FACULDADE DE MEDICINA
UNIVERSIDADE D
COIMBRA

Mestrado Integrado em Medicina Dentária

Coronal dentin structural changes induced by different endodontic irrigation protocols

Maria Luísa de Carvalho Ribeiro

Orientador: Prof. Doutor Paulo Jorge Palma
Co-orientador: Doutor Rui Isidro Falacho

Coimbra, 2021

Coronal dentin structural changes induced by different endodontic irrigation protocols

Ribeiro ML[1], Falacho RI[2], Palma PJ[3]

1) Dentistry Student, Faculty of Medicine, University of Coimbra, Portugal
2) Invited Assistant Lecturer, Institute of Oral Implantology and Prosthodontics, Faculty of Medicine, University of Coimbra, Portugal
3) Invited Assistant Professor, Institute of Endodontics, Faculty of Medicine, University of Coimbra, Portugal

Área de Medicina Dentária
Faculdade de Medicina da Universidade de Coimbra
Av. Bissaya Barreto, Blocos de Celas
3000-075 Coimbra, Portugal
Tel.: +351 239 249 151/2
Fax.: +351 239 402 910

Email: marialuisacarvalhoribeiro16@gmail.com

Table of contents

Resumo

Objetivo: Avaliar os efeitos decorrentes da aplicação de diferentes protocolos de irrigação na ultraestrutura da dentina coronária.

Materiais e Métodos: Dois discos de dentina, obtidos a partir de um terceiro molar permanente humano, foram seccionados em 5 fragmentos dentinários. As amostras foram aleatoriamente divididas em cinco grupos de estudo (n=1), de acordo com os seguintes protocolos de irrigação: G1: água destilada (controlo); G2: 3% NaOCl; G3: 3% NaOCl + 17% EDTA; G4: 3% NaOCl + 17% EDTA + 2% CHX e G5: 3% NaOCl/9% ácido etidrónico (HEDP). A ultraestrutura dentinária foi posterioremente analisada com recurso a microscopia eletrónica de varrimento (SEM).

Resultados: O grupo controlo mostrou uma remoção incompleta da *smear layer,* com alguns túbulos obliterados e sem evidência de erosão. As amostras tratadas com NaOCl e NaOCl + EDTA mostraram uma remoção heterogénea da *smear layer,* com observação adicional de erosão da dentina peritubular e intertubular no grupo NaOCl + EDTA. A aplicação sequencial de NaOCl e EDTA, com ou sem irrigação subsequente com CHX, resultou em graus semelhantes de erosão dentinária. O grupo NaOCl + EDTA + CHX pareceu promover uma completa remoção da *smear layer* e abriu claramente os túbulos. Foi observada erosão da dentina intertubular (com pouco ou nenhum efeito a nível peritubular), modificação clara da topografia, bem como uma remoção eficaz da *smear layer* nas amostras tratadas com NaOCl/HEDP.

Conclusão: Todos os protocolos de irrigação, com exceção da água destilada e do NaOCl aplicado isoladamente, resultaram em alterações da ultraestrutura da dentina coronária. Verificou-se uma maior eficácia na remoção da *smear layer* após a aplicação sequencial de NaOCl, EDTA e CHX. Adicionalmente, a irrigação com NaOCl e EDTA, com ou sem irrigação subsequente com CHX, resultou em graus semelhantes de erosão dentinária. A associação de NaOCl com EDTA causou erosão da dentina peritubular e intertubular, enquanto que a combinação NaOCl/HEDP promoveu pouca ou nenhuma erosão da dentina peritubular, com modificação topográfica da dentina intertubular e promoveu uma remoção eficaz da *smear layer*.

Palavras-Chave: Ácido etidrónico, clorhexidina, dentina coronária, EDTA, hipoclorito de sódio.

Abstract

Aim: To evaluate the effects of different irrigation protocols on coronal dentin ultrastructure.

Methods: Two discs obtained from one third human permanent molar were sectioned into five dentinal fragments. Specimens were then randomly distributed among five study groups (n=1) according to the following irrigation protocols: G1: distilled water (control group); G2: 3% NaOCl; G3: 3% NaOCl + 17% EDTA; G4: 3% NaOCl + 17% EDTA + 2% CHX and G5: 3% NaOCl/9% etidronic acid (HEDP). Subsequent scanning electron microscopy (SEM) analysis was performed for dentinal ultrastructure assessment.

Results: Control group showed incomplete smear layer removal, with some obliterated dentinal tubules and no signs of degradation or erosion. Samples treated with NaOCl and NaOCl + EDTA exhibited heterogeneous smear layer removal, with erosion of both peritubular and intertubular dentin observed in the NaOCl + EDTA group. Sequential irrigation with NaOCl and EDTA, with or without subsequent CHX application, resulted in similar dentin erosion degree. NaOCl + EDTA + CHX group seemed to promote complete smear layer removal and plainly opened the tubules. Although few to no peritubular effects, intertubular dentinal erosion, with a clear topographic modification and effective smear layer removal, were observed following irrigation with NaOCl/HEDP.

Conclusion: All irrigation protocols led to structural changes in coronal dentin ultrastructure, except distilled water and isolated NaOCl application. Sequential irrigation with NaOCl, EDTA, and CHX allowed the most efficient smear layer removal. Moreover, irrigation with NaOCl and EDTA, with or without subsequent CHX application, resulted in similar dentin erosion degree. EDTA following NaOCl irrigation induced erosion of both peritubular and intertubular dentin while NaOCl/HEDP combined solution produced few to no peritubular erosion with a topographic change in intertubular dentin and an effective smear layer removal.

Keywords: Chlorhexidine, coronal dentin, EDTA, etidronic acid, sodium hypochlorite.

