1. Introduction

The sequential application of sodium hypochlorite (NaOCl) and ethylenediamine tetraacetic acid (EDTA) provides a predictable means for clinicians who encompass removal of canal wall smear layers as part of their root canal cleaning and shaping objectives. Nevertheless, canal wall erosion, characterised by dissolution of intertubular and peritubular

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dentine and coalescence of widened dentinal tubules, has been associated with the use of this dual irrigant regime. As these surface features were acquired exclusively using scanning electron microscopy, little is known about the subsurface characteristics of canal wall erosion. Decline in mechanical properties of a bulk specimen is unlikely to be caused only by surface alteration of the material. Thus, the recent report that prolonged NaOCl–EDTA irrigation results in deterioration of the mechanical properties of radicular dentine requires clarification of the subsurface extent of canal wall erosion.

As EDTA demineralises radicular dentine, it is logical to assume that canal wall erosion is caused by prolonged use of EDTA or its sustained release from dentinal tubules. Indeed, attempts have been made to inactivate the chelating effects of EDTA or its sustained release from dentinal tubules. If this hypothesis is correct, increasing the initial NaOCl exposure time while keeping the final EDTA exposure time constant should increase the likelihood of inducing canal wall erosion. As the mechanical properties of mineralised dentine are adversely affected by its prolonged contact with 3–5% NaOCl, it is more likely that NaOCl and EDTA interact synergistically to augment penetration of EDTA into the intertubular and peritubular dentine to expedite their desmineralisation. If this hypothesis is correct, increasing the initial NaOCl exposure time while keeping the final EDTA exposure time constant should increase the likelihood of inducing canal wall erosion. Thus, the objective of the present study was to test the hypothesis that the use of EDTA as a final irrigant causes canal wall erosion only after prolonged use of 5.25% NaOCl as the initial irrigant.

2. Materials and methods

2.1. Flexural strength

Flexural strength evaluation was performed using mid-coronal dentine obtained from 30 non-caries human third molars to ensure collection of enough testable substrates and consistency in dentine tubular orientation. A dentine disk was prepared perpendicular to the longitudinal axis of each tooth with a slow-speed Isomet saw (Buehler Ltd., Lake Bluff, IL, USA) under copious water cooling. A 7 mm × 3 mm × 0.2 mm beam was prepared from the centre of each disk to ensure that dentinal tubules were oriented parallel to the plane of maximum stress during three-point flexure. The dimensions of each beam were measured to the nearest 0.01 mm.

The dentine beams were randomly divided into three groups (N = 10). Beams in the control group were tested under water without prior immersion in active root canal irrigants. The first experimental group was designed to simulate root canal treatment in single-rooted anterior teeth with large canals wherein the NaOCl remains in the pulp chamber for 10 min during canal instrumentation. Accordingly, the beams were first immersed in 5.25% NaOCl (The Clorox Company, Oakland, CA, USA) for 10 min, rinsed with deionised water and immersed in 17% EDTA (Sigma–Aldrich, St. Louis, MO, USA) for 2 min. The beams were thoroughly rinsed with deionised water again to prevent continuous demineralisation of the dentine surfaces. The second experimental group was designed to simulate a more complex treatment scenario in root canal treatment of posterior teeth wherein the NaOCl remains in the pulp chamber for 60 min during canal negotiation and shaping. Accordingly, the beams were immersed in 5.25% NaOCl for 60 min, rinsed with deionised water, immersed in 17% EDTA for 2 min and then with deionised water.

Flexural testing was performed using a miniature three-point flexure device with a 5-mm support span. Each 7-mm long beam was placed on top of the support span and loaded to fracture under water using a universal testing machine (Vitrodyne V100, Liveco Inc., Burlington, VT, USA) at a crosshead speed of 1 mm/min. Flexural strength (in MPa) was calculated using the formula $\sigma = \frac{3PL}{2bd^3}$, where $P$ = load at fracture, $L$ = length of support span [mm], $b$ = beam width [mm] and $d$ = beam thickness [mm]. As the data from the three groups were not normally distributed, they were statistically analysed using Kruskal–Wallis ANOVA and Dunn’s multiple comparison test at $\alpha = 0.05$.

