Chlorhexidine improves the mechanical properties of root dentin

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ABSTRACT

Objective: To evaluate the maximum flexural strength (MFS), elastic modulus (E), Knoop microhardness (KHN), and surface roughness (Ra) of root dentin treated with 2% chlorhexidine (CHX) solution. **Materials and methods:** Fifty bovine incisors with closed apex were selected. Thirty roots were cut into dentin bars (DB) and divided into three groups (n=10): control (no treatment), G1 (2.5% NaOCl + 17% EDTA) and G2 (2% CHX + H2O). The DB were submitted to the three-point flexural strength test to obtain the MFS and E. Twenty roots were cut longitudinally into two half-halves and placed in acrylic resin. The pulp surfaces were sanded and polished. They were rinsed according to the group (G1 or G2; n = 20 per group) and subjected to the KHN and Ra tests. SEM analysis was made. Data were analysed by one- and two-way ANOVA and Tukey post hoc test (a = 0.05). **Results:** The values of MFS ($P < 0.05$) and E ($P < 0.01$) were higher for G2. The KHN reduced after both endodontic irrigation treatments ($P < 0.001$), with no differences between them ($P = 0.115$). Ra was higher for G1 ($P < 0.001$). **Conclusions:** 2% CHX improved the mechanical properties of MFS, E and Ra of root dentin

Keywords: Chlorhexidine. Dentin. Sodium Hypochlorite. Elastic Modulus.

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Introduction

One of the aims of endodontic treatment is to promote the elimination of septic content of the root canal, which can be achieved with an efficient chemomechanical treatment.[1] The infected contents are located beyond the pulp cavity, at the depths of the predentin and root dentin.[2] Thus, irrigating solutions must have antimicrobial activity. An ideal irrigating agent must provide, besides lubrication, the dilution and elimination of necrotic and vital pulp tissue, as well as help to remove the smear layer. There is no irrigating agent that has all these properties, and it is necessary to use combinations of solutions to achieve these objectives. Thus, ethylenediaminetetraacetic acid (EDTA) has been used as an irrigating agent for the removal of the smear layer.[3]

Sodium hypochlorite solution (NaOCl) at concentrations that generally range from 0.5 to 5.25%[4] is considered the gold standard of irrigating agents.[5,6] However, because it has a potent solvent effect on pulp tissues, it dissolves and removes both pulp tissue and collagen from the organic dentin matrix. This is an aggravating factor for the weakening of the root dentin[7,8] increasing the possibility of fractures.[7,9] However, studies have shown that chlorhexidine (CHX) inactivates the metalloproteinases of the dentin organic matrix, helping to preserve this structure and protecting dentin adhesion and mineral ion retention.[10-14]

The change in the mineral content of the substrate may reflect on the mechanical properties, such as maximum flexural strength (MFS), elastic modulus (E),[15] Knoop microhardness (KHN), and surface roughness (Ra).[16]

Thus, the objective of this study was to evaluate the behaviour of the mechanical properties of MFS, E, KHN, and Ra of bovine root dentin after irrigation with 2% CHX solution.

Material and Methods

Fifty bovine incisors with closed apex were selected and maintained in 0.1% sodium azide (± 4°C) until use.[17] The teeth were sectioned with a diamond disc adapted to the metallographic saw (Isomet 1000-Buehler Ltd, Lake Bluff, IL, USA) at the cementoenamel junction and 5 mm short of the root apex.

MFS and E

Thirty teeth were deoronated and their roots sectioned in dentin bars (DB) with dimensions (10 x 1 x 1 mm) measured by digital paquimeter (Mitutoyo Corp, Tokyo, Japan). The DB of each root were immersed in irrigating solutions (2 mL) conditioned in polypropylene tubes according to the group (n=10): control (no treatment), G1 (2.5% NaOCl + 17% EDTA;), and G2 (2% CHX + H_2O;). With the exception of the control group, they were agitated in an ultrasonic tub (BioFree-Gnatus, Ribeirão Preto, SP, Brazil) (Table 1).

After treatment, the three-point bending flexural test (0.5 mm/min) was performed in the universal testing machine (Instron 3342, Canton, MA, USA) to obtain MFS (MPa) and E (GPa) directly from the strain x deformation diagram (Instron® Bluehill®, Barueri, SP, Brazil). For data analysis, one-way ANOVA was used, followed by the Tukey test.

KHN and Ra

The specimens (sp) were prepared from 20 roots cut longitudinally into two half-halves (± 15 mm). They were worn by 400 sandpaper (Microcut®, Silicon Carbide, Buehler) producing 40 dentin plates (1 mm thick), which were individually inserted in self-curing acrylic resin with pulp surfaces exposed. The sp were sanded again (400, 600, and 1200, Microcut®, silicon carbide, AutoMet 2000 Power Heads, Buehler) and polished with felt discs (Microcloth Psa, Buehler) and diamond pastes (3, 1, and ¼ micra; Diamond polishing compound Metadi II, Buehler). The sp were cleaned with deionised H_2O for 5 minutes in an ultrasonic vat and exposed to the irrigating solutions according to Table 1.

