RESEARCH ARTICLE

# The effects of natural collagen cross-linking agent “Proanthocyanidin” on the flexure strength of the radicular dentin

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## ABSTRACT

**INTRODUCTION**: Sodium hypochlorite (NaOCl) and ethylenediamine tetra-acetic acid (EDTA) have been widely used as efficient irrigation solutions to remove the smear layer. However, the synergistic influence of NaOCl and EDTA could cause harmful changes in the mechanical properties of the tooth structure. Chemical cross-linkers have been reported to further stabilize collagen fibrils in several connective tissues by promoting additional hydrogen bonding and/or the formation of covalent inter- and -intra-molecular cross-links.

**OBJECTIVE**: To evaluate the effects of Proanthocyanidin as a collagen cross-linking agent on the flexure strength of root dentin.

**METHODS**: Twenty-four dentin bars were prepared by longitudinal sectioning of the midcoronal part of each root. Based on the final irrigating regimen, samples were divided into 3 groups (n=8): G1: distilled water, G2: 5.25% sodium hypochlorite (NaOCl)/17% ethylenediaminetetraacetic acid (EDTA), G3: 7% Proanthocyanidin. Flexural testing was performed by subjecting the dentin bars using three points flexure device. The load at fracture was recorded directly from the load testing machine in Newton.

**RESULTS**: the highest value was observed in group 3 with no significant difference with group1, while group 2 showed significantly the lowest value.

**CONCLUSION**: Proanthocyanidin significantly improved the flexural strength of the root dentin bars.

**Key words**: Root canal irrigants, flexure strength, collagen cross-linking, Proanthocyanidin.

## INTRODUCTION

Sodium hypochlorite (NaOCl) and ethylenediamine tetra-acetic acid (EDTA) have been widely used as efficient irrigation solutions to remove the smear layer.[1] NaOCl acts to dissolve the organic part of the smear layer, while EDTA can eliminate inorganic elements.[2] However, the synergistic influence of NaOCl and EDTA could cause harmful changes in the mechanical properties of the tooth structure, i.e., hardness, fracture resistance, flexure strength and fatigue strength, consequently rendering these endodontically treated teeth more susceptible to vertical root fracture.[3]

Dentin is composed of two phases: an inorganic phase of hydroxyapatite crystals and an organic phase of predominantly type-I collagen. Type-I collagen is present in tissues as fibrils that are stabilized by lysyl-oxidase-mediated covalent intermolecular cross-linking.[4]

Chemical cross-linkers have been reported to further stabilize collagen fibrils in several connective tissues by promoting additional hydrogen bonding and/or the formation of covalent inter- and -intra-molecular cross-links that prevent the collagen molecules from sliding past each other under mechanical stress and also reduce biodegradation by endogenous proteases.[5] It has been reported that applying cross-linking agents to dentin collagen enhances ultimate tensile strength and stiffness.[6]

Grape seed extract (GSE), primarily composed of proanthocyanidins, has been reported to be a natural cross-linking agent used for these purposes.[5] GSE is widely used in the



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medical field as natural antioxidants, free radical scavengers, antibacterial, antiallergic and antigenotoxic effects, and to inhibit platelet aggregation and capillary permeability.[7] Moreover, in dentistry, it has been recently reported that GSE-induced cross-linking improved dentin's mechanical properties and bond strength.[8] Accordingly, strengthening dentin collagen fibrils by cross-linking agents to increase mechanical properties and bond strength may be an important application in endodontic dentistry. Hence, the rationale of this study was to stabilize the demineralized radicular dentin using the collagen cross-linking agent (proanthocyanidin) as a final irrigant.

The aim of this study was to evaluate the effects of Proanthocyanidin as a collagen cross-linking agent on the flexure strength of root dentin.

## METHODS

**Fabrication of dentin bars**: Twenty-four standardized dentin bars (10 mm × 1mm × 1mm) were prepared by longitudinal sectioning of the midcoronal part of each root, figure 1. Measurements were confirmed with a digital vernier at three locations along the specimen length, no enamel or cementum was included in the dentin bars. Each bar was examined under a stereomicroscope at 40x magnification to check the presence of cracks or defects that identify potential damage. The specimen that did not meet the criteria was replaced. The specimens were randomly allocated into three groups relative to the applied irrigation regime.

**Immersion of dentin bars with test solutions**: The irrigating solutions were placed into separate containers at room temperature. The amounts of irrigants and interval of immersion in each container corresponded to the proposed irrigation protocols in this study, table 1. In all groups, the immersion solutions were renewed for every specimen.

