Effects of Irrigating Solutions on Dentin

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Irrigating solutions play an important role in the eradication of intracanal microbes and debris dissolution during endodontic treatment. Different combinations of solutions and protocols have been advocated, with sodium hypochlorite (NaOCl), ethylenediamine tetra acetic acid (EDTA), and chlorhexidine (CHX) remaining the most widely used ones by many clinicians. Although these solutions provide efficient inorganic dissolution and antimicrobial capacity, their use has also been reported to cause undesired effects on root dentin composition and mechanical and biomechanical properties, such as microhardness, surface roughness, bond strength, and matrix metalloproteinase (MMP) activity.

Keywords: endodontics ; irrigating solutions ; matrix metalloproteinases

1. Introduction

To achieve an effective chemomechanical preparation of the root canal system space, several irrigant solutions have been adopted in root canal treatment, which include, but are not limited to, the following: ethylenediamine tetra acetic acid (EDTA); citric acid (CA); sodium hypochlorite (NaOCl); chlorhexidine (CHX); iodine potassium iodide; hydrogen peroxide; local anesthetic; and saline and/or water [1].

Irrespective of the various irrigating solutions available, a systematic review revealed that there is no difference between these several solutions, and the deficit in well-conducted clinical studies should be taken into consideration when considering a “no difference” result as opposed to taking it for granted [2].

Although irrigating solutions may differ in their actions and chemical properties [3], from the range of characteristics an ideal irrigant should possess, only a few of them, when used alone, offer some spectrum of the ideal properties [2]. Some of these include germicidal, fungicidal, nontoxic, nonirritating, stability in solution, noninterference with tissue repair, prolonged antimicrobial effect, and relatively inexpensive [4][5].

2. Effect of Sodium Hypochlorite

NaOCl is a broad-spectrum antimicrobial solution used in endodontics to eliminate biofilms of species and microorganisms, such as enterococcus, actinomyces, and candida species [6]. Consequently, it is widely recognized for its effective antibacterial activity, and at minimal concentrations, it destroys bacteria rapidly [7]. Furthermore, it possesses tissue dissolution ability and endotoxin deactivation, and it is nonallergenic [3]. It is also inexpensive, has a long shelf-life, and is easily available [8][9][10]. Regardless of the many advantages of NaOCl, its relative toxicity, inability to remove the smear layer, and unpleasant taste have been condemned [11][12].

It has been recorded that a concentration of NaOCl as high as 10% has been used by dentists [13]. Although an increase in the solution concentration enhances its tissue dissolution effect [14], it could also adversely affect dentin properties [15]. Coupled with an increase in the concentration of hypochlorite solution, other factors that may enhance the tissue dissolution effect of the irrigant include an increase in pH, prolonged exposure time, increase in temperature, and ultrasonic agitation [3][16][17].

As a nonspecific oxidizing agent, the adverse effect of NaOCl is also concentration dependent as with its antimicrobial and tissue dissolving effects [18]. For this reason, it has been utilized for hard tissue deproteination [19]. As an oxidizing agent, its ability to fragment peptide chains and to chlorinate protein terminal groups to produce N-chloramines [20][21] is responsible for its deleterious effects on the dentin surface [22]. Reports have also demonstrated that sodium hypochlorite changes the mechanical properties of dentin by the degradation of the dentin’s organic constituents, and this is because the organic constituent of dentin is 22% of its weight and the irrigant can easily deplete it if dentine is demineralized [22][23].

It was noted from a study carried out on bovine dentin that alterations of the chemical and physical properties occur within the timespan of endodontic treatment [24]. Based on the study of the effect of NaOCl on human root dentin by Marending et al. [22], it was demonstrated that a hypochlorite solution dissolves the organic constituent of dentin but leaves the inorganic constituents unaltered. However, because of the varying methodologies and experimental parameters (i.e., lack of standardization), studies have revealed contradicting results concerning the effect of sodium hypochlorite on the organic constituent of dentine [22].

Compared to a physiological saline solution, the mechanical properties of dentin, such as flexural strength and the modulus of elasticity, have been reported to become remarkably reduced after exposure to a greater than 3% weight per volume of NaOCl for 2 h [25][26]. Additionally, a 2.5% NaOCl solution decreases the flexural strength of dentin after 24 min exposure [27], while with a 5.25% NaOCl solution, both the elastic modulus and flexural strength of dentin decrease after a 2 h exposure [25]. Even a lower 0.5% hypochlorite solution has a comparatively lesser effect on the flexural strength and modulus of the elasticity of dentin when compared to the 6.0% concentration, which is used by several clinicians in the United States of America [25]. The changes in the flexural strength and elastic modulus caused by a high concentration of NaOCl may lead to a decrease in the properties by 50% [22]. On the other hand, a contradicting study by Machnick et al. [28] observed that the flexural strength and elastic modulus were not affected by the sodium hypochlorite concentration.