KHN was measured with three indentations spaced 0.2 mm (100 gf/20s; Shimadzu, HMV-2T, Kyoto, Japan) using the software C.A.M.S. Win 5.0. The Ra of each sp was obtained from the mean of six readings in two different directions (three vertical and three horizontal) using a digital rugosimeter (Surftest 301; Mitutoyo America Corporation, Suzano, SP, Brazil) calibrated for a distance of 0.5 mm (0.1mm/s - American National Standards Institute, 2009) and with a reading range of 0.25 mm (ISO 3650). The obtained data were submitted to Tukey test and two-way ANOVA (a = 0.05). The control's (KHNi and Rai) means were obtained before irrigation. After the treatment, the tests were repeated (KHNf and Raf).

Table 1. Agitation time and exchange of irrigating solutions during treatment protocol.

	MFS					KHN and Ra				
Groups	**Control**	**G1**		**G2**		**Control (KHNi) (Rai)**	**G1 (KHNf) (Raf)**		**G2 (KHNf) (Raf)**	
% Solution	-	**NaOCl 2,5%**	**EDTA 17%**	**CHX 2%**	**H_2O**	**H_2O**	**NaOCl 2,5%**	**EDTA 17%**	**CHX 2%**	**H_2O**
Agitation time	-	80’	5’	100’	25’	5’	80’	5’	100’	25’
Solution exchange	-	20’	1’	20’	5’	-	20’	1’	20’	5’

MFS = Maximum flexural strength. KHN = Knoop microhardness. Ra = Surface roughness. KHNi = Initial Knoop microhardness. KHNf = Final Knoop microhardness. Rai = Initial surface roughness. Raf = Final surface roughness.
_ (Without solution). *30 mL of the solutions were used at each exchange.

Scanning Electron Microscopy (SEM)

Two DB from each group were randomly selected for analysis. The DB were mounted on aluminum specimen mount stubs covered with conductive carbon adhesive tabs (Ted Pella, Redding, CA, USA) and analyzed under a Field Emission Scanning Electron Microscope TM3030 (Hitachi, Toronto, Canada) at 15 kV.

Results

Figure 1 shows that the mean of the MFS values in G2 were higher when compared to the control ($P < 0.05$) and G1 ($P < 0.01$). The E values were lower for G1 ($P < 0.01$). After both treatment protocols, the KHN of the root dentin reduced ($P < 0.001$), without difference between the groups ($P = 0.115$); Ra increased, with higher values for G1 ($P < 0.001$). SEM shows the protective capacity and cleaning of 2% CHX + H_2O on the dentin surface compared to the control group which has a smear layer on its surface (Figure 2A, B, E and F). In addition, Figure 2B and C shows the demineralization potential of 2.5% NaOCl + 17% EDTA by means of more open dentinal tubules with removal of its peritubular dentin.

