Effects of Reducing Agents on Birefringence Dentin Collagen after Use of Different Endodontic Auxiliary Chemical Substances

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Abstract

Introduction: The aim of this study was to evaluate the effect of 10% ascorbic acid or 10% sodium ascorbate on organic matrix collagen of bovine dentin root canal walls after irrigation with 5.25% sodium hypochlorite (NaOCl), 17% ethylenediaminetetraacetic acid (EDTA), or 0.9% sodium chloride. **Methods:** Eighty bovine incisors were randomly divided into 8 groups (n = 10): group 1, 0.9% sodium chloride (control); group 2, 5.25% NaOCl + 17% EDTA (NaOCl + EDTA); group 3, 5.25% NaOCl + 17% EDTA + 10% ascorbic acid (NaOCl + EDTA + AA); group 4, 5.25% NaOCl + 17% EDTA + 10% sodium ascorbate (NaOCl + EDTA + SA); group 5, 5.25% NaOCl (NaOCl); group 6, 17% EDTA; group 7, 10% ascorbic acid (AA); and group 8, 10% sodium ascorbate (SA). Teeth were chemomechanically prepared, submitted to histologic processing, and stained with Sirius Red dye to be analyzed under polarized light microscopy. Absorbance assay was also performed to confirm the loss of collagen. **Results:** NaOCl + EDTA and NaOCl groups presented a significantly different birefringence pattern compared with the control group ($P < .05$). The measurement of the optical retardations of NaOCl + EDTA + SA indicated that this group was not statistically different from the control group. Although the measurement of the optical retardations of NaOCl + EDTA + AA was statistically different from the control group, the results were significantly higher than for NaOCl + EDTA. The birefringence of EDTA, AA, and SA groups was not statistically different from that of control group. The absorbance assay of NaOCl + EDTA and NaOCl groups confirmed the loss of collagen ($P < .05$). **Conclusions:** It is possible to conclude that 5.25% NaOCl, whether associated or not with 17% EDTA, causes birefringence alterations and loss of dentin collagen. These alterations reduced the ability of Sirius Red to bind with collagen fiber molecules. The reductions in the optical retardation values could be reversed by the application of either 10% ascorbic acid or 10% sodium ascorbate after 5.25% sodium hypochlorite and 17% EDTA irrigation. *(J Endod 2011;37:1406–1411)*

Key Words

Auxiliary chemical solutions, birefringence, collagen, reducing agents

One of the primary objectives of endodontic therapy is the microbial reduction, which in turn promotes the normal healing process of the periodontal tissues (1). However, elimination of microorganisms from infected root canals is a hard task, mainly considering the complex anatomy of the root canal pulp space (2). The chemomechanical preparation concept relates to the use of chemically active irrigating solutions in combination with mechanical cleansing (3).

Among the auxiliary chemical substances used in endodontics during chemomechanical preparation, sodium hypochlorite (NaOCl) in different concentrations has been the most widely recommended irrigant solution (4). This endodontic irrigant has the ability to destroy a broad spectrum of microbes and to dissolve organic materials; however, it is known that NaOCl solution has cytotoxic effects and that it alters the organic components of dentin, especially collagen, which contributes considerably to the mechanical properties of dentin (3, 5).

The effects of NaOCl on resin-dentin bond strength have been extensively investigated (6–9). It is thought that NaOCl leads to oxidation of some component in the dentin matrix, and this might interfere with the penetration of monomers within the demineralized dentin structure. In addition, NaOCl breaks down to sodium chloride and oxygen. Oxygen from such chemicals causes strong inhibition of the interfacial polymerization of resin bonding materials. The generation of oxygen bubbles at the resin-dentin interface might also interfere with resin infiltration into the tubules and intertubular dentin (6, 8, 10).

The residual NaOCl could be neutralized by the application of biocompatible reducing agents (eg, ascorbic acid and sodium ascorbate) to the oxidized dentin, which restores the redox potential of dentin and converts the microenvironment of the dentin from an oxidized substrate to a reduced substrate, thus facilitating complete polymerization of resin bonding materials (11–13).

According to Lai et al (14), although the reducing agents reverse the compromised bond strength, this phenomenon might be system-specific. However, the authors affirmed that clinical implication of this would be that by means of the use of an antioxidant such as

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sodium ascorbate, clinicians can acid-etch and bond immediately to endodontically treated teeth that were irrigated with NaOCl, without compromising the clinical performance or longevity of these restorations.

Ethylenediaminetetraacetic acid (EDTA) is another substance often used with NaOCl as chelating agent to rid the root canal system of the smear layer, which consists of dentin particles embedded in an amorphous mass of organic material that forms on the canal walls during the instrumentation procedure (15). In radicular dentin, EDTA reacts with the calcium ions of hydroxyapatite crystals, causing changes in the microstructure of the dentin and changes in the calcium to phosphorus ratio. Changes in the mineral content ratio might alter the original proportion of organic and inorganic components, which in turn reduces the microhardness, increases the permeability and solubility of the root canal dentin, and increases the adherence and adhesion force of *Enterococcus faecalis* to dentin, important bacteria that are associated with post-treatment endodontic infections (16–18).

Dentin collagen plays an important role in the effective bonding procedure. The reduction of the bond strength seen between adhesive systems and dentin walls might occur because of the removal of collagen fibrils from the dentin surface by NaOCl and might impede the formation of a consistent hybrid layer. One of the factors related to bonding failures in the root canal system is the dentin collagen integrity, especially after treatment with endodontic irrigants (6–9). Dentin collagen alteration after treatment with chemical substances can be observed by polarized light microscopy (PLM).