Corresponding to the study by Lee et al. [29], who revealed that cracks were on the dentin surfaces after exposure to 5% NaOCl, Marending et al. 2007 [22] also noted crack lines in the dentin specimens after exposure to a 5% hypochlorite solution for 2 h. These results support the hypothesis that the microhardness of root dentin is dependent on the concentration of the hypochlorite used [24]. The microhardness of dentin was depleted after immersion in 1.0% sodium hypochlorite for 15 min [30] and 5.0% hypochlorite solution for 60 s [31], which also corroborates the findings that there is a greater effect on the microhardness when an increased concentration is used.

According to Marending et al. [22], the permeability of dentin is also altered by exposure to NaOCl. The study revealed that dentin permeability was significantly increased after exposure of dentin bars to a 1.0% hypochlorite solution but most especially to a 5.0% concentration. This experiment was carried out by subjecting the dentin specimens treated with NaOCl in a basic fuchsin dye. Changes of the sealing ability and adhesion of the resin-based cement to the dentinal collagen are also consequences of the effect of NaOCl on the dentin collagen [32][33][34]. This effect may produce equal or superior bonding results for some dentin-bonding systems [35][36][37][38][39].

Ari et al. [40] also revealed that 2.5% to 5.25% of NaOCl caused a remarkable increase in the surface roughness, while other data recorded no effect on roughness [41]. A study using the in silico approach observed an increase in stress and strain concentrations with the use of a hypochlorite solution [28][42]. There was a 15.9% increase in tensile strain and a 33.5% increase in compressive strain after a high hypochlorite solution was used as an irrigant [25].

Reports also show that alterations of the dentin matrix are a result of all NaOCl concentrations [40][43]. Zhang et al. [44] reported that a 5.25% hypochlorite solution caused greater dentinal erosion compared to a 1.3% concentration, and the mechanism by which NaOCl removed the dentin organic phase was by infiltration into the apatite-encapsulated collagen matrix. This was possible due to the low molecular weight of the irrigant [44]. Another study on the tissue dissolution and modifications in the dentin composition by different NaOCl concentrations observed that the uninterrupted degeneration of the dentin surface collagen was evident by the time-dependent effect in the increase in the amide II/phosphate ratio [45]. Preceding studies in line with this outcome also proved that the removal of the dentin organic phase was time-dependent [44]. Furthermore, Tartari et al. [45] also confirmed that the degeneration of dentin collagen by a hypochlorite solution was concentration dependent. In this study, both the concentration and time-dependent effects of NaOCl were evident by the decrease in the carbonate/phosphate ratio after 30 s immersion in all concentrations. Apart from the degenerating effects on collagen, another study demonstrated the effect of NAOC on chondroitin sulfate and more specifically on type I collagen, while others demonstrated the loss of immunoreactivity of both glycosaminoglycans and type I collagen after treatment with sodium hypochlorite [46].

The consequences of damage to the collagen matrix of dentine included a less resilient and a more fragile substrate [22][47], which may encourage the generation of fatigue cracks when cyclic forces are applied and ultimately cause a decrease in the resistance to crown and root fractures [44][48]. The deproteination ability of the hypochlorite solution led to an unequal effect thus producing an unbound hydroxyapatite and collagen-sparse dentine subsurface rich in apatite [47][49]. The deproteination effect also caused additional dentin changes evident by the “moth-eaten” appearance seen on a scanning electron microscope (SEM) [35][50]. The microscopy analysis revealed undamaged intertubular dentin surface as effects of NaOCl on the dental tissue [51].

In as much as there have been insufficient studies on the direct quantitative effects of low concentrations (0.5–2.25%) and short exposure periods (1–10 min) of NaOCl on dentin deproteination, 0.5% sodium hypochlorite causes a lesser effect on dentin deproteination than 1.0% and 2.25% concentrations [52]. Deproteination by 5.0% NaOCl for 10 min decreased the bond strength between the fiber posts and dentin surfaces [53].

To maintain an aseptic environment in the root canal while trying to limit dentin deproteination, it is advised that an ideal NaOCl concentration at a suitable exposure time is used [47]. Hence the use of hypochlorite concentrations, such as 1.0% and 2.5%, which have been found to promote organic tissue dissolution as well as limited destruction to the dentin structure, have been advised [45]. Although preceding studies revealed that 0.5% NaOCl is effective only for the removal of bacteria on the dentin surface layer, other studies recommended the use of a low 0.5% hypochlorite solution concentration as a routine irrigating solution but at a longer exposure period because it achieved excellent antimicrobial activity and had an insignificant effect on dentin deproteination [41].