**Three-point bending testing of dentin bars**:- Flexural testing was performed by subjecting the dentin bars using a miniature three points flexure device. The dentin bar was oriented so that the direction of the stress was applied parallel to the dentinal tubules, figure 2.

Each beam was placed on top of the support span (6 mm) and loaded at the mid-point through the loading head and shaft using a universal testing machine at a crosshead speed of 0.5 mm/min, figure 3. The load at fracture was recorded directly from the load testing machine in Newton (N). All the dentin bars were kept moist during testing with distilled water.

**Calculation of flexural strength**: The flexural strength values were recorded in Newton and converted to megapascal using the following equation:[9]

$FS = (3F_{max} L)/(2bh^2)$   Where;

FS: the flexural strength (MPa),

***F***max: the necessary load for fracture (N),

***L***: the distance between the supports (6 mm),

***b*** and ***h*** are the test specimen's width and height (mm), respectively.

## RESULTS

The minimum, maximum, mean and standard deviation of flexural strength values, meas-

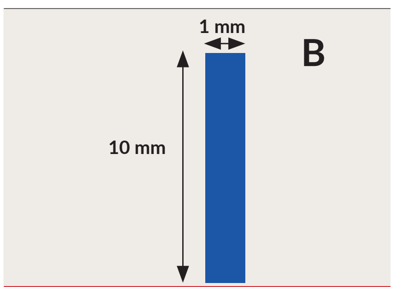


**Figure 1** | longitudinal view of dentin bar.

**Table 1** | Immersion protocol of dentin bars.

| Steps | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| 1 | DW only | 1 ml of NaOCl (5.25%) for 30 sec. | 1 ml of NaOCl (5.25%) for 30 sec. |
| 2 | | 5 ml of DW and dried with pp. | 5 ml of DW and dried with pp. |
| 3 | | 1 ml of EDTA (17%) for 30 sec. | 1 ml of EDTA (17%) for 30 sec. |
| 4 | | 1 ml of EDTA (17%) for 30 sec. | 1 ml of EDTA (17%) for 30 sec. |
| 5 | | 5 ml of DW and dried with pp. | 5 ml of DW and dried with pp. |
| 6 | | 1 ml of NaOCl (5.25%) for 30 sec | 1 ml of NaOCl (5.25%) for 30 sec |
| 7 | | 5 ml of DW and dried with pp. | 5 ml of DW and dried with pp. |
| 8 | | | 1 ml of PA for 30 sec |
| 9 | | | 1 ml of PA for 30 sec and dried with pp. |

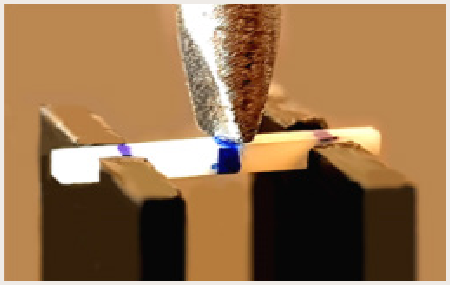

**Figure 2** | Three-points bending test using universal testing machine.

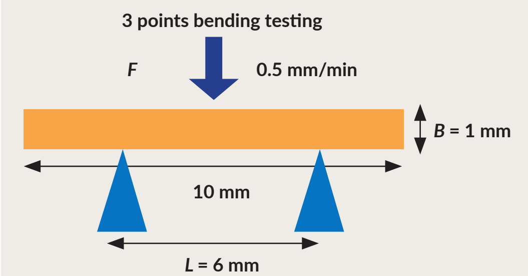


**Figure 3** | Schematic view of three-points bending force on the prepared specimen.

ured in megapascal (MPa) for the three experimental groups, are summarized in table 2.

Table 2 shows that the highest flexural strength value was observed in group 3 (147.37 ± 20.37), followed by group 1 (136.12 ± 19.50), while group 2 has the lowest mean value (113.62 ± 18.59).

For Inferential statistics, one way ANOVA test (table 3) was applied to find differences among the groups. ANOVA test revealed that there were statistically significant differences among the groups.

Intergroup multiple comparisons were made by the LSD test ( see table 4), which also revealed no statistically significant differences between Group 1 and Group 3, while Group 2 differed significantly with other groups.

Statistical analysis revealed that irrigation protocols had a significant effect on flexural strength of radicular dentin.