Introduction

Non-surgical endodontic treatment (TENC) aims to treat or prevent apical periodontitis.[1] The success of TENC relies, among several factors, on effective cleaning, disinfection, and shaping of the root canal system.[2] The complex anatomy of the root canal space limits the action of mechanical preparation, thus requiring simultaneous chemical preparation during endodontic therapy to reach areas left untouched by the instruments.[2,3]

After mechanical preparation, dentin surface is covered with smear layer, which presents an amorphous, irregular, and granular aspect and is composed of inorganic (dentin chips containing hydroxyapatite) and organic material (necrotic or vital pulp tissue, odontoblastic remnants, coagulated proteins, blood cells, nerve fibers, collagen, tissular fluid, saliva, bacteria, and their by-products).[4] Complete removal of smear layer previously to root canal filling results in an improved adaptation of obturation materials, decreases apical and coronal microleakage, and facilitates the diffusion of irrigant solutions and intracanal medications within the root canal system.[5] It is also believed that removing smear layer exposes the attached microbiota and their toxins from root canal walls, ultimately reducing the potential of bacterial survival and reproduction.[6] Thus, an ideal irrigation solution should be able to ensure smear layer removal.[7]

The most commonly used irrigating solutions include sodium hypochlorite (NaOCl) in concentrations ranging from 2.5% to 5.25%, 17% ethylenediaminetetraacetic acid (EDTA) and 10% citric acid (CA) as chelating agents, as well as 2% chlorhexidine (CHX).[6,8] Recently, etidronic acid (HEDP) has been suggested as an alternative to EDTA. This weak chelator can be mixed with NaOCl, to be used as a single irrigating solution.[8] As none of these irrigants displays all ideal properties, simultaneously targeting organic and inorganic components of smear layer, it becomes mandatory to combine different solutions by developing irrigation protocols to overtake the shortcomings inherent to each irrigant solution and fulfill the chemical preparation objectives.[9]

Antisepsis of the root canal space is thus ensured by combining distinct irrigating agents, which operate at the level of organic (proteolytic and antiseptic agents) and/or inorganic substrates (demineralizing/chelating substances).[6,8]

Dentin presents a unique and complex substrate that constitutes the bulk of tooth structure and absorbs mechanical loads.[8] It comprises approximately 45% inorganic phase, 33% organic matrix, and 22% water.[10] Microscopically, this mineralized connective tissue is bursting with organic substances or extracellular matrix. Type I collagen fibers present the main organic component, playing an essential role in distributing the functional stress applied to the tooth. The inorganic phase of dentin encloses hydroxyapatite crystals and other mineral

salts, such as carbonates and amorphous calcium phosphates. Microstructurally, dentin contains inverted-cone-shaped dentinal tubules, which contain odontoblastic processes, collagen fibers in deeper regions, and dentinal fluid, making it sensitive to structural and biological alterations.[8]

During endodontic treatment, irrigation solutions may affect mechanical and structural dentin characteristics.[8,11,12] Previous studies identify both mineral content and chemical composition changes, as well as dentin erosion, as consequences of root canal disinfection.[7,11,13–15] The deleterious effects of chemical preparation in root dentin are currently well-described and depend on concentration, time of exposure and, regarding chelating agents, on pH.[8,16,17] Most studies refer impairment of micro and nanohardness.[7,9,12,15,18] Findings indicating a negative impact on strength properties are also reported.[18,19] A possible reduction of dentin modulus of elasticity following EDTA and NaOCl is also described[20] and some authors outline an impairment on adhesion to root dentin.[21–23]

Despite extensive reports on the effects in root dentin, structural changes of coronal dentin following irrigation protocols remain unclear, even though this dentin is exposed to irrigants deposited in the pulp chamber with the same or higher volume and duration as root dentin.[9,24] Coronal dentin has a distinct number, diameter, and direction of dentinal tubules. Hence, the heterogeneity of the dentinal substrate impairs the extrapolation of irrigation protocols' effects from root to coronary dentin. Considering the inevitability of using root canal irrigants in endodontics and recognizing the negative impact of their use on the mechanical properties of endodontically treated teeth, it becomes crucial to assess the effects of these substances also in coronal dentin, which will be the primary substrate for adhesion, thus being responsible for the long-term stability of the endodontic and restorative treatment.

The present *in-vitro* pilot study aims to evaluate the effects of different irrigation protocols on coronal dentin ultrastructure. The alternative hypothesis is that irrigation protocols cause structural changes in coronal dentin ultrastructure.

Methods

This study was approved by the Ethical Committee of the Faculty of Medicine, University of Coimbra (notification CE-001/2013). One intact third human permanent molar extracted for orthodontic reasons was selected. The tooth was clinically and radiographically free of caries, cracks, restorations, or other abnormal features and had no previous root-canal treatment. The tooth surface was cleaned using periodontal scalers and polished with pumice and water to remove adherent organic material or calculus previously to immersion. Subsequently, it was stored in 0.5% chloramine T at 7ºC for five weeks before initiating the experimental procedures.

1. Dentin discs preparation

The tooth's radicular portion was embedded in autopolymerizing acrylic resin (Autopolimerizable Acrylic Resin; Schmidt Laboratory, Madrid, Spain; Lot: 47975, Expiration date: 2024/11) up to the cementoenamel junction. Two coronal dentin discs (one vestibular and one lingual) were obtained through longitudinal sectioning parallel to the long axis of the tooth. The section was performed with a low-speed (300 rpm at 0.050 mm/s) diamond disc (Accutom-5; Struers, Ballerup, Denmark) under continuous water cooling. Dentin surfaces were subsequently manually prepared using abrasive sandpaper in an ascending grit series (120, 240, and 600 grits according to ISO/DTS 11405) under constant irrigation to create smear layer. Vestibular and lingual discs were then sectioned in 3 and 2 dentin fragments, respectively, producing a total of 5 fragments. Disk sectioning into fragments was performed with a high-speed diamond bur under water cooling.