3. Effect of Decalcifying Agents

The various levels of actions of decalcifying agents on mineral dentin depend on the concentration, immersion time, and decalcifying capability [45]. Irrespective of the concentration and time, EDTA and other chelating agents can cause harmful effects. Exposure to 17% EDTA for 3 min can dissolve the inorganic smear layer of dentin, and because EDTA has been found to remove the smear layer [41], other chelating agents, such as etidronic acid (HEDP), tetrasodium ethylenediamine tetraacetic acid ($EDTANa_4$), and peracetic acid (PAA), have also been used as substitutes of EDTA [45] to remove the smear layer. It has been noted that in 5 min, HEDP and $EDTANa_4$ completed this action [54][55], while in 1 min, PAA could remove the inorganic components of the smear layer [56].

The study by Tartari and Bachmann [45] revealed that changes in the components of dentin were evident by the ratios of amide III/phosphate (PO_4^{3-} v3) and carbonate (CO_3^{2-} v2)/phosphate (PO_4^{3-} v3) bands. At different rates, increments in the amide II/phosphate ratio indicated dentin demineralization and dentin surfaces sparse in apatite but rich in collagen, while a decrease in the phosphate and carbonate apatite bands by the different decalcifying agents at different rates was also observed. At 9% and 18%, HEDP did not change the amide III/phosphate ratio; however, at 5% and 10%, $EDTANa_4$ minimally reduced the phosphate group compared to the other decalcifying agents. At a 2% concentration, PAA eliminated the phosphate group and exposed the collagen matrix, which ultimately caused a significant increase in the amide III/phosphate ratio. The results by Tartari and Bachmann [45] reveal that HEDP and alkaline $EDTANa_4$ have little effect on dentin demineralization, while $EDTAHNa_3$ and PAA, at concentrations greater than 0.5%, may have a greater demineralizing ability and should be prudently used [57].

The process by which EDTA removes calcium ions (Ca^{2+}) from mineral tissues is shown to destroy the dentin matrix [58], and it has been noted that calcium was depleted from the dentin surface to a depth of almost 150µm after exposure to 17% EDTA for 2 h [59]. Although EDTA destroys the dentin matrix [58], its solo use as an irrigant could inhibit dentin dissolution because of the accumulation of an organic matrix on the canal surface [46].

EDTA has been found to be a major cause of dentinal erosion [60], and because the erosion of the canal wall is a result of prolonged used of the solution due to its ability to demineralize root dentin [61][62], efforts have been made to weaken the constant chelating effect of EDTA for longer periods [63][64]. The use of a lower EDTA concentration (1%) was recommended by Şen et al. [64] to prevent extreme root canal dentin erosion but also to provide sufficient smear layer removal. Apart from EDTA's effect on the dentinal wall, 19% citric acid has also been reported to enlarge the superficial part of dentinal tubules [65][66].

Although the bond strength between endodontic sealers, such as AH plus (Dentsply DeTrey, Konstanz, Germany), and dentin is enhanced due to the chemical bond between the material and the amino groups of dentin that is exposed by the demineralizing effect of strong decalcifying agents ($EDTAHNa_3$ & PAA) [34], the generation of a weak bond and an increase in interfacial degradation could occur due to insignificant penetration of the material into the demineralized dentin caused by significant decalcification in the walls of the root canal [54][67][68][69][70].

Additionally, deleterious effects on dentin's surface roughness provoked by EDTA exposure has been reported [25][71]. Ari et al. in 2004 [40] also reported a substantial increase in the surface roughness on root canal dentine by 17% EDTA. Other dentin properties, such as micro and nanohardness, have also been reported to be changed using chelating agents [41], and this corroborates the studies by Saleh et al. in 1999 [31] and Cruz-Filho et al. in 2001 [72] that demonstrated a decrease in dentin's microhardness by EDTA.

Moreover, the teeth fracture resistance was found to be markedly reduced by 17% EDTA when compared to 5% EDTA after a 10 min exposure, even though after a 1 min immersion, it was reported that 5% EDTA had a greater effect on the reduction of teeth fracture resistance when compared to 17% EDTA [73]. Additionally, demonstrations by Uzunoglu et al. [73] revealed that when compared to roots rinsed with distilled water only, 17% or 5% EDTA produced a higher fracture resistance of the roots after 1 min exposure, which was explained by the ability to remove the smear layer, and this has been shown to improve the bond strength of resin-based sealers to dentin [73].

Following dentin exposure to 17% EDTA for 2 h, the flexural strength and the modulus of elasticity were reduced by one third and by half, respectively [74]. Because mechanical properties, especially strength, are less likely to be affected after the use of EDTA for shorter periods, it is recommended to reduce the influence of high-concentration EDTA (15% or 17%) for it to be used during shorter periods (approximately 2 min) [73][75][76][77][78][79][80].

