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Impact of chemical agents for surface treatments on microhardness and flexural strength of root dentin

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Abstract: This study assessed the cross-sectional Knoop microhardness and flexural strength of root dentin exposed to different surface treatments with chemical agents after biomechanical preparation. Root canals from human canines were biomechanically treated and divided into eight groups (n=10) to receive one of the following dentin treatments: I. Deionized water (control); II. 5.25% Sodium hypochlorite (NaOCl); III. NaOCl + 10% Sodium ascorbate (SA); IV. SA; V. 2% Chlorhexidine gel (CHX); VI. 37% Phosphoric acid gel (PA) + CHX; VII. PA; and VIII. PA + NaOCl. The roots were sectioned to obtain specimens that were evaluated for cross-sectional Knoop microhardness and flexural strength using a three-point bending test. ANOVA and Tukey's test were performed. The microhardness in the control group was significantly higher ($p < 0.05$) than in the groups exposed to chemical agents, which in turn were statistically similar ($p > 0.05$) to each other. Regarding flexural strength, PA+NaOCl provided statistical higher values than PA+CHX and CHX. However, there was no significant difference between the control group and those groups subjected to surface treatment ($p > 0.05$). Dentin microhardness was reduced after exposure to NaOCl, CHX, PA, SA and their associations and the flexural strength of radicular dentin was not affected by the chemical agents.

Keywords: Chemical Compounds; Physical Properties; Dentin.

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Introduction

Most clinical failures in teeth restored with fiberglass posts occur due to post debonding.[1] The durability and stability of resin bonded interfaces on dentin from the post space walls can be reached by improving the bond strength of the resin material to the root dentin[1,2] during the cementation and by preserving the integrity of resin-dentin bonds.[1] For this purpose, root dentin pretreatment with chemical agents has been suggested.[3,4] However, despite the possible bond strength improvement,[3,4,5,6,7] the mechanical properties of the treated dentin could be affected by the chemical substances.

The phosphoric acid etching of dentin surface removes hydroxyapatite, exposes the hydrated collagen network allowing hydrophilic adhesive penetration to form the hybrid layer.[8] Nevertheless, degradation of resin-dentin bonds occurs over time due to hydrolysis of the collagen fibrils within the hybrid layer that are not fully infiltrated by resin monomers.[1,9]

Stable hybrid layer and preservation of adhesion can be reached by controlling the degradation of the denuded collagen matrix.[10] Therefore, chlorhexidine digluconate (CHX) can inhibit the expression of endogenous matrix metalloproteinases (MMPs)[1,4,10] that are involved in the degradation of collagen in resin-dentin interfaces.[11] Additionally, by applying NaOCl on the acid-demineralized dentin,[12] the exposed collagen is removed,[5] and the wettability of the bonding substrate[9] and resin infiltration may be facilitated.

The exposure to antioxidant agents (*e.g.*, 10% sodium ascorbate) has been proposed to increase the bond strength of resin materials to the dentin to reverse the oxidizing effect of NaOCl-treated dentin surface,[13] thereby neutralizing the residual chemical[14] and reestablishing the compromised bond strength.[15]

Although the application of the aforementioned agents or their associations can preserve or improve the adhesion of resin materials to radicular dentin,[3,4,5,6,7,16,17] changes in organic and mineral content caused by these chemicals[18] could negatively affect the physical properties of dentin, thus causing root fragility.

Studies revealed a reduction in microhardness of radicular dentin exposed to NaOCl[19,20] and CHX[20] used as irrigating solutions. The degradation of the dental structure after NaOCl irrigation compromised the flexural strength.[21,22,23] However, it is not well established whether dentin pretreatment with chemical substances prior to adhesive cementation of fiber posts affects the mechanical properties of the biomechanically treated dentin.

Therefore, the aim of this study was to assess the impact of dentin surface treatment with chemical agents on cross-sectional Knoop microhardness and flexural strength of dentin after biomechanical preparation of root canals. The hypothesis was that the surface treatments do not affect the dentin microhardness and flexural strength negatively.

Methodology

The study protocol was approved by the local Ethics Committee (190.326).

Specimen preparation

Eighty maxillary canines were selected that had straight roots, completely formed apices, single canals, no calcifications or resorptions that had been confirmed radiographically; these canines were stored in 0.1% thymol solution. The clinical crowns were removed, and the root length was standardized to 14 mm.

Instrumentation of the root canals was performed with Protaper system (Dentsply Maillefer –Ballaigues-Switzerland) at the working length determined visually by subtracting 1 mm from the root length of a size 15 K-file (Dentsply Maillefer, Ballaigues, Switzerland) at the apical foramen. Root canals were irrigated with 2 mL of 2.5% sodium hypochlorite between each file, followed by 5 mL of 17% EDTA for 5 min. Final irrigation was completed with 10 mL of distilled water for 1 min, and the canals were dried with absorbent paper points (Dentsply Ind. e Com. Ltda., Petrópolis, Brazil).