2. Dentin surface irrigation

Dentin samples were randomly assigned to five study groups (n=1) immediately after sectioning, according to the irrigation protocols shown in Table 1.

Table 1. Study groups.

Study group		**Irrigation protocol**
G1 (Control)	DW	Distilled water
G2	NaOCl	3% NaOCl
G3	NaOCl/EDTA	3% NaOCl 17% EDTA
G4	NaOCl/EDTA/CHX	3% NaOCl 17% EDTA 0.9% NaCl 2% CHX
G5	NaOCl/HEDP	3% NaOCl/9% HEDP

Dentin surface of specimens from groups 2, 3, and 4 was immersed in 3% NaOCl pre-heated at 37ºC (Digital Thermostatic Water Bath; Nahita, Beriain-Navarra, Spain) for 30 minutes. Subsequently, specimens from both 3 and 4 groups were introduced in 1 mL of 17% EDTA for 1 minute. Additionally, after immersion in saline solution (2 rounds of 1 minute in 1 mL), a final immersion in 1 mL of 2% chlorhexidine for 2 minutes was performed in group 4. Samples from the control group and group 5 were irrigated for 30 minutes with distilled water or a combination of 3% NaOCl and 9% HEDP at 37ºC, respectively. All distilled water, NaOCl, and NaOCl/HEDP solutions were renewed with 1 mL of irrigant solution every 2 minutes. Lastly, the exposed dentin was rinsed with water for 1 minute and dried using absorbing paper. The combined solution was freshly mixed before initiating the irrigation protocol by using a sterile spatula to mix 20 mL of NaOCl with the powder contained in 2 capsules of Dual Rinse® HEDP (Table 2) for 2 minutes.

Irrigation procedures were performed within a glass recipient on top of a vibrating base. All previously described experimental procedures were performed in a controlled environment of 22.1ºC and 43% humidity.

Table 2. Irrigant solutions specifics.

Irrigant solution	Manufacturer	Lot / Expiration date
Sodium hypochlorite 3%	Henry Shein Inc. Melville, USA	NH3-0221-8KV6 01/2022
CanalPro™ EDTA 17%	Coltène/Whaledent GmbH + Co.KG Langenau, Germany	171430 03/2023
CanalPro™ CHX 2%	Coltène/Whaledent GmbH + Co.KG Langenau, Germany	171012 11/2021
Dual Rinse® HEDP	Medcem GmbH Bahnhofstrasse, Weinfelden	DR210419 03/2024

3. Scanning Electron Microscopy (SEM)

For SEM analysis, immediately after irrigation procedures completion, all dentin specimens were firstly fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer (PBS) (0.1M, pH 7.4) for 48 hours at 4ºC. Samples were then rinsed three times with PBS 0.1M for 30 minutes each. For dehydration, the fixed specimens were immersed in ascending ethanol concentration solutions (30%, 50%, 70%, 80%, 95%, 100%, 100%) for 20 minutes each, followed by hexamethyldisilazane (HMDS) drying until its complete evaporation. Samples were then covered with absorbent paper and stored with silica at room temperature. Subsequently, specimens of each group were fractured into halves to allow transversal and longitudinal SEM

visualization of the dentin tubules. Afterwards specimens were set up on aluminum stubs using carbon glue and sputter-coated with gold-palladium for SEM (SEM S-4100; Hitachi, Chiyoda, Tokyo, Japan) observation at 15.0 kV to assess dentin ultrastructure. Photomicrographs were obtained under 2.000x and 10.000x magnification.

Results

Figures 1 to 5 show the representative SEM photomicrographs of the coronal dentin after irrigation.

Samples from the control group showed incomplete smear layer removal, with some obliterated dentinal tubules (Figure 1A). In cross-section, dentin tubule orifices were visible, with peritubular and intertubular dentin surfaces exhibiting a smooth aspect with no signs of degradation or erosion (Figure 1B).

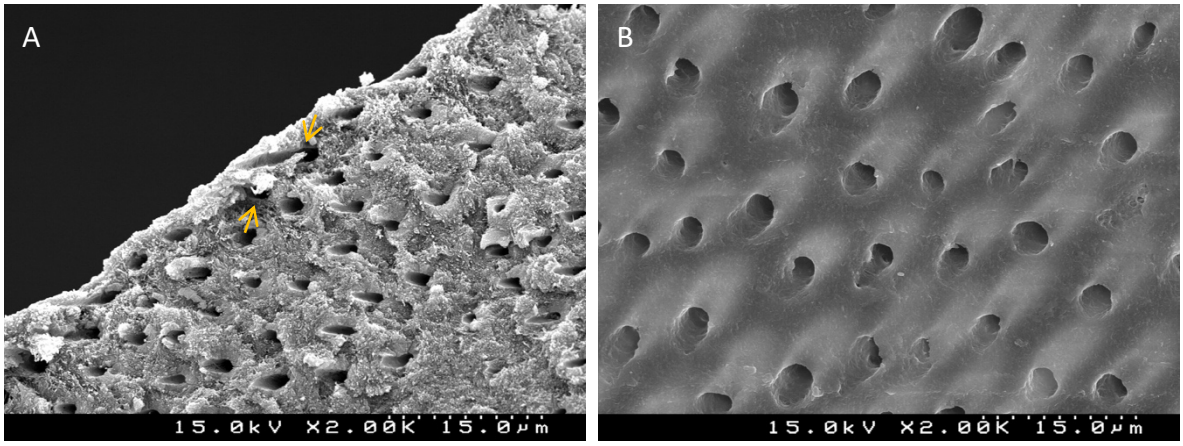

Figure 1. Representative SEM photomicrographs of coronal dentin after irrigation with distilled water (2.000x): (A) longitudinal section – incomplete smear layer removal with some obliterated tubules (arrows); (B) cross section – dentin tubules orifices visible and no signs of erosion.