4. Effect of Chlorhexidine

Chlorhexidine (CHX), a cationic biguanide with a broad spectrum, is the most used antiseptic product [7]. It is used as a disinfectant, preservative, and antiseptic in the medical, pharmaceutical, and dental fields [81][82]. The bis-biguanide is a strong basic salt, and in its original form (chlorhexidine acetate and hydrochloride), it is most stable, but due to its insolubility in water [3][83], chlorhexidine digluconate became its replacement [84].

Based on the effect of CHX on the mechanical properties of dentin, only one study proved there was no effect [80]. The association of chlorhexidine molecules with the surface of dentin was responsible for the activity of CHX [85]. The removal of the smear layer by this interaction also increased chlorhexidine activity [86][87].

Ari et al. [40] observed that the microhardness of dentin was markedly decreased by all irrigating solutions (NaOCl, H_2O_2, EDTA, etc.) except CHX. They noted that the surface roughness of dentin was not affected by 0.2% CHX gluconate, and their results also reveal that this solution was the only one, amongst the other irrigating solutions, that did not affect the constituents of dentin. Due to its nontoxic effect, no negative effect on microhardness, and roughness of dentin, it was suggested that 0.2% CHX gluconate was a suitable irrigant to be used in endodontics [40]. Further, chlorhexidine did not influence the fracture resistance of endodontically treated teeth after irrigation, while insufficient smear layer removal using chelating agents may inhibited its beneficial effects [73]. It has also been noted that the demineralization of dentin and exposure of dentinal tubules by 17% EDTA reinforced the effect of chlorhexidine [88].

Compared to other irrigating solutions, CHX showed the greatest bond strength values [89]; by reducing the wetting angle and raising the surface energy, it improved the penetration and bond strength of AH Plus (Dentsply, Petrópolis, Rio de Janeiro, Brazil) and wettability of dentin [90]. Worthy of note is the fact that CHX can prevent the destruction of collagen by host-derived proteases MMPs [69][91].

References

1. Darcey, J.; Jawad, S.; Taylor, C.; Roudsari, R.V.; Hunter, M. Modern endodontic principles part 4: Irrigation. Dent. Update 2016, 43, 20–33.
2. Fedorowicz, Z.; Nasser, M.; Sequeira-Byron, P.; de Souza, R.F.; Carter, B.; Heft, M. Irrigants for non-surgical root canal treatment in mature permanent teeth. Cochrane Database Syst. Rev. 2012, 9, 1–55.
3. Zehnder, M. Root canal irrigants. J. Endod. 2006, 32, 389–398.
4. Tay, F.R.; Pashley, D.H.; Loushine, R.J.; Doyle, M.D.; Gillespie, W.T.; Weller, R.N.; King, N.M. Ultrastructure of smear layer-covered intraradicular dentin after irrigation with BioPure MTAD. J. Endod. 2006, 32, 218–221.
5. Andrabi, S.M.U.N.; Kumar, A.; Tewari, R.K.; Mishra, S.K.; Iftekhar, H. An in vitro SEM study on the effectiveness of smear layer removal of four different irrigations. Iran. Endod. J. 2012, 7, 171–176.
6. Rahimi, S.; Janani, M.; Lotfi, M.; Shahi, S.; Aghbali, A.; Pakdel, M.V.; Ghasemi, N. A review of antibacterial agents in endodontic treatment. Iran. Endod. J. 2014, 9, 161–168.
7. Haapasalo, M.; Endal, U.; Zandi, H.; Coil, J.M. Eradication of endodontic infection by instrumentation and irrigation solutions. Endod. Top. 2005, 10, 77–102.
8. Heling, I.; Rotstein, I.; Dinur, T.; Szwec-Levine, Y.; Steinberg, D. Bactericidal and cytotoxic effects of sodium hypochlorite and sodium dichloroisocyanurate solutions in vitro. J. Endod. 2001, 27, 278–280.