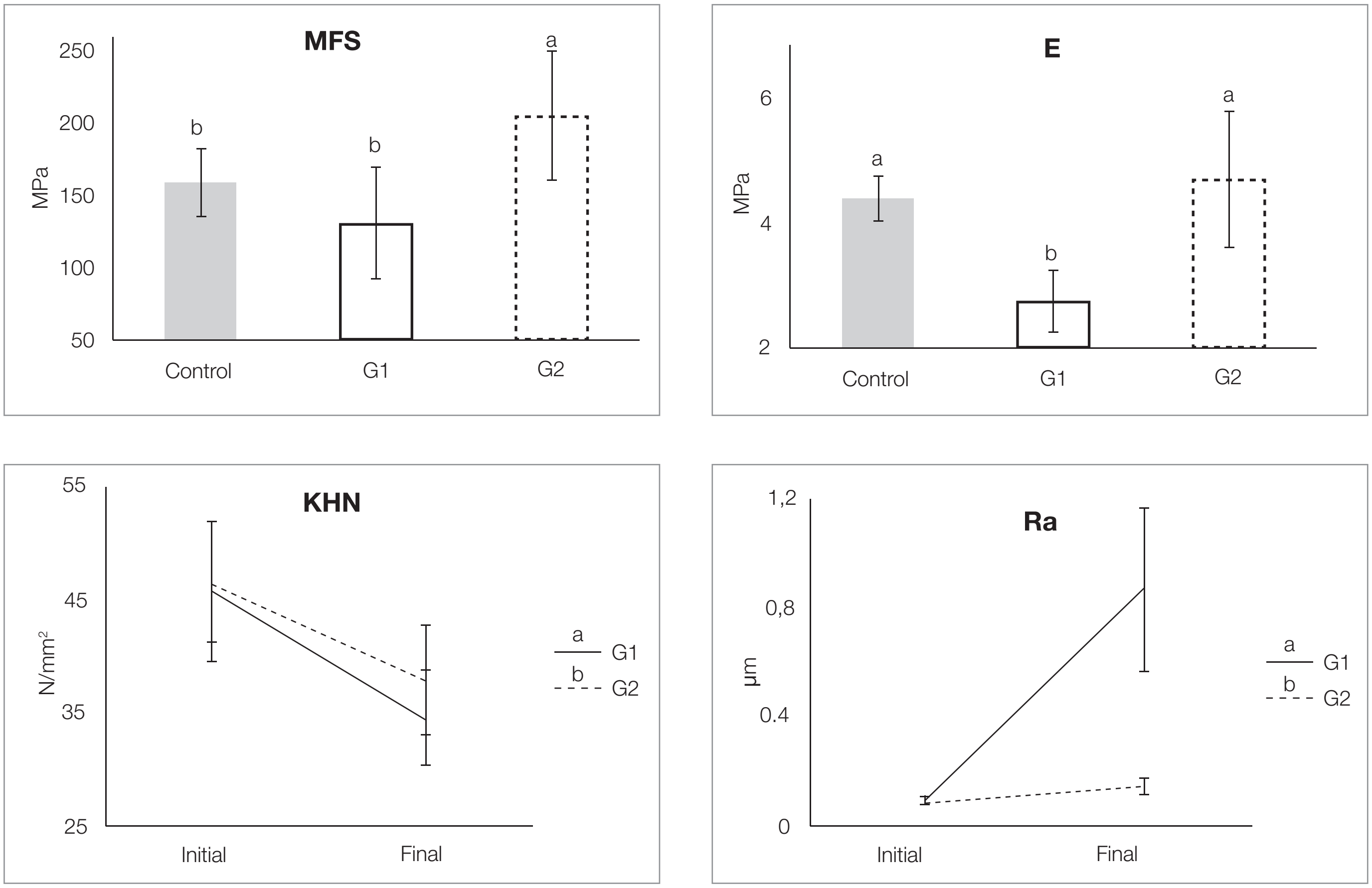

Figure 1. Mean and standard deviation of the MFS, E, KHN and Ra of the root dentin after the treatment protocols. Different lowercase letters indicate statistical difference amongst groups.