In the NaOCl group, an inconsistent pattern of smear layer removal was observed, with most areas either totally or partially covered with dense, abundant smear layer and few areas where dentin tubule orifices could be observed (Figure 2).

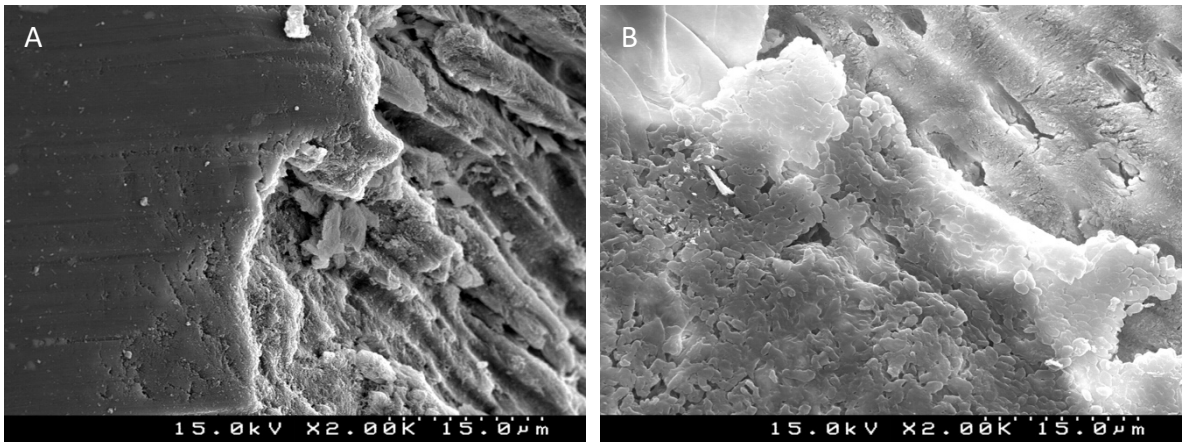

Figure 2. Representative SEM photomicrographs of coronal dentin after irrigation with NaOCl (2.000x): (A) longitudinal section – dense smear layer; (B) cross section – visible tubule orifices.

Samples treated with NaOCl/EDTA exhibited heterogeneous smear layer removal, with some tubule orifices partially occluded (Figure 3B). Erosion of peritubular and intertubular dentin, with widening of the dentinal tubule openings, was evident in this group (Figure 3A,C). In some regions, the magnitude of erosion resulted in intertubular dentin thinning or even disappearance between tubule openings (Figure 3C).

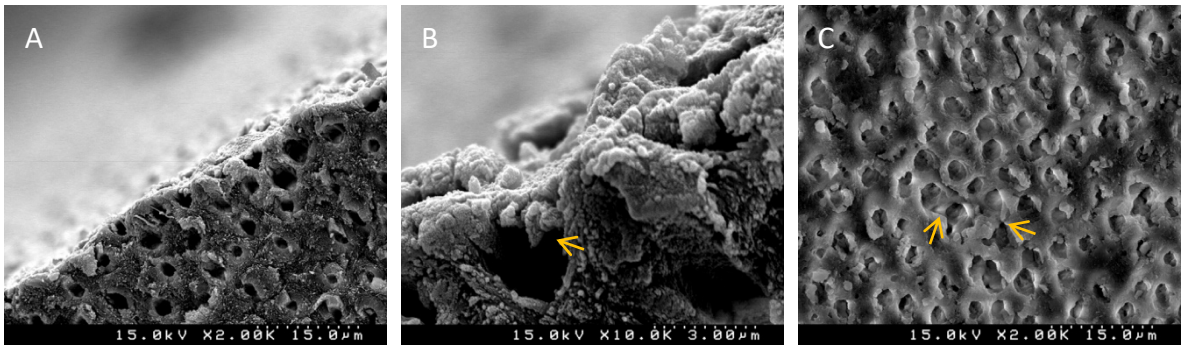

Figure 3. Representative SEM photomicrographs of coronal dentin after irrigation with NaOCl + EDTA: (A) longitudinal section – heterogeneous smear layer removal and widening of the tubule openings (2.000x); (B) longitudinal section – tubule orifices partially occluded with smear layer (arrow) (10.000x); (C) cross section – erosion of peritubular and intertubular dentin with intertubular dentin disappearance between tubule openings (arrows) (2.000x).

The dentin ultrastructure analysis of NaOCl/EDTA/CHX group was similar to that of NaOCl/EDTA group but with a discernable reduction in smear layer. Plainly opened tubules, in which smear layer appeared to have been entirely removed, were observed (Figure 4B,C). Opened calyx-shaped tubule orifices are found in longitudinal photomicrographs under high magnification (Figure 4A,B).

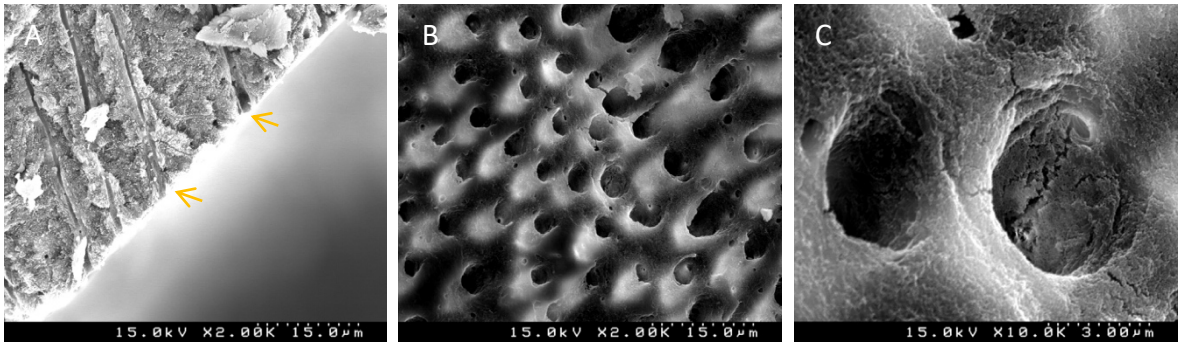

Figure 4. Representative SEM photomicrographs of coronal dentin after irrigation with NaOCl + EDTA + CHX: (A) longitudinal section – calyx-shaped tubule orifices (arrows) (2.000x); (B) cross section – completely smear layer removal (2.000x); (C) cross section – plainly opened tubules (10.000x).