PLM can selectively visualize anisotropic compounds or structures. Anisotropic objects might exhibit a number of phenomena, one of which is birefringence. Birefringence is the characteristic of an object to transmit plane-polarized light at different velocities in different directions. Quantitative analysis of birefringence by PLM is a useful method to investigate the macromolecular orientation and organization of collagen fibers in connective tissues (19). Alterations of collagen's birefringence pattern might occur as a result of disorganization in its ultrastructure. The collagen's birefringence might be increased by dying the tissue with Sirius Red stain. Sirius Red molecules bind in parallel with collagen fiber molecules, which enhanced their birefringence under PLM (20, 21).

In previous work (22), it was possible to observe that 5.25% NaOCl, whether or not associated with 17% EDTA, causes alteration in the dentin collagen by a quantitative analysis of birefringence by PLM. More information is needed regarding the consequences of the use of the ascorbic acid/sodium ascorbate in dentin collagen after endodontic irrigation with 5.25% NaOCl. Thus, the aim of the present study was to evaluate the effect of 10% ascorbic acid or 10% sodium ascorbate on organic matrix collagen of bovine dentin root canal walls after irrigation with 5.25% NaOCl, 17% EDTA, or 0.9% sodium chloride.

Materials and Methods

Eighty bovine incisors, stored in a 0.2% thymol solution and frozen to maintain freshness, were selected for this study. The teeth were randomly divided into 8 groups (n = 10) according to the auxiliary chemical substance used: group 1, 0.9% sodium chloride (control); group 2, 5.25% NaOCl + 17% EDTA (NaOCl + EDTA); group 3, 5.25% NaOCl + 17% EDTA + 10% ascorbic acid (NaOCl + EDTA + AA); group 4, 5.25% NaOCl + 17% EDTA + 10% sodium ascorbate (NaOCl + EDTA + SA); group 5, 5.25% NaOCl (NaOCl); group 6, 17% EDTA; group 7, 10% ascorbic acid (AA); and group 8, 10% sodium ascorbate (SA).

The teeth were prepared according to Moreira et al (22). The apexes of the teeth were sealed with wax (Wilson; Polidental Indústria e Comércio Ltda, Cotia, SP, Brazil), and the whole outer surface of the tooth was covered with a layer of cyanoacrylate (Super Bonder-Loctite, Itapevi, SP, Brazil).

Crowns were separated from roots by a horizontal cut made 3 mm under the amelocemental junction with a Carborundum disk (Vipi, Pirassununga, SP, Brazil). The root samples were fixed with wax and screwed to a metallic apparatus. To prevent any leakage of endodontic irrigants around the external surface of the root, a polyether impression material (Impregum Soft; 3M ESPE, Sumaré, SP, Brazil) was used to seal the area around the root canal entrance and the metallic apparatus.

Pulp tissue and predentin were removed by using #6 Gates-Glidden burs (Maillefer, Ballaigues, Switzerland) and #130 file (Maillefer). During instrumentation, irrigation protocols were performed according to the previously specified groups. All groups used a total volume of 10 mL for irrigation. All root canals were then filled with the respective auxiliary chemical substances for different periods of time as shown in Table 1.

Five milliliters of sodium chloride was used, followed by the renewal of the NaOCl and sodium chloride solutions every 3 minutes and 1.5 minutes for EDTA solution during the period in which the auxiliary chemical substances were placed in contact with the root canal dentin. As an inert solution, sodium chloride was chosen for this step to allow the washing and removal of any traces of the auxiliary chemical substances previously used. After rinsing with 10 mL of sodium chloride, the canals were dried with Capillary Tips (Ultradent Products Inc, South Jordan, UT) and paper points. Then the samples were rinsed with 10 mL of freshly prepared 10% ascorbic acid (pH 2.05) or 10% sodium ascorbate (pH 7.15) for 10 minutes, followed by the renewal of the solutions every 5 minutes and a final rinsing with 10 mL of sodium chloride. Samples were stored in 10% formaldehyde for 48 hours. After this period, each sample was submitted to histologic processing to be analyzed under PLM.

Polarized Light Microscopy

After fixation, the samples were decalcified in 5% nitric acid and 4% formaldehyde during a period of 3–5 weeks. The samples were dehydrated with alcohol, cleared in xylene, and embedded in paraffin, and 7-μm-thick slices were obtained. The sections were stained with 1% Sirius Red in saturated picric acid (picrosirius) and mounted in Vetec Synthetic Canada Balsam (Vetec, Duque de Caxias, Rio de Janeiro, Brazil).

TABLE 1. Irrigation Times and Protocols for Tested Auxiliary Chemical Substances

	Irrigation protocols	Total times of auxiliary chemical substance in contact with root canal (min)
Group 1	0.9% sodium chloride solution	30
Group 2	5.25% NaOCl + 17% EDTA	30 3
Group 3	5.25% NaOCl + 17% EDTA + 10% ascorbic acid	30 3 10
Group 4	5.25% NaOCl + 17% EDTA + 10% sodium ascorbate	30 3 10
Group 5	5.25% NaOCl	30
Group 6	17% EDTA	3
Group 7	10% ascorbic acid	10
Group 8	10% sodium ascorbate	10