9. Mahmoudpour, A.; Rahimi, S.; Sina, M.; Soroush, M.H.; Shahisa, S.; Asl-Aminabadi, N. Isolation and identification of Enterococcus faecalis from necrotic root canals using multiplex PCR. J. Oral Sci. 2007, 49, 221–227.
10. Nadalin, M.R.; Perez, D.E.D.C.; Vansan, L.P.; Paschoala, C.; Souza-Neto, M.D.; Saquy, P.C. Effectiveness of different final irrigation protocols in removing debris in flattened root canals. Braz. Dent. J. 2009, 20, 211–214.
11. Spangberg, L.; Engstrom, B. Toxicity and antimicrobial effect of endodontic antiseptics in vitro. Oral Surg. Oral Med. Oral Pathol. 1973, 36, 856–870.
12. McComb, D.; Smith, D.C.; Beagrie, G.S. The results of in vivo endodontic chemomechanical instrumentation—A scanning electron microscopic study. Int. Endod. J. 1976, 9, 11–18.
13. Matsumoto, T.; Nagai, T.; Ida, K.; Ito, M.; Kawai, Y.; Horiba, N.; Sato, R.; Nakamura, H. Factors affecting successful prognosis of root canal treatment. J. Endod. 1987, 13, 239–242.
14. Baumgartner, J.C.; Cuenin, P.R. Efficacy of several concentrations of sodium hypochlorite for root canal irrigation. J. Endod. 1992, 18, 605–612.
15. Xu, H.; Ye, Z.; Zhang, A.; Lin, F.; Fu, J.; Fok, A.S. Effects of concentration of sodium hypochlorite as an endodontic irrigant on the mechanical and structural properties of root dentine: A laboratory study. Int. Endod. J. 2022, 55, 1091–1102.
16. Christensen, C.E.; McNeal, S.F.; Eleazer, P. Effect of lowering the pH of sodium hypochlorite on dissolving tissue in vitro. J. Endod. 2008, 34, 449–452.
17. Stojicic, S.; Zivkovic, S.; Qian, W.; Zhang, H.; Haapasalo, M. Tissue dissolution by sodium hypochlorite: Effect of concentration, temperature, agitation, and surfactant. J. Endod. 2010, 36, 1558–1562.
18. Dychdala, G.R. Chlorine and chlorine compounds. In Disinfection, Sterilization, and Preservation, 4th ed.; Block, S.S., Ed.; Lea & Febiger: Philadelphia, PA, USA, 1991; pp. 131–151.
19. Johnson, G.S.; Mucalo, M.R.; Lorier, M.A. The processing and characterization of animal-derived bone to yield materials with biomedical applications Part 1: Modifiable porous implants from bovine condyle cancellous bone and characterization of bone materials as a function of processing. J. Mater. Sci. Mater. Med. 2000, 11, 427–441.
20. Stoward, P.J. A histochemical study of the apparent deamination of proteins by sodium hypochlorite. Histochemistry 1975, 45, 213–226.
21. Davies, J.M.; Horwitz, D.A.; Davies, K.J. Potential roles of hypochlorous acid and N-chloroamines in collagen breakdown by phagocytic cells in synovitis. Free Radic. Biol. Med. 1993, 15, 637–643.
22. Marending, M.; Luder, H.U.; Brunner, T.J.; Knecht, S.; Stark, W.J.; Zehnder, M. Effect of sodium hypochlorite on human root dentine–mechanical, chemical and structural evaluation. Int. Endod. J. 2007, 40, 786–793.
23. Pascon, F.M.; Kantovitz, K.R.; Sacramento, P.A.; Nobre-dos-Santos, M.; Puppin-Rontani, R.M. Effect of sodium hypochlorite on dentine mechanical properties. A review. J. Dent. 2009, 37, 903–908.
24. Slutzky-Goldberg, I.; Maree, M.; Liberman, R.; Heling, I. Effect of sodium hypochlorite on dentin microhardness. J. Endod. 2004, 30, 880–882.
25. Sim, T.P.C.; Knowles, J.C.; Ng, Y.L.; Shelton, J.; Gulabivala, K. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. Int. Endod. J. 2001, 34, 120–132.
26. Grigoratos, D.; Knowles, J.; Ng, Y.L.; Gulabivala, K. Effect of exposing dentine to sodium hypochlorite and calcium hydroxide on its flexural strength and elastic modulus. Int. Endod. J. 2001, 34, 113–119.
27. Marending, M.; Paque, F.; Fischer, J.; Zehnder, M. Impact of irrigant sequence on mechanical properties of human root dentin. J. Endod. 2007, 33, 1325–1328.
28. Machnick, T.K.; Torabinejad, M.; Munoz, C.A.; Shabahang, S. Effect of MTAD on flexural strength and modulus of elasticity of dentin. J. Endod. 2003, 29, 747–750.
29. Lee, B.S.; Hsieh, T.T.; Chi, D.C.H.; Lan, W.H.; Lin, C.P. The role of organic tissue on the punch shear strength of human dentin. J. Dent. 2004, 32, 101–107.
30. Oliveira, L.D.; Carvalho, C.A.T.; Nunes, W.; Valera, M.C.; Camargo, C.H.R.; Jorge, A.O.C. Effects of chlorhexidine and sodium hypochlorite on the microhardness of root canal dentin. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 2007, 104, e125–e128.
31. Saleh, A.A.; Ettman, W.M. Effect of endodontic irrigation solutions on microhardness of root canal dentine. J. Dent. 1999, 27, 43–46.
32. HS Delgado, A.; Belmar Da Costa, M.; Polido, M.C.; Mano Azul, A.; Sauro, S. Collagen-depletion strategies in dentin as alternatives to the hybrid layer concept and their effect on bond strength: A systematic review. Sci. Rep. 2022, 12,

13028.

