandil HE, Labib AH, Alhadainy HA. Effect of different irrigant solutions on microhardness and smear layer removal of root canal dentin. *Tanta Dent J* 2014; 11(1):1–11.
- Violich DR, Chandler NP. The smear layer in endodontics - a review. *Int Endod J* 2010; 43(1):2–15.
- Lima Nogueira BM, da Costa Pereira TI, Pedrinha VF, de Almeida Rodrigues P. Effects of Different Irrigation Solutions and Protocols on Mineral Content and Ultrastructure of Root Canal Dentine. *Iran Endod J* 2018; 13(2):209–15.
- Baldasso FER, Roletto L, Silva VDD, Morgental RD, Kopper PMP. Effect of final irrigation protocols on microhardness reduction and erosion of root canal dentin. *Braz Oral Res* 2017; 31:e40.
- Torabinejad M, Khademi AA, Babagoli J, Cho Y, Johnson WB, Bozhilov K, et al. A new solution for the removal of the smear layer. *J Endod* 2003; 29(3):170–5.
- Shabahang S, Torabinejad M. Effect of MTAD on Enterococcus faecalis-contaminated root canals of extracted humanteeth. *J Endod* 2003; 29(9):576–9.
- Najeeb S, Khurshid Z, Zafar MS, Ajlal S. Applications of Light Amplification by Stimulated Emission of Radiation (Lasers) for Restorative Dentistry. *Med Princ Pract* 2016; 25(3):201–11.
- Viapiana R, Sousa-Neto MD, Souza-Gabriel AE, Alfredo E, Silva-Sousa YT. Microhardness of radicular dentin treated with 980-nm diode laser and different irrigant solutions. *Photomed Laser Surg* 2012; 30(2):102–6.
- Hmud R, Kahler WA, George R, Walsh LJ. Cavitation effects in aqueous endodontic irrigants generated by near-infrared lasers. *J Endod* 2010; 36(2):275–8.
- Gutknecht N, Franzen R, Schippers M, Lampert F. Bactericidal effect of a 980-nm diode laser in the root canal wall dentin of bovine teeth. *J Clin Laser Med Surg* 2004; 22(1):9–13.
- Wang X, Sun Y, Kimura Y, Kinoshita J, Ishizaki NT, Matsumoto K. Effects of diode laser irradiation on smear layer removal from root canal walls and apical leakage after obturation. *Photomed Laser Surg* 2005; 23(6):575–81.
- Prabhakar AR, Basavraj P, Basappa N. Comparative evaluation of Morinda citrifolia with chlorhexidine as antimicrobial endodontic irrigants and their effect on micro-hardness of root canal dentin: An in vitro study. *Int J Oral Health Science* 2013; 3(1):5–9.
- Patil CR, Uppin V. Effect of endodontic irrigating solutions on the micro-hardness and roughness of root canal dentin: an in vitro study. *Indian J Dent Res* 2011; 22(1):22–7.
- De Moor RJG, Meier M. High-Power Lasers in Endodontics-Fiber Placement for Laser-Enhanced Endodontics: in the Canal or at the Orifice? *J LAHA* 2014; (1)20–8.
- Saha SG, Sharma V, Bharadwaj A, Shrivastava P, Saha MK, Dubey S, et al. Effectiveness of Various Endodontic Irrigants on the Micro-Hardness of the Root Canal Dentine: An in vitro Study. *J Clin Diagn Res* 2017; 11(4):ZC01–4.
- Fuentes V, Toledano M, Osorio R, Carvalho RM. Microhardness of superficial and deep sound human dentin. *J Biomed Mater Res A* 2003; 66(4):850–3.
- Duvvi SAB, Adarsha MS, Usha HL, Ashwini P, Murthy CS, Shivekshith AK. A comparative Assessment of Different Concentrations of Sodium Hypochlorite and Calcium Hypochlorite on Microhardness Of Root Canal Dentine –An In Vitro Study. *Int J Oral Care* 2018; 6(1):54–8.

18. Kolosowski KP, Sodhi RN, Kishen A, Basrani BR. Qualitative Time-of-Flight Secondary Ion Mass Spectrometry Analysis of Root Dentin Irrigated with Sodium Hypochlorite, EDTA, or Chlorhexidine. *J Endod* 2015; 41(10):1672-7.
19. Cobankara FK, Erdogan H, Hamurcu M. Effects of chelating agents on the mineral content of root canal dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; 112(6):e149-54.
20. Driscoll CO, Dowker SE, Anderson P, Wilson RM, Gulabivala K. Effects of sodium hypochlorite solution on root dentine composition. *J Mater Sci Mater Med* 2002; 13(2):219-23.
21. Kinney JH, Balooch M, Marshall SJ, Marshall GW Jr, Weihs TP. Hardness and Young's modulus of human peritubular and intertubular dentine. *Arch Oral Biol* 1996; 41(1):9-13.
22. Qian W, Shen Y, Haapasalo M. Quantitative analysis of the effect of irrigant solution sequences on dentin erosion. *J Endod* 2011; 37(10):1437-41.
23. Pérez-Heredia M, Ferrer-Luque CM, González-Rodríguez MP, Martín-Peinado FJ, González-López S. Decalcifying effect of 15% EDTA, 15% citric acid, 5% phosphoric acid and 2.5% sodium hypochlorite on root canal dentine. *Int Endod J* 2008; 41(5):418-23.
24. Tartari T, de Almeida Rodrigues Silva E Souza P, Vila Nova de Almeida B, Carrera Silva Júnior JO, Fáciola Pessoa O, Silva E Souza Junior MH. A new weak chelator in endodontics: effects of different irrigation regimens with etidronate on root dentin microhardness. *Int J Dent* 2013;2013:743018.
25. Cruz-Filho AM, Sousa-Neto MD, Saquy PC, Pécora JD. Evaluation of the effect of EDTAC, CDTA, and EGTA on radicular dentin microhardness. *J Endod* 2001; 27(3):183-4.
26. Singla MG, Garg A, Gupta S. MTAD in endodontics: an update review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; 112(3):e70-6.
27. Saleh AA, Ettman WM. Effect of endodontic irrigation solutions on microhardness of root canal dentine. *J Dent* 1999; 27(1):43-6.
28. Shetty K, Satish SV, Kilaru KR, Reza KM, Basavanagowda, Patil S. Effect of EDTA, ETIDRONIC Acid, PHYTIC Acid and Er:YSGG laser on calcium loss of root dentin using atomic absorption spectrophotometer. *J Appl Dent Sci* 2016; 2(4):18-24.
29. de Macedo HS, Colucci V, Messias DC, Rached-Júnior FJ, Fernandes FS, Silva-Sousa YT, et al. Effect of Nd:YAG (1064-nm) and Diode Laser (980-nm) EDTA Agitation on Root Dentin Ultrastructure Properties. *Photomed Laser Surg* 2015; 33(7):349-56.
30. Sayin TC, Serper A, Cehreli ZC, Otlu HG. The effect of EDTA, EGTA, EDTAC, and tetracycline-HCl with and without subsequent NaOCl treatment on the microhardness of root canal dentin. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; 104(3):418-24.
31. Arslan H, Ayrancı LB, Karatas E, Topçuoğlu HS, Yavuz MS, Kesim B. Effect of agitation of EDTA with 808-nanometer diode laser on removal of smear layer. *J Endod* 2013; 39(12):1589-92.