2.2. Transmission electron microscopy

Two methacrylate resin-based sealers, RealSeal and RealSeal SE (SybronEndo, Orange, CA, USA) were used to facilitate ultrastructural examination of the radicular dentine after irrigation with the aforementioned experimental protocols. These mild sealers rely more on the ability of EDTA rather than their inherent self-etching capacities to bond to radicular dentine. They were used in lieu of non-bondable sealers to create continuous sealer–dentine interfaces that were amendable to ultramicrotomy without separation of the thin sections. Five single-rooted human teeth were used. A polyvinylsiloxane (PVS) mould was prepared for each tooth by inserting the root into mixed, putty-type PVS. Each root was split into two longitudinal halves, which were re-assembled back into the mould. Working length was established 0.5 mm short of the apex. Instrumentation was performed using a crown-down technique. The canals were prepared initially up to size 40, 0.06 taper using nickel–titanium rotary instruments (Profile rotary instruments, Dentsply Maillefer, Ballaigues, Switzerland). The apical seat was further prepared to size 60 using stainless steel hand files. During cleaning and shaping, five roots were irrigated with the first experimental irrigation protocol. The other five roots were irrigated using the second experimental protocol. The Irrigants were delivered to within 2 mm of the working length using 30-gauge ProRinse irrigation needles (Dentsply Tulsa Dental, Tulsa, OK, USA). As there was continuous seepage of the irrigants from the fractured roots, the 60 min NaOCl irrigation protocol was modified by immersing the entire assembly in NaOCl for 50 min after an initial 10-min period of syringe-mediated NaOCl irrigation.

The root-halves were removed from the mould after irrigation. Half of the fractured canal was filled with RealSeal and the other half with RealSeal SE, according to the
manufacturer's instructions. A 2-mm thick transverse section containing the sealer–dentine interface was prepared from each root-half. Sectioning was performed at 5 mm from the apical foramen, corresponding to the middle third of the canal. The specimens were fixed, dehydrated in an ascending ethanol series and embedded in epoxy resin.26 Undemineralised, 90–120 nm thick sections were examined without staining using a JEM-1230 transmission electron microscope (JEOL, Tokyo, Japan) operated at 110 keV.

3. Results

Flexural strengths (mean ± standard error of the mean, in MPa) for the control, first (10-min NaOCl) and second (60-min NaOCl) experimental groups were 218.0 ± 7.5, 220.9 ± 20.8 and 22.1 ± 2.7, respectively. Exposure of the dentine beams to NaOCl for 60 min significantly reduced dentine flexural strength (p < 0.001) when compared with those beams that had not been immersed in active irrigants. By contrast, exposure of the dentine beams to NaOCl for 10 min did not result in a significant decline in flexural strength (Fig. 1).

Fig. 1 – Flexural strengths of mineralised dentine beams (3 mm × 7 mm × 0.2 mm thick) that were tested to failure under water. Values are mean ± standard error of the mean (N = 10). Groups designated by the same letters above the error bars are not statistically significant (p > 0.05).

Fig. 2 – TEMs of instrumented root dentine that had been treated with 5.25% NaOCl for 10 min and 17% EDTA for 2 min. Specimens were bonded with RealSeal (RS) that utilised a separate self-etching primer (P), or RealSeal SE (SE), a self-adhesive sealer. M: mineralised dentine. (A) The RS–dentine interface revealed mild etching (open arrowhead) of the dentine surface without erosion of the subsurface dentin. (B) High magnification of Fig. 2A showing a completely demineralised zone (between open arrowheads). (C) The SE–dentine interface showed a similar mild surface etching effect (open arrowhead). (D) High magnification of Fig. 2C revealed a partially demineralised zone (between open arrowheads) with remnant apatite crystallites.
Instrumented canal walls in the 10-min NaOCl experimental group were completely devoid of smear layer, irrespective of whether they were bonded with RealSeal that utilised a self-etching primer (Fig. 2A) or the self-adhesive RealSeal SE (Fig. 2C). For both methacrylate resin-based sealers, a 0.5 μm thick, uniform demineralisation front could be identified along the dentine surface (Fig. 2B and D). There was no erosion of the subsurface peritubular and intertubular dentine.

Instrumented canal walls in the 60-min NaOCl experimental group exhibited severe surface and subsurface erosion that extended 25–30 μm beneath the resin–dentine interfaces (Fig. 3A and C). This tunneling erosive pattern resulted in a weakened dentine infrastructure that was characterised by dissolution of the peritubular dentine, enlarged and interconnected tubular spaces and extensive loss of the intertubular dentine. Irrespective of the type of adhesive sealer (Fig. 3B and D), 0.5 μm thick, demineralisation fronts were present on all eroded dentine surfaces and subsurfaces.