Intertubular dentinal erosion was observed in samples treated with NaOCl/HEDP combined solution, although few to no peritubular effects were found. A clear topographic modification with surface roughening and an apparent increase in intertubular dentin area is shown in Figure 5.

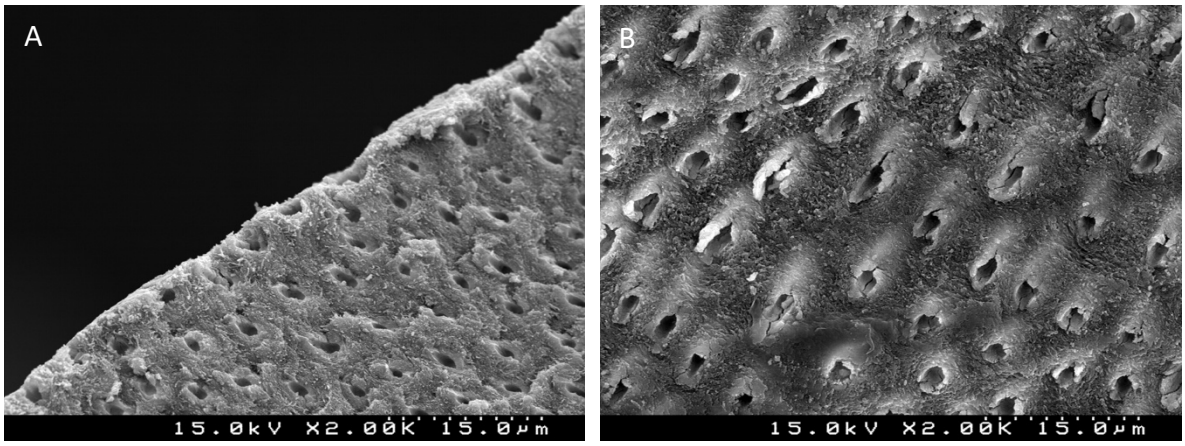

Figure 5. Representative SEM photomicrographs of coronal dentin after irrigation with NaOCl/HEDP (2.000x): (A) longitudinal section; (B) cross section. Topographic modification with surface roughening.

Discussion

This study assessed the structural changes caused by different irrigation protocols in coronal dentin. The alternative hypothesis was accepted since irrigation protocols led to structural changes in coronal dentin ultrastructure.

Irrigation is a *sine qua non* step for the success of non-surgical endodontic treatment. Sodium hypochlorite remains the most widely recommended irrigant in endodontics due to its antimicrobial properties and unique capacity to promote pulp tissue dissolution.[8,25] It has been reported that, regarding root dentin, NaOCl alone does not effectively remove the smear layer, regardless of the different concentrations and application time.[15,26,27] In this study, 3% NaOCl at 37ºC for 30 minutes was associated with an incomplete smear layer removal, with areas either totally or partially covered with a dense smear layer. Accordingly, Pedersen *et al.*[24] found that, in coronal dentin, smear layer was still present in samples exclusively irrigated with NaOCl. Previous studies described the remaining smear layer as thick and completely obliterating the dentinal tubules, whereas in this study, few tubule openings were still visible. This difference might have originated in the longer irrigation time and irrigant's agitation related to using a vibrating base in the present study, which may have contributed to a more efficient mechanical smear layer removal.[15,24,26] Similarly, a higher degree of smear layer removal was found in the control group when compared to data reported in the literature. Distilled water agitation with a vibrating base, common to all irrigation protocols, together with constant solution renewal, might also explain these results. Another possible explanation may reside in the fact that SEM images were obtained from deep dentin, in which more dentinal tubules are present. The use of abrasive sandpaper to create smear layer tends to have more open dentinal tubules than a dental bur as well as produces a less smear layer.[28] This could also explain both higher degree of smear layer removal and opened dentinal tubules.

The use of a chelating agent in addition to NaOCl has been advocated to allow complete smear layer removal.[27] A combination of NaOCl and EDTA is recommended to efficiently remove both organic and inorganic components of the smear layer. However, changes in both organic and inorganic dentin phases, as well as dentin erosion, have been described when NaOCl was used in association with the chelating agent.[15,26] These findings are in agreement with those obtained from the present study, in which EDTA used subsequently to NaOCl caused erosion of both peritubular and intertubular dentin, with widening of the dentinal tubule openings. Conversely, previous reports indicate no erosive effect after using EDTA associated with NaOCl.[18,29] These results' unconformity might be explained by different NaOCl and EDTA application times, which were higher in the present study, as well as different methodologies, as our study tried to simulate clinical conditions where irrigants are applied with an abundant

flow. Furthermore, the fact that most literature results refer to root dentin could also be an explanation.[15,18,26,27,29]

According to the literature, when NaOCl is used before EDTA, the hydroxyapatite coating seems to protect the underneath collagen fibers from the dissolving action of NaOCl. On the contrary, when NaOCl is used subsequently to the EDTA, it can directly degrade collagen previously exposed by the demineralizing agent. Thus, the erosive effects are more intense when NaOCl is used subsequently to EDTA.[30]

Although the application time of irrigants presents an important factor, there is no consensus regarding the optimal contact time during which irrigants should be applied in the root canal to ensure complete removal of the smear layer. It has been suggested that 1-minute EDTA irrigation effectively removes the smear layer, while a 10-minute application leads to excessive peritubular and intertubular dentin erosion. Hence, EDTA is not recommended for a more extended application than 1 minute to avoid undesirable amplified dentin erosion.[31] Considering this, in the present experimental study, EDTA was applied only for 1 minute in both groups 3 and 4.