33. Lisboa, D.S.; Santos, S.V.D.; Griza, S.; Rodrigues, J.L.; Faria-e-Silva, A.L. Dentin deproteinization effect on bond strength of self-adhesive resin cements. Braz. Oral Res. 2013, 27, 73–75.

34. Neelakantan, P.; Sharma, S.; Shemesh, H.; Wesselink, P.R. Influence of irrigation sequence on the adhesion of root canal sealers to dentin: A Fourier transform infrared spectroscopy and push-out bond strength analysis. J. Endod. 2015, 41, 1108–1111.

35. Inai, N.; Kanemura, N.; Tagami, J.; Watanabe, L.G.; Marshall, S.J.; Marshall, G.W. Adhesion between collagen depleted dentin and dentin adhesives. Am. J. Dent. 1998, 11, 123–127.

36. Kanca, J., 3rd; Sandrik, J. Bonding to dentin. Clues to the mechanism of adhesion. Am. J. Dent. 1998, 11, 154–159.

37. Wakabayashi, Y.; Kondou, Y.; Suzuki, K.; Yatani, H.; Yamashita, A. Effect of dissolution of collagen on adhesion to dentin. Int. J. Prosthodont. 1994, 7, 302–306.

38. Gwinnett, A.J. Altered tissue contribution to interfacial bond strength with acid conditioned dentin. Am. J. Dent. 1994, 7, 243–246.

39. Vargas, M.A.; Cobb, D.S.; Armstrong, S.R. Resin-dentin shear bond strength and interfacial ultrastructure with and without a hybrid layer. Oper. Dent. 1997, 22, 159–166.

40. Ari, H.; Erdemir, A.; Belli, S. Evaluation of the effect of endodontic irrigation solutions on the microhardness and the roughness of root canal dentin. J. Endod. 2004, 30, 792–795.

41. Dotto, L.; Onofre, R.S.; Bacchi, A.; Pereira, G.K. Effect of root canal irrigants on the mechanical properties of endodontically treated teeth: A scoping review. J. Endod. 2020, 46, 596–604.

42. Belli, S.; Eraslan, O.; Eraslan, O.; Eskitascioglu, M.; Eskitascioglu, G. Effects of Na OC l, EDTA and MTAD when applied to dentine on stress distribution in post-restored roots with flared canals. Int. Endod. J. 2004, 47, 1123–1132.

43. Pérez-Heredia, M.; Ferrer-Luque, C.M.; González-Rodríguez, M.P.; Martín-Peinado, F.J.; 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, 418–423.

44. Zhang, K.; Kim, Y.K.; Cadenaro, M.; Bryan, T.E.; Sidow, S.J.; Loushine, R.J.; Tay, F.R. Effects of different exposure times and concentrations of sodium hypochlorite/ethylenediaminetetraacetic acid on the structural integrity of mineralized dentin. J. Endod. 2010, 36, 105–109.

45. Tartari, T.; Bachmann, L.; Maliza, A.G.A.; Andrade, F.B.; Duarte, M.A.H.; Bramante, C.M. Tissue dissolution and modifications in dentin composition by different sodium hypochlorite concentrations. J. Appl. Oral Sci. 2016, 24, 291–298.

46. Oyarzún, A.; Cordero, A.M.; Whittle, M. Immunohistochemical evaluation of the effects of sodium hypochlorite on dentin collagen and glycosaminoglycans. J. Endod. 2002, 28, 152–156.

47. Di Renzo, M.; Ellis, T.H.; Sacher, E.; Stangel, I. A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: II. Deproteination. Biomaterials 2001, 22, 793–797.

48. Kruzic, J.J.; Ritchie, R.O. Fatigue of mineralized tissues: Cortical bone and dentin. J. Mech. Behav. Biomed. Mater. 2008, 1, 3–17.

49. Driscoll, C.O.; Dowker, S.E.P.; Anderson, P.; Wilson, R.M.; Gulabivala, K. Effects of sodium hypochlorite solution on root dentine composition. J. Mater. Sci. Mater. Med. 2002, 13, 219–223.

50. Perdigão, J.A.; Thompson, J.Y.; Toledano, M.; Osorio, R. An ultra-morphological characterization of collagen-depleted etched dentin. Am. J. Dent. 1999, 12, 250–255.

51. Kaya, S.; Yiğit-Özer, S.; Adigüzel, Ö. Evaluation of radicular dentin erosion and smear layer removal capacity of Self-Adjusting File using different concentrations of sodium hypochlorite as an initial irrigant. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 2011, 112, 524–530.