4. Discussion

The results obtained from the present study support the hypothesis that the use of EDTA as a final irrigant causes canal wall erosion only when NaOCl is used adjunctively as the initial irrigant. The reduction in mean flexural strength in the 60-min NaOCl experimental group to 1/10 of that in the control group has to be interpreted with caution, as both sides of the 200 μm thick dentine flexure specimens were simultaneously exposed to irrigants. Clinically, we expect a milder scenario with irrigants diffusing only from the pulpal side of the canal space. Generation of a 25–30 μm thick erosion zone from either side could have reduced the intact dentine to only 140–150 μm thick. This ultrastructure-based approximation is likely to underestimate the extent of damage, as NaOCl may penetrate far deeper into the intact dentine, causing biochemical damage to the organic components of the mineralised collagen27–30 that are not recognisable at the ultrastructural level.

![Fig. 3](image_url) - TEMs of instrumented root dentine that had been treated with 5.25% NaOCl for 60 min and 17% EDTA for 2 min before the application of RealSeal (RS) or RealSeal SE (SE). P: self-etching primer; M: mineralised dentine. (A) The RS–dentine interface exhibited severe surface and subsurface erosion (between open arrows) with extensive loss of peritubular and intertubular dentine and enlarged, interconnected tubular spaces. A thin demineralisation front was present on all eroded dentine surfaces (open arrowheads). (B) High magnification of Fig. 3A. The thickness of the demineralisation fronts (open arrowheads) in the eroded regions was similar to that depicted Fig. 2B. (C) The SE–dentine interface exhibited a similar tunneling erosive pattern (between open arrows) and thin demineralisation fronts (open arrowheads). (D) High magnification of Fig. 3C showing a thin layer of demineralised dentine (open arrowheads) on all the eroded dentine surfaces.
The subsurface erosive pattern identified from the radicular dentine specimens in the 60-min NaOCl group has not been reported in the literature. It had never been observed when EDTA or NaOCl was used separately, even when either solution was in prolonged contact with mineralised dentine. Although apatite-encapsulated mineralised dentine is less vulnerable initially to the destructive effects of NaOCl, its organic phase will eventually be deproteinised by NaOCl in a time-dependent manner, resulting in increases in brittleness and permeability. Even with these destructive NaOCl-induced events, preservation of the apatite phase within the mineralised dentine implies that erosive disintegration of intertubular and peritubular dentine will not occur.

Conversely, when NaOCl and EDTA are used in sequence, the two irrigants can interact synergistically to induce an erosive effect. Unlike dentine demineralisation that creates uniform demineralisation fronts propagating from the dentine surface downwards, deproteinisation proceeds non-uniformly along deproteinisation channels within the dentinal tubules. Removal of the organic phase in mineralised dentine by NaOCl enhances the permeability of peri-tubular and intertubular dentine to EDTA, which in turn, demineralises the apatite phase and expedites NaOCl infiltration and destruction. Once all the exposed collagen fibrils are dissolved, the deproteinisation process reverts to that observed for the apatite-encapsulated collagen, repeating the vicious cycle. This series of events also explains why the thickness of the demineralisation front in the 60-min NaOCl group did not increase but remained consistently at 0.5 μm along the dentine surface and eroded subsurface sites (Fig. 3B and D). Although peritubular dentine is noncollagenous, it is rich in glutamic acid-containing proteins which are also susceptible to the deproteinisation. Being hypermineralised, the more rapid demineralisation rate of peritubular dentine probably expedites the diffusion of NaOCl into the intertubular dentine.

5. Conclusion

Within the limits of this study, it may be concluded that the apparent aggressiveness of EDTA in causing erosion of radicular dentine is attributed to the prolonged initial contact of the dentine by NaOCl. The 60-min period of NaOCl application should not be viewed as an overzealous use of the disinfecting irrigant. On the contrary, this time period is clinically realistic in the treatment of teeth with multiple canals wherein the pulp chamber dentine is constantly bathed in NaOCl during negotiation and shaping of the canals. Indeed, clinicians are employing different means to improve the debridement efficacy within the root canal system. They include frequent replenishing of irrigants, warming of NaOCl to improve its tissue dissolution efficacy and the use of different agitation devices to enhance irrigant flow and replacement along the canal walls. In the presence of thin pulp chamber dentine or canal walls, the synergistic action between NaOCl and EDTA may render these rooted-treated teeth more prone to vertical fracture.

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References