Dentin treatment with chemical agents

The root apices were sealed with sticky wax (Wilson Polidental, Cotia, Brasil), and the dentin treatment was performed by flooding the root canal with the substance designed for each group (n=10), as follows:

1. GI) Deionized water (DW - control group): 10 mL of deionized water was inserted into the canal lumen for 10 min;
2. GII) 5.25% NaOCl (NaOCl): 10 mL of NaOCl was inserted into the canal lumen for 5 min[3];
3. GIII) 5.25% NaOCl followed by 10% sodium ascorbate (NaOCl+SA): NaOCl was applied as described in GII, and 10 mL of sodium ascorbate was injected into the canal lumen for 10 min[5];
4. GIV) 10% sodium ascorbate (SA). Sodium ascorbate was applied as described in GIII;
5. GV) 2% chlorhexidine digluconate gel (CHX): Chlorhexidine gel was applied to fill completely the root canal for 5 min[3,4];
6. GVI) 37% phosphoric acid gel (PA): phosphoric acid gel was used to fill the root canal completely for 15 s;
7. GVII) 37% phosphoric acid gel followed by 2% chlorhexidine gel (PA+CHX): phosphoric acid gel was used as described in GVI. The gel was removed from the root canal by washing with deionized water for 15 s. Chlorhexidine gel was applied as described in GV;
8. GVIII) 37% phosphoric acid gel followed by 5.25% NaOCl (PA+NaOCl): phosphoric acid was applied to the root canal as described in GVI. NaOCl was injected as described in GII.

Following the treatment, the root canals were washed with deionized water and stored in distilled water at 37 ºC for 24 h.

Grooves were prepared along the long axis of the roots that were longitudinally cleaved in a buccolingual direction to expose the entire canal extension.[24] One half was used to assess microhardness while the other half was subjected to the flexural test.

Cross-sectional Knoop microhardness measurement

A 3 mm-width segment from the cervical-middle region of each half was sectioned. The coronal portions of the specimens were ground with 400-, 600- and 1200-grit silicon carbide papers (3M, Sumaré, Brazil) and polished on cloths with a 0.3-µm alumina suspension (Alpha Micropolish, Buehler, Lake Bluff, USA). Dentin microhardness was measured with a Knoop indenter at ×40 magnification (Shimadzu HMV-2000; Shimadzu Corporation, Kyoto, Japan) under a 10-g load and a 10-second dwell time. Three Knoop microhardness indents were made in a linear fashion at 200 µm from the root canal lumen along the horizontal line and spaced 200 µm apart. The mean values of all three measuring points were then averaged.

Flexural strength of dentin

Each half of the root was longitudinally sectioned in a dentin bar (10 mm long x 1.4 mm wide x 1.5 mm thick) from the cervical-middle area. The three-point bending flexural test was performed using an universal testing machine (Instron 4444; Instron Corporation, Canton, USA), with the distance between the center support points fixed at 8 mm. The load cell was applied perpendicular to the long axis of the specimen with a 0.5 mm/min crosshead speed until fracture. The flexural strength (σ_f) of the dentin was calculated using the following equation: $= \sigma_f = 3F_{max} L / 2bd_2$ such that F_{max} is the maximum load in Newton, L is the distance between the support points in mm (8 mm), *b* is the specimen wide (1.4 mm) and *d* is the specimen thickness (1.5 mm).

Statistical analysis

After checking the assumption of homogeneity of variance and normal distribution, microhardness and flexural resistance data were statistically analyzed by ANOVA and Tukey's test when significant differences were detected. The SPSS 17 software (SPSS Inc., Chicago, USA) was used to perform the statistical analyses at a 5% significance level.

Results

Cross-Sectional Microhardness

One-way ANOVA revealed a significant difference between dentin treatments ($p < 0.05$).

The root dentin exposed to the deionized water had significantly higher ($p < 0.05$) microhardness than those treated with any other substance. These other groups did not have any statistically significant differences ($p > 0.05$) as shown in Table 1.

Flexural Strength

One-way ANOVA showed a significant difference in flexural strength ($p < 0.05$).

Tukey´s test confirmed that PA+NaOCl provided higher ($p < 0.05$) flexural strength than CHX and PA+CHX, which had the lowest values. The other groups presented intermediate values, and there was no statistically significant difference between them ($p > 0.05$). All chemical agents provided flexural strength similar to the deionized water ($p > 0.05$), as depicted in Table.

Table. Microhardness and flexural strength (MPa) values provided by each dentin treatment.

Dentin Treatments	Microhardness	Flexural Strength
DW	104.1 (21.2) A	221 (29.5)[ab]
NaOCl	53.6 (10.5) B	209 (30.4)[ab]
NaOCl + SA	53.2 (10.6) B	197 (29.4)[ab]
SA	55.4 (12.9) B	189 (20.0)[ab]
CHX	54.6 (12.5) B	139 (15.5)[b]
PA	45.2 (9.3) B	185 (27.7)[ab]
PA + CHX	43.2 (10.8) B	150 (32.4)[b]
PA + NaOCl	45.5 (8.1) B	246 (41.6)[a]

[a]Values are means ± SD
[b]Significant differences are expressed by different letters ($p < 0.05$), within each column.