52. Hu, X.; Ling, J.; Gao, Y. Effects of irrigation solutions on dentin wettability and roughness. J. Endod. 2010, 36, 1064–1067.

53. da Cunha, L.F.; Furuse, A.Y.; Mondelli, R.F.L.; Mondelli, J. Compromised bond strength after root dentin deproteinization reversed with ascorbic acid. J. Endod. 2010, 36, 130–134.

54. De-Deus, G.; Namen, F.; Galan, J., Jr.; Zehnder, M. Soft chelating irrigation protocol optimizes bonding quality of Resilon/Epiphany root fillings. J. Endod. 2008, 34, 703–705.

55. Tartari, T.; Oda, D.F.; Zancan, R.F.; da Silva, T.L.; De Moraes, I.G.; Duarte, M.A.H.; Bramante, C.M. Mixture of alkaline tetrasodium EDTA with sodium hypochlorite promotes in vitro smear layer removal and organic matter dissolution

during biomechanical preparation. Int. Endod. J. 2017, 50, 106–114.

56. De-Deus, G.; Souza, E.M.; Marins, J.R.; Reis, C.; Paciornik, S.; Zehnder, M. Smear layer dissolution by peracetic acid of low concentration. Int. Endod. J. 2011, 44, 485–490.

57. Cobankara, F.K.; 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, e149–e154.

58. Qian, W.; Shen, Y.; Haapasalo, M. Quantitative analysis of the effect of irrigant solution sequences on dentin erosion. J. Endod. 2011, 37, 1437–1441.

59. Kawasaki, K.; Ruben, J.; Tsuda, H.; Huysmans, M.C.D.N.J.M.; Takagi, O. Relationship between mineral distributions in dentine lesions and subsequent remineralization in vitro. Caries Res. 2000, 34, 395–403.

60. Torabinejad, M.; Cho, Y.; Khademi, A.A.; Bakland, L.K.; Shabahang, S. The effect of various concentrations of sodium hypochlorite on the ability of MTAD to remove the smear layer. J. Endod. 2003, 29, 233–239.

61. Niu, W.; Yoshioka, T.; Kobayashi, C.; Suda, H. A scanning electron microscopic study of dentinal erosion by final irrigation with EDTA and NaOCl solutions. Int. Endod. J. 2002, 35, 934–939.

62. Slutzky-Goldberg, I.; Liberman, R.; Heling, I. The effect of instrumentation with two different file types, each with 2.5% NaOCl irrigation on the microhardness of root dentin. J. Endod. 2002, 28, 311–312.

63. Perez, F.; Rouqueyrol-Pourcel, N. Effect of a low-concentration EDTA solution on root canal walls: A scanning electron microscopic study. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 2005, 99, 383–387.

64. Şen, B.H.; Ertürk, Ö.; Pişkin, B. The effect of different concentrations of EDTA on instrumented root canal walls. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod. 2009, 108, 622–627.

65. Goldberg, F.; Abramovich, A. Analysis of the effect of EDTAC on the dentinal walls of the root canal. J. Endod. 1977, 3, 101–105.

66. Di Lenarda, R.; Cadenaro, M.I.L.E.N.A.; Sbaizero, O.R.F.E.O. Effectiveness of 1 mol L−1 citric acid and 15% EDTA irrigation on smear layer removal. Int. Endod. J. 2000, 33, 46–52.

67. Perdigão, J.; Eiriksson, S.; Rosa, B.T.; Lopes, M.; Gomes, G. Effect of calcium removal on dentin bond strengths. Quintessence Int. 2001, 32, 142–146.

68. Pashley, D.H.; Tay, F.R.; Yiu, C.K.Y.; Hashimoto, M.; Breschi, L.; Carvalho, R.M.; Ito, S. Collagen degradation by host-derived enzymes during aging. J. Dent. Res. 2004, 83, 216–221.

69. De Munck, J.; Van den Steen, P.E.; Mine, A.; Van Landuyt, K.L.; Poitevin, A.; Opdenakker, G.; Van Meerbeek, B. Inhibition of enzymatic degradation of adhesive-dentin interfaces. J. Dent. Res. 2009, 88, 1101–1106.

70. Schwartz, R.S. Adhesive dentistry and endodontics. Part 2: Bonding in the root canal system—The promise and the problems: A review. J. Endod. 2006, 32, 1125–1134.

71. Eldeniz, A.U.; Erdemir, A.; Belli, S. Effect of EDTA and citric acid solutions on the microhardness and the roughness of human root canal dentin. J. Endod. 2005, 31, 107–110.