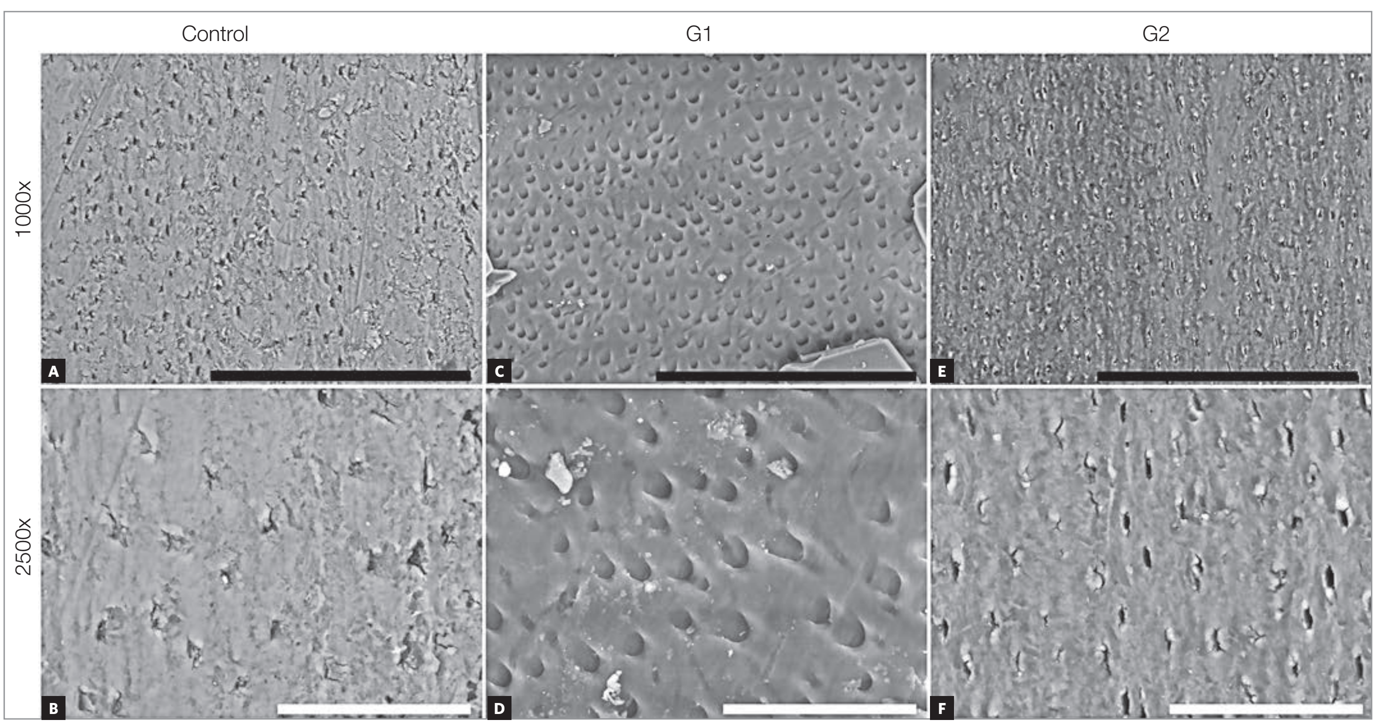

Figure 2. Representative SEM images from dentin bars treated with irrigating solutions and control at 1000x and 2500x magnification. (**A** and **B**) Control: No treatment. (**C** and **D**) G1: NaOCl and 17% EDTA treatment. (**E** and **F**) G2: CHX and H2O treatment. Black scale bar - 100 µm; White scale bar - 30µm.

Discussion

This study demonstrated that 2% CHX obtained higher values than 2.5% NaOCl in the MFS and E evaluations, confirming the protective capacity of CHX on the dentin structure.[13] In addition, it ratified the harmful potential of NaOCl solution on the dentin substrate.[8,18] The flexural strength of the dentin depends in part on an intimate connection between its two main components: the hydroxyapatite crystals and the collagen network.[19]

NaOCl is a potent mineral ion sequestrant[20] and CHX, in contrast, has shown action in the mineral and organic maintenance of the dentin structure,[12] especially in the collagen that contributes substantially to the mechanical properties of this structure.[21]

Our results reinforce recent studies.[22,23] In this sense, Moreira et al.[18] showed a uniform fibrillar network in dentin treated with 2% CHX, differently from that treated with 5.25% NaOCl and 17% EDTA. The authors further credit EDTA for structural demineralisation characterised by the diminution of the intertubular dentin and widening of the dentinal tubules. In agreement with these findings, in our study it was possible to see these statements (Fig 2), verifying more open dentinal tubules by 2.5% NaOCl and 17% EDTA action. On the other hand, in groups that used 2% CHX solution and H_2O, and no irrigating agent it was verified maintenance of peritubular dentin structure.[20]

The E of dentin is dependent on the quality of hydroxyapatite and type I collagen, and a negative alteration in this microstructure may lead to modifications of this property[24]. In this study, G2 did not alter the DB root's E, maintaining the same values of the control that did not use any previous solution.[8]

It is important to remember that both 2.5% NaOCl solution and 17% EDTA solution have the ability to denature organic proteins and remove the mineral content, whether combined or isolated.[25] This change in the mineral concentration of dentin directly affects its microhardness.[24] Our study showed that the vibration of the irrigating solutions decreased dentin KHN, without significant differences between them. Since the KHN is a surface analysis, it is believed that these findings are due to the long time of vibration to which all surfaces of the sp were exposed to CHX, explaining the absence of difference with NaOCl.[26] On the other hand, Patil and Uppin[27] indicate CHX as an appropriate irrigating solution due to its harmless effect on the microhardness of root dentin, confirming other studies showing little or no demineralising effect of CHX on the dentin structure.[16,28] KHN tests are not usually used to predict root fracture,[29] however, it may be related to other mechanical properties, such as those discussed in this study.

Previous studies have shown the absence of modifications in Ra after treatment with 0.2% CHX.[16,27] Even though both groups demonstrated increased Ra values, with higher NaOCl results statistically significant ($P < 0.001$). It is believed that such disagreement is due to the low concentrations of the CHX solution, lower volumes, and shorter contact times of the substrates with the solution when compared to the present study.

Ra is related to the topography of the dentin surface and influences the wetting of the sealing cements, besides favouring adhesion by mechanical bonding of the cements to the substrate. The increase of dentin Ra is desirable because it increases the surface area to be adhered.[30] However it is discussed about the benefit achieved by NaOCl as since it is a known oxidising agent that negatively affects the process of adhesion of the intraradicular dentin to the root canal filling materials. It impairs the monomeric penetration, compromising the bond strength and, consequently, the quality and durability of post-core cementation.[31,32] Thus, the benefits of the higher Ra values of NaOCl in relation to CHX are questionable, in detriment to the oxygen release that potentiates adhesive failures.[33]

Conclusion

In conclusion, the irrigation protocol with 2% CHX solution and H_2O showed to preserve the mechanical properties of MFS, E and Ra of the root dentin.

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