Discussion

Changes in mechanical properties of dentin can influence the behavior of the dentin/restoration interface.[25] Additionally, the root resistance to fracture can be adversely affected by reduction in microhardness[26] and flexural strength.[21] The present study assessed the microhardness and flexural strength of root dentin exposed to chemical agents.

The hypothesis that the surface treatments do not negatively affect the dentin microhardness was rejected. Lower microhardness values were obtained by specimens exposed to chemical agents other than to deionized water. As microhardness is sensitive to the composition and surface changes of the tooth structure,[27] alterations in the structure of root dentin achieved by chemical agents can reduce the microhardness, as observed in the present and previous investigations.[19,20] In this study, although the dentin had already been exposed to irrigations with NaOCl and EDTA during biomechanical treatment of root canals, chemical surface treatments provided additional structural changes, thereby compromising the dentin microhardness. The reduction of dentin hardness following NaOCl treatment indicates potent direct effects on the organic and mineral content of dentin structures.[18]

The current results corroborate the findings from Oliveira *et al*.[20] that verified reduced microhardness of dentin treated with 2% CHX gel. CHX is a cationic compound that has the ability to bind anionic molecules, such as the phosphates present in the hydroxyapatite structure.[18] Considering that phosphate is present in the calcium carbonate complex of dentin, CHX can induce changes in the Ca/P ratio,[18] which could explain the reduced values of microhardness in root dentin exposed to CHX.

There is a lack of studies evaluating the effect of sodium ascorbate on the mechanical properties of dentin. Although reducing agents have been shown to not cause any additional changes in the structure[13] and micromorphology of the dentin surface, a slightly increased roughness and more clearly visible collagen fibrils were verified.[28] The possible presence of exposed organic material can explain the decreased microhardness found in this investigation. Additionally, sodium ascorbate dissociates in ascorbic acid with low pH, which could be contributed to demineralization of the dentin surface.

The compromised microhardness of the dentin treated with phosphoric acid was expected due to removal of the smear layer and the superficial part of the dentin, opening dentinal tubules, demineralizing the dentin surface and increasing the microporosity of the intertubular dentin.[7] Subsequent studies may clarify the clinical relevance of the decreased microhardness.

Regarding the flexural strength, the radicular dentin treated with chemical agents did not differ from those exposed to deionized water, thereby supporting the hypothesis that the substances used in the dentin treatment do not compromise the flexural strength. These findings are in contrast to previous studies, which reported reduced flexural strength due to degradation of surface structures treated with NaOCl.[21,22,23] Considering that the alterations in the mechanical properties of dentin are time dependent,[22] the shorter application time used in the present investigation (10 min) compared to other studies can justify the absence of negative effects of NaOCl on flexural strength.

Direct comparisons of the current findings with the literature could not be performed due to the lack of studies evaluating the flexural properties of dentin exposed to CHX, phosphoric acid, sodium ascorbate and their associations. To simulate the clinical protocol, the agents were applied to the dentin previously subjected to biomechanical treatment and irrigation with 2.5% NaOCl and 17% EDTA. Moreira *et al*.[29] suggested a possible interaction between CHX-based cavity disinfectants and endodontic irrigants, such as those used in this study during the biomechanical preparation, which could have promoted some structural alterations and affected the flexural strength.

The irrigation period has a crucial effect on dentin microhardness[19] and flexural strength.[23] Previous investigations indicated that short application times did not lead to significant changes in dentin mechanical properties.[19,23] Considering that the agents evaluated in this study are used for the pretreatment of dentin in order to improve the adhesion process, the exposure of dentin to chemical substances was conducted for time periods to restore and improve the bond strength.[2,3,4,5,6] The periods of exposure to treatment agents were based on previous investigations of dentin pretreatment with NaOCl,[3] CHX,[3,4] phosphoric acid[2] and sodium ascorbate.[5]

Other chemical agents and treatment protocols seemed to be important factors affecting the mechanical properties of the root dentin. However, additional studies should be conducted to evaluate the behavior of root dentin exposed to chemical agents and mainly the clinical relevance of these effects.

The present findings suggest that although Knoop microhardness of root dentin was adversely affected by chemical substances, the change in the hardness did not interfere with the flexural strength of the substrate. Considering that the flexural strength parameter determines the fracture resistance of a sample,[30] the lack of difference from the control group (treated with deionized water) indicates that the fracture resistance was not affected by the chemical agents.

Conclusions

Within the limitations of an *in vitro* study, it may be concluded that the microhardness of dentin was reduced after exposition to NaOCl, chlorhexidine, phosphoric acid, sodium ascorbate and associations, while flexural strength was not affected by the chemical agents.

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