Final CHX immersion in group 4 (NaOCl + EDTA + NaCl + CHX) showed similar results when comparing with group 3 (NaOCl + EDTA), but with a marked reduction in smear layer. According to the literature, CHX is a potent antiseptic that causes no adverse effects on the mechanical properties of root canal dentin.[20] It holds benefits as a root canal irrigant, including affinity to dental hard tissues with an extended antimicrobial activity and potential to remineralizing the demineralized dentin.[25] However, despite its usefulness as a final irrigant, CHX cannot be advocated as the main irrigant because of its incapacity to dissolve pulp tissue remnants. In the present study, 2% CHX was included since it simulates the concentration applied in endodontic irrigation.[25] The higher levels of smear layer removal observed in the present study, when compared to group 3 (NaOCl + EDTA), are thought to stem from a purely mechanical debriding action of the added two minutes irrigation with NaCl and two more minutes with CHX under vibration, not from the chemical reactivity of CHX.

Recently, a new chelator has been suggested as an alternative to EDTA.[25] Etidronic acid (HEDP) is a biocompatible chelator that can be used in combination with sodium hypochlorite without short-term properties loss in both compounds. Therefore, a NaOCl/HEDP combination was newly introduced as a single irrigant during and after instrumentation.[32] It has also been reported that the addition of etidronate powder to NaOCl increases surface tension of the mixed solution leading to less penetration on dentin which may limit its adverse effects.[33] Zehnder *et al.*[32] suggested that HEDP is a less aggressive chelating agent than EDTA, with minimal demineralization potential, although also efficient for smear layer removal. Concerning

root dentin, peritubular and intertubular erosion has been found following irrigation with 9% HEDP and 2.5% NaOCl/9% HEDP.[29] Our results in coronal dentin demonstrated that the tested combined solution (3% NaOCl/9% HEDP) caused intertubular erosion with a topography change in which dentinal tubules maintain their original width. However, dentin suffers a superficial roughening and, thus, an apparent increase in surface area speculated to allow for an improved interface between root or coronal dentin interface with either cement or adhesives. These findings can be attributed to the possibly higher wettability and penetration ability of HEDP compared with other irrigants.[33] The optimal concentrations of HEDP that can remove the smear layer and exert antibacterial activity were reported to be 9-18%[34], with lower concentrations being recommended to carry out the tested continuous chelation protocol.

The present study aimed at providing a scenario close to the clinical context and overcome some of the limitations potentially associated with *in vitro* testing.

It has been advocated in the literature that irrigant delivery and, especially, the activation of irrigation solutions are both crucial for further improving cleanliness and disinfection of the entire root canal system. Different methods of activation (manual agitation, manual-dynamic irrigation, sonic or ultrasonic activation and laser) have been suggested and, overall, they appear to have better results when compared with conventional syringe needle irrigation alone.[35] Thus, in agreement with these facts, a vibrating base was used to simulate irrigants activation.

Although dentin discs immersion in the irrigation solutions does not directly reproduce clinical conditions, this technique attempts at simulating the irrigant flow to which dentin is subjected inside the root canal or in the access cavity during the endodontic procedure.[12]

Although intrinsic to the nature of a pilot study, the small sample size presents additional limitations. To mitigate these and other procedural limitations, methodology standardization among different groups was taken into account by preparing similarly dimensioned dentin discs and using the same volume of irrigants, under the same technical and environmental conditions.

Resistance against fracture and the durability of coronal restorations might play a major role in the survival of endodontically treated teeth.[24] Chemical solutions used during root canal preparation may alter the structure, but also the chemical composition of dentin. Changes in dentin ultrastructure and composition can lead to a reduction in fracture resistance and affect interaction with materials used for coronal sealing.[7,36] Also, bonding effectiveness between adhesive systems and dentinal substrate depends on several variables, including dentin

inorganic and organic integrity.[37] Thus, the choice of an adequate irrigant solution to be used during the endodontic therapy assumes a crucial relevance.

Conclusion

Within the limitations of the present *in vitro* study, all irrigation protocols led to structural changes in coronal dentin ultrastructure, except distilled water and isolated NaOCl application. Sequential irrigation with NaOCl, EDTA and CHX allowed for the most efficient smear layer removal. Moreover, irrigation with NaOCl and EDTA, with or without subsequent CHX application, resulted in similar dentin erosion degree. The use of EDTA following NaOCl irrigation induced erosion of both peritubular and intertubular dentin and enlargement of dentinal tubules. NaOCl/HEDP irrigation protocol produced few to no peritubular erosion, while promoting an effective smear layer removal and a topographic change in intertubular dentin, which appears to improve surface area.

Future studies are needed to complement the qualitative structural analysis with data regarding the chemical composition of coronal dentin after irrigation protocols to further assess the impact of these alterations on its mechanical properties, including microhardness, flexural strength and modulus of elasticity, as well as on the bond strength of materials used for coronal sealing.

Acknowledgments

Ao longo da viagem que foram os últimos 5 anos aprendi que, ao lado das pessoas certas, a caminhada é melhor, mais fácil, mais leve... Termino com a certeza de que sem qualquer um de vocês não teria chegado até aqui.

Ao Professor Doutor Paulo Palma, pela oportunidade de realizar este trabalho e pela confiança que depositou em mim.