72. Cruz-Filho, A.M.; Sousa-Neto, M.D.; Saquy, P.C.; Pécora, J.D. Evaluation of the effect of EDTAC, CDTA, and EGTA on radicular dentin microhardness. J. Endod. 2001, 27, 183–184.

73. Uzunoglu, E.; Yilmaz, Z.; Erdogan, O.; Görduysus, M. Final irrigation regimens affect fracture resistance values of root-filled teeth. J. Endod. 2016, 42, 493–495.

74. Vollenweider, M.; Brunner, T.J.; Knecht, S.; Grass, R.N.; Zehnder, M.; Imfeld, T.; Stark, W.J. Remineralization of human dentin using ultrafine bioactive glass particles. Acta Biomater. 2007, 3, 936–943.

75. Zaparolli, D.; Saquy, P.C.; Cruz-Filho, A.M. Effect of sodium hypochlorite and EDTA irrigation, individually and in alternation, on dentin microhardness at the furcation area of mandibular molars. Braz. Dent. J. 2012, 23, 654–658.

76. Ghisi, A.C.; Kopper, P.M.P.; Baldasso, F.E.; Stürmer, C.P.; Rossi-Fedele, G.; Steier, L.; Vier-Pelisser, F.V. Effect of super-oxidized water, sodium hypochlorite and EDTA on dentin microhardness. Braz. Dent. J. 2014, 25, 420–424.

77. Souza, E.M.; Calixto, A.M.; e Lima, C.N.; Pappen, F.G.; De-Deus, G. Similar influence of stabilized alkaline and neutral sodium hypochlorite solutions on the fracture resistance of root canal–treated bovine teeth. J. Endod. 2014, 40, 1600–1603.

78. Tiwari, S.; Nikhade, P.; Chandak, M.; Sudarshan, C.; Shetty, P.; Gupta, N.K. Impact of Various Irrigating Agents on Root Fracture: An in vitro Study. J Contemp. Dent. Pract. 2016, 17, 659–662.

79. Khoroushi, M.; Ziaei, S.; Shirban, F.; Tavakol, F. Effect of intracanal irrigants on coronal fracture resistance of endodontically treated teeth undergoing combined bleaching protocol: An in vitro study. J. Dent. 2018, 15, 266–274.

80. Cecchin, D.; Farina, A.P.; Souza, M.A.; Albarello, L.L.; Schneider, A.P.; Vidal, C.M.P.; Bedran-Russo, A.K. Evaluation of antimicrobial effectiveness and dentine mechanical properties after use of chemical and natural auxiliary irrigants. J. Dent. 2015, 43, 695–702.
81. Russell, A.D.; Day, M.J. Antibacterial activity of chlorhexidine. J. Hosp. Infect. 1993, 25, 229–238.
82. Josic, U.; Maravic, T.; Mazzitelli, C.; Del Bianco, F.; Mazzoni, A.; Breschi, L. The effect of chlorhexidine primer application on the clinical performance of composite restorations: A literature review. J. Esthet. Restor. Dent. 2021, 33, 69–77.
83. Kanisavaran, Z.M. Chlorhexidine gluconate in endodontics: An update review. Int. Dent. J. 2008, 58, 247–257.
84. Jaju, S.; Jaju, P.P. Newer root canal irrigants in horizon: A review. Int. J. Dent. 2011, 2011, 851359.
85. Mohammadi, Z.; Abbott, P.V. The properties and applications of chlorhexidine in endodontics. Int. Endod. J. 2009, 42, 288–302.
86. Gamal, A.Y.; Mailhot, J.M. Effects of EDTA gel preconditioning of periodontally affected human root surfaces on chlorhexidine substantivity–An SEM study. J. Periodontol. 2007, 78, 1759–1766.
87. Wang, Z.; Shen, Y.; Haapasalo, M. Effect of smear layer against disinfection protocols on Enterococcus faecalis–infected dentin. J. Endod. 2013, 39, 1395–1400.
88. Turk, T.; Kaval, M.E.; Sarikanat, M.; Hülsmann, M. Effect of final irrigation procedures on fracture resistance of root filled teeth: An ex vivo study. Int. Endod. J. 2017, 50, 799–804.
89. Erdemir, A.; Ari, H.; Güngüneş, H.; Belli, S. Effect of medications for root canal treatment on bonding to root canal dentin. J. Endod. 2004, 30, 113–116.
90. Prado, M.; de Assis, D.F.; Gomes, B.P.; Simao, R.A. Effect of disinfectant solutions on the surface free energy and wettability of filling material. J. Endod. 2011, 37, 980–982.
91. Breschi, L.; Maravic, T.; Comba, A.; Cunha, S.R.; Loguercio, A.D.; Reis, A.; Mazzoni, A. Chlorhexidine preserves the hybrid layer in vitro after 10-years aging. Dent. Mater. 2020, 36, 672–680.