**Table 2** | descriptive statistics of flexural strength (MPa) of the three groups.

| Groups | No. | Min. | Max. | Mean | ±SD |
|---|---|---|---|---|---|
| Group 1 | 8 | 108 | 162 | 136.12 | 19.50 |
| Group 2 | 8 | 90 | 144 | 113.62 | 18.59 |
| Group 3 | 8 | 117 | 171 | 147.37 | 20.37 |

**MPa**: Megapascal, **No.**: Number, **Min.**: Minimum, **Max.**: Maximum

**Table 3** |One way ANOVA comparing effect of irrigants on flexural strength

| Groups | F-test | P-value | Significance |
|---|---|---|---|
| Group 1<br>Group 2<br>Group 3 | 6.210 | 0.008 | Highly significant |

**Table 4** | LSD test for comparison of significance among the groups

| Compared Groups | | Mean Difference | SE | P-value | Sig. |
|---|---|---|---|---|---|
| G 1 | G 2 | 22.50000 | 9.75206 | 0.031 | S |
| G 1 | G 3 | 11.25000 | 9.75206 | 0.262 | NS |
| G 2 | G 3 | 33.75000 | 9.75206 | 0.002 | HS |

## DISCUSSION

American Society for Testing and Materials stated that the flexural properties of rigid or semi-rigid materials could be determined by using a three-point loading system, utilizing central loading on a supported beam.[10] Many studies in dental literature used a three-point test to investigate the effects of different chemicals on the flexural strength of tooth structures.[9,10,11]

In contrast to previous studies[9,12,13] which used irrigation solutions for longer periods, the current study's irrigation protocols were conducted according to clinical protocols to get more clinically relevant results.

From the obtained data, it can be concluded that NaOCl/EDTA (group 2) significantly reduced the flexural strength of dentin bars compared to other groups. Return to the proposed irrigation protocol of the present study, NaOCl was used as the first irrigant; the inorganic crystals seem to protect the organic matrix, and the effect of the NaOCl on dentin is limited. However, when EDTA was used as a chelating agent, the hydroxyapatite crystals (HAp) were quickly dissolved, exposing the underlying collagen fibres. If NaOCl is used again at this stage, it can directly attack the protein (collagen) and, in a relatively short time, cause a detrimental effect on the flexural strength of dentin bars. This is in accordance with the widely accepted belief that the synergistic action of these irrigants might considerably negatively affect the mechanical properties of dentin, namely flexural strength. This explanation was adopted by several studies.[14,15,16]

On the contrary, Marcelino et al. in 2014[17] concluded that the flexural strength of the radicular dentin, treated with NaOCl, did not differ from those of the control group. These conflicting results may be due to variations in methodology as they applied NaOCl solely, while in the study at hand NaOCl/EDTA were used sequentially, causing the dissolving of organic and inorganic constituents of tooth materials.

It is worth mentioning that the dentin bars which had been immersed in NaOCl/EDTA appeared bleached and 'chalky' in texture. This phenomenon was earlier reported and explained by Sim et al.[18] and Reddy et al.[19] This appearance translated into a significant decrease in flexural strength and rigidity compared to the other two groups. The change in the physical properties could be explained by the loss of collagen and HAp within the dentin.

Pronthocyanidin significantly improved the flexural strength of the dentin bars of group 3. Although there was no significant difference with the control group, PA showed the highest flexural strength values among the experimental groups. This result was supported by several pieces of research that assessed the potential effects of PAs on the biomechanics of tooth structure.[20,21,22] These studies revealed that PAs can stabilize and enhance the short- and long-term results of the mechanical properties of collagen.

Based on the results of this study, future clinical studies could be verified in this line of thought that the endodontic treatment using PA as the final step in irrigation protocol may help prevent root fractures via enhancement of the flexural strength of radicular dentin.

## CONCLUSION

Proanthocyanidin significantly improved the flexural strength of the root dentin bars after being negatively affected by NaOCl/EDTA, making it insignificantly different from the control group.

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**Abbreviations list**: Analysis of variance (**ANOVA**), Distilled water (**DW**), Ethylenediaminetetraacetic acid (**EDTA**), Fisher test (**F-test**), Grape seed extract (**GSE**), High significant (**HS**) Hydroxyapatite crystals (**Hap**), Least significant difference (**LSD**), Megapascal (**MPa**) Newton (**N**), Non- significant (**NS**), Proanthocyanidin (**PA**), Probability value (**P-value**), Significant (**Sig**), Sodium hypochlorite (**NaOCl**), Standard deviation (**SE**), Standard error (**SD**).

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