Ao Doutor Rui Falacho pelo esforço incansável, pela dedicação, amizade, disponibilidade, paciência, apoio e incentivo ao longo do último ano. A sua exigência contribuiu para o meu crescimento, tanto pessoal como profissional e o profissionalismo, paixão e excelência com que exerce esta profissão são, sem dúvida, uma inspiração. Obrigada por todo o conhecimento que tão bem me transmitiu e por ter sido das pessoas que mais acreditou em mim. Sem dúvida que muito do que sou hoje e ambiciono ser enquanto profissional, a si o devo. Estar-lhe-ei para sempre grata!

À Dra. Joana Marques, por toda a disponibilidade e dedicação. Os seus concelhos, palavras encorajadoras e amizade foram determinantes na conclusão deste percurso!

Aos meus amores de Coimbra, pelos últimos cinco anos! Por termos caminhado sempre lado a lado, pelas memórias, pelos bons momentos que me proporcionaram, pelas noites, os jantares, os brindes, as queimas e latadas, as viagens. Agradeço-vos a amizade, o carinho, o companheirismo. Fizeram-me feliz todos os dias e levo-vos para sempre comigo! Às minhas meninas, Carol e Inês, por terem sido o meu pilar todos os dias ao longo deste último ano. Não há palavras para agradecer o amor que sempre me deram! Serão para sempre o melhor significado de Coimbra e a minha melhor recordação dela!

Às de sempre, Madalena, Sílvia e Mariana, por me relembrarem quão bom é voltar a casa, pelos fins de semana, pelas saídas, por vivermos as conquistas umas das outras como se fossem as nossas e por serem a prova de que, apesar da distância e dos meses sem nos vermos, há coisas que permanecem e vão sempre permanecer exatamente onde foram deixadas.

Ao Rui, pelo apoio incondicional, pela compreensão e paciência, pelo carinho, pelo amor! Por todos os momentos em que acreditaste mais em mim do que eu mesma, por seres a minha força todos os dias, por todos os conselhos e por nunca desistires de mim. Para ti as palavras nunca serão suficientes!

Aos meus pais e mana, por serem o pilar da minha vida! Agradeço-vos o amor incondicional, a confiança, a preocupação e todo o apoio que sempre me deram. Obrigada por me terem proporcionado todas as ferramentas para alcançar tudo o que desejei e por me terem deixado

voar, sempre com a certeza e segurança de que estariam lá quando voltasse. São e sempre serão o meu exemplo a seguir! A vocês devo tudo o que sou hoje e estarei para sempre grata por tudo.

À minha família, tios, primas, vovó Mimi, a vocês agradeço a preocupação e o incentivo que sempre me deram em todos os momentos da minha vida. Obrigada por estarem sempre presentes e acreditarem sempre em mim!

Aos que, apesar de terem partido a meio desta jornada, foram sempre a minha maior força! A vocês o meu eterno obrigada, daqui... até ao Céu!

Por fim a Coimbra, eterna cidade dos estudantes, por me ter acolhido e ensinado a crescer ao longo dos últimos cinco anos. Guardo em mim todas as memórias, desde a primeira serenata até ao último cortejo, ao último toque da cabra, à última balada.

Eternamente agradecida!

References

1. Dahlkemper P., Ang D., Goldberg R., Rubin R., Schultz G., Sheridan B., et al. Guide to Clinical Endodontics. Am Assoc Endodontists. 2016;88(6):1–39.
2. Peters OA. Current challenges and concepts in the preparation of root canal systems: a review. J Endod. 2004 Aug;30(8):559–67.
3. Siqueira JFJ, Rôças IN, Santos SRLD, Lima KC, Magalhães FAC, de Uzeda M. Efficacy of instrumentation techniques and irrigation regimens in reducing the bacterial population within root canals. J Endod. 2002 Mar;28(3):181–4.
4. Kokkas AB, Boutsioukis AC, Vassiliadis LP, Stavrianos CK. The influence of the smear layer on dentinal tubule penetration depth by three different root canal sealers: an in vitro study. J Endod. 2004 Feb;30(2):100–2.
5. Violich DR, Chandler NP. The smear layer in endodontics - a review. Int Endod J. 2010 Jan;43(1):2–15.
6. Di Lenarda R, Cadenaro M, Sbaizero O. Effectiveness of 1 mol L-1 citric acid and 15% EDTA irrigation on smear layer removal. Int Endod J. 2000 Jan;33(1):46–52.
7. Aranda-Garcia AJ, Kuga MC, Chavéz-Andrade GM, Kalatzis-Sousa NG, Hungaro Duarte MA, Faria G, et al. Effect of final irrigation protocols on microhardness and erosion of root canal dentin. Microsc Res Tech. 2013;76(10):1079–83.
8. Rath PP, Yiu CKY, Matinlinna JP, Kishen A, Neelakantan P. The effect of root canal irrigants on dentin: a focused review. Restor Dent Endod. 2020;45(3):1–11.
9. Zaparolli D, Saquy PC, Cruz-Filho AM. 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(6):654–8.
10. Perdigão J. Current perspectives on dental adhesion: (1) Dentin adhesion - not there yet. Jpn Dent Sci Rev. 2020 Nov;56(1):190–207.
11. Cobankara FK, Erdogan H, Hamurcu M. Effects of chelating agents on the mineral content of root canal dentin. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2011;112(6):e149–54. Available from: http://dx.doi.org/10.1016/j.tripleo.2011.06.037
12. 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 Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2007;104(3):418–24.

13. Mai S, Kim YK, Arola DD, Gu L sha, Kim JR, Pashley DH, et al. Differential aggressiveness of ethylenediamine tetraacetic acid in causing canal wall erosion in the presence of sodium hypochlorite. J Dent. 2010;38(3):201–6. Available from: http://dx.doi.org/10.1016/j.jdent.2009.10.004

14. Saghiri MA, Delvarani A, Mehrvarzfar P, Malganji G, Lotfi M, Dadresanfar B, et al. A study of the relation between erosion and microhardness of root canal dentin. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology. 2009;108(6):e29–34. Available from: http://dx.doi.org/10.1016/j.tripleo.2009.07.056

15. Baldasso FER, Roleto L, Silva VD da, Morgental RD, Kopper PMP. Effect of final irrigation protocols on microhardness reduction and erosion of root canal dentin. Braz Oral Res. 2017;31:e40.

16. Cury JA, Bragotto C, Valdrighi L. The demineralizing efficiency of EDTA solutions on dentin. I. Influence of pH. Oral Surg Oral Med Oral Pathol. 1981 Oct;52(4):446–8.

17. Zhang K, Kim YK, Cadenaro M, Bryan TE, Sidow SJ, Loushine RJ, et al. Effects of Different Exposure Times and Concentrations of Sodium Hypochlorite/Ethylenediaminetetraacetic Acid on the Structural Integrity of Mineralized Dentin. J Endod. 2010;36(1):105–9. Available from: http://dx.doi.org/10.1016/j.joen.2009.10.020

18. Bosaid F, Aksel H, Makowka S, Azim AA. Surface and structural changes in root dentine by various chelating solutions used in regenerative endodontics. Int Endod J. 2020;53(10):1438–45.

19. Cecchin D, Soares Giaretta V, Granella Cadorin B, Albino Souza M, Vidal C de MP, Paula Farina A. Effect of synthetic and natural-derived novel endodontic irrigant solutions on mechanical properties of human dentin. J Mater Sci Mater Med. 2017;28(9):1–6. Available from: http://dx.doi.org/10.1007/s10856-017-5960-1

20. Durmus B, Hale AA, Oguz E, Sema B. The effect of different irrigation protocols on elastic modulus of dentine and biomechanics of single-rooted premolar tooth: A nano-indentation and finite element analysis study. Niger J Clin Pract. 2019;22(1):101–7.

21. Morris MD, Lee KW, Agee KA, Bouillaguet S, Pashley DH. Effects of sodium hypochlorite and RC-prep on bond strengths of resin cement to endodontic surfaces. J Endod. 2001 Dec;27(12):753–7.

22. Prati C, Chersoni S, Pashley DH. Effect of removal of surface collagen fibrils on resin-dentin bonding. Dent Mater. 1999 Sep;15(5):323–31.

23. Panighi M, G'Sell C. Influence of calcium concentration on the dentin wettability by an

adhesive. J Biomed Mater Res. 1992 Aug;26(8):1081–9.

24. Pedersen ND, Uzunoglu-Özyürek E, Dogan Buzoglu H. Influence of different irrigation protocols on microhardness and flexural strength values of young and aged crown dentin. Gerodontology. 2020;37(1):53–8.

25. Zehnder M. Root canal irrigants. J Endod. 2006 May;32(5):389–98.

26. Baldasso FER, Cardoso LR, Silva VD da, Morgental RD, Kopper PMP. Evaluation of the effect of four final irrigation protocols on root canal dentin components by polarized light microscopy and scanning electron microscopy. Microsc Res Tech. 2017;80(12):1337–43.

27. Mancini M, Armellin E, Casaglia A, Cerroni L, Cianconi L. A Comparative Study of Smear Layer Removal and Erosion in Apical Intraradicular Dentine With Three Irrigating Solutions: A Scanning Electron Microscopy Evaluation. J Endod. 2009;35(6):900–3. Available from: http://dx.doi.org/10.1016/j.joen.2009.03.052

28. Akter RS, Ahmed Z, Yamauti M, Carvalho RM, Chowdhury AFMA, Sano H. Effects of remaining dentin thickness, smear layer and aging on the bond strengths of self-etch adhesives to dentin. Dent Mater J. 2021;40(2):538–46.

29. Ulusoy, Mantı A, Çelik B. Nanohardness reduction and root dentine erosion after final irrigation with ethylenediaminetetraacetic, etidronic and peracetic acids. Int Endod J. 2020;53(11):1549–58.

30. 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. Available from: http://dx.doi.org/10.1016/j.joen.2011.06.005

31. Çalt S, Serper A. Time-dependent effects of EDTA on dentin structures. J Endod. 2002;28(1):17–9.

32. Zehnder M, Schmidlin P, Sener B, Waltimo T. Chelation in root canal therapy reconsidered. J Endod. 2005 Nov;31(11):817–20.

33. Giardino L, Del Fabbro M, Morra M, Pereira T, Bombarda de Andrade F, Savadori P, et al. Dual Rinse(®) HEDP increases the surface tension of NaOCl but may increase its dentin disinfection efficacy. Odontology. 2019 Oct;107(4):521–9.

34. Ulusoy Öİ, Zeyrek S, Çelik B. Evaluation of smear layer removal and marginal adaptation of root canal sealer after final irrigation using ethylenediaminetetraacetic, peracetic, and etidronic acids with different concentrations. Microsc Res Tech. 2017 Jul;80(7):687–92.

35. Gu L, Kim JR, Ling J, Choi KK, Pashley DH, Tay FR. Review of contemporary irrigant agitation techniques and devices. J Endod. 2009 Jun;35(6):791–804.

36. Santos JN, Carrilho MR de O, De Goes MF, Zaia AA, Gomes BPF de A, Souza-Filho FJ de, et al. Effect of chemical irrigants on the bond strength of a self-etching adhesive to pulp chamber dentin. J Endod. 2006 Nov;32(11):1088–90.

37. Moreira DM, Almeida JFA, Ferraz CCR, Gomes BPF de A, Line SRP, Zaia AA. Structural analysis of bovine root dentin after use of different endodontics auxiliary chemical substances. J Endod. 2009 Jul;35(7):1023–7.