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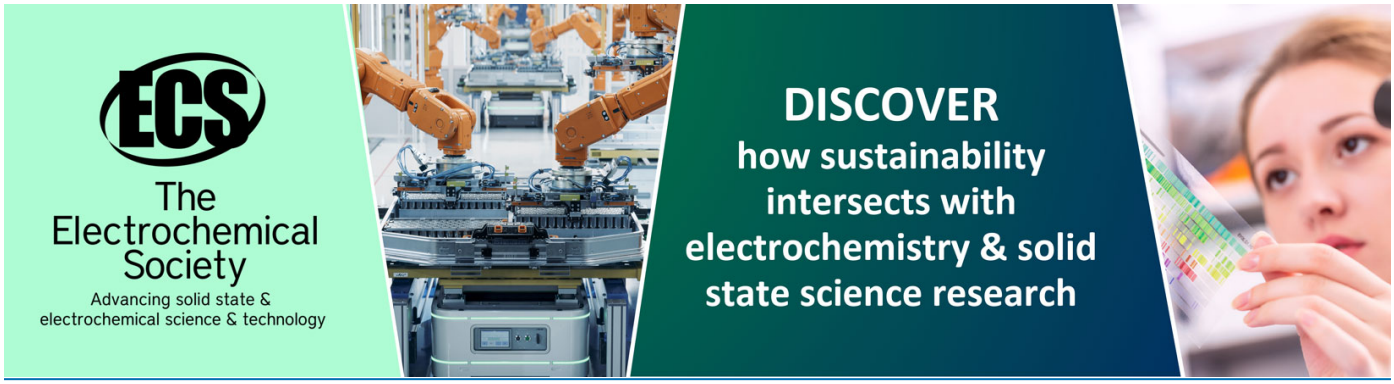

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Effect of grape seed extract solution on the flexural strength of root canal dentin

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Abstract. Grape seed extract (GSE) contains proanthocyanidin as a collagen crosslink, which determines the mechanical properties of dentin. This study aimed to analyze the effect of GSE solution on the flexural strength of root canal dentin. Ninety root canal dentin slabs were divided into three groups, and immersed in a GSE solution , in NaOCl and in aqua bidest . Dentin flexural strength was measured using a universal testing machine . The highest flexural strength was found in the GSE solution group , and the lowest in the NaOCl group. There were no significant differences between the GSE group and the aqua bidest group ($p > 0.05$). Thus, the flexural strength of root canal dentin was significantly higher in the GSE-solution group than in the NaOCl solution group.

1. Introduction

The rationale for root canal therapy is to obtain a clean root canal system that is free of microbiota, which can then be hermetically filled. The ideal root canal irrigant should include a broad-spectrum antimicrobial solution and have the added ability to stimulate hard tissue repair [1,2].

The most common hard tissue found in a tooth is dentin. Dentin comprises approximately 22% of organic material, primarily consisting of type-1 collagen, which acts as a scaffolding for dentin mineralized materials and determines the viscoelasticity by forming a rigid, robust, and space-filling biomaterial. These inter-and intra-molecular crosslinks form the basis for mechanical properties of dentin.

Currently, sodium hypochlorite (NaOCl) is the primary irrigator used during root canal treatment. NaOCL is widely recommended because of its ability to dissolve necrotic tissue remnants and its excellent antimicrobial potency [3,5].

NaOCL, a nonspecific oxidizing agent, fragments long peptide chains and to chlorinates protein terminal groups; the resulting N-chloramines are then broken down into other species. Further, NaOCL is capable of removing necrotic tissue remnants. However, it may cause changes to the organic and mechanical properties of dentin (microhardness, roughness, elastic modulus, flexural and fatigue strength) because of its proteolytic action. Sim et al. (2001) have suggested that the exposure of dentin to a solution of > 3% NaOCL significantly decreases the elastic modulus, and flexural strength, and weakens the root canal [6].

The destruction of the collagen matrix in mineralized tissue may precipitate the development of fatigue cracks during cyclic stresses (Kruzic and Ritchie, 2008). Marending et al. (2007) demonstrated that a 24-min exposure of teeth to 2.5% NaOCl caused a significant drop in the flexural strength, which might result in fracture. To date, NaOCl remains the gold standard for root canal irrigation because of its antimicrobial properties, ability to lubricate tissue, solvent activity, low cost, and easy availability. However, it has some drawbacks such as toxicity to vital tissues and patient allergies [7].

Given the disadvantages of NaOCL reported in previous studies, various natural-based root canal irrigants have been developed. Specifically, grape seed extract, (GSE) contains 74%-78% of proanthocyanidin and 6% of flavanol monomer. Proanthocyanidin has been widely reported for its clinical application in dentistry. According to a study conducted by Impalgia (2012), proanthocyanidin or tannic acid can induce collagen crosslinking in biological tissues. Collagen crosslinking influences the mechanical properties of dentin by reducing enzymatic degradation, and by increasing the resin-dentin bond strength. Angelina (2013) found that proanthocyanidin from GSE can enhance the removal of dentine smear layer on 1/3 of the root canal dentinal wall. Soetanto (2014) studied proanthocyanidin of GSE solution (provided as an herbal drink) as an antibacterial agent targeted against *Enteroeoccus* faecalis biofilm [3,8].

2. Materials and Methods

Thirty one-rooted human premolar teeth extracted for orthodontic purposes were collected and utilized in the present investigation. Before experimentation, all teeth were kept in a saline solution. All teeth were decoronated at cement-enamel junction and sectioned to a standardized length of 7 mm at the apical region. Samples were then kept in a glass container filled with a saline solution. Dentin slabs were prepared by sectioning the root into rectangular beams and trimmed to a final rectangular dimension of 1-mm thickness x 4-mm width x 7-mm length, using a low-speed carborundum disk under running water.

Samples were randomly divided into three groups as follow: Group 1: root canal dentin slabs immersed in 2.9% GSE solution, Group 2: root canal dentin slabs immersed in NaOCl solution, and Group 3: root canal dentin slabs immersed in distilled water. All samples were immersed in each solution for 30 min each day for a total of 3d.

Mechanical properties were assessed using 7 x 4 x 1-mm dentin slab to get dentinal tubule direction that parallels the plane that would receive maximum pressure in 3-point bending with a span of 5 mm. Dentin slabs were divided into three groups (n = 30) according to the materials tested. Each slab was placed on the top of two supports and compressed while being immersed in running water until the sample was fractured using the universal testing machine (AG-5000E Shimadzu) set at a crosshead speed of 1 mm/min. Flexural strength was calculated using the 3PL/2bd2 formula, where P is the maximum load (N), L is the support span (mm), b is the width of the specimen, and d is the thickness of the specimen.

Normally distributed data were analyzed with one-way analysis of variance using SPSS version 20, with $\alpha = 0.05$ level of significance. Abnormally distributed data were analyzed using theKruskal Wallis test. All data are presented as a mean ± the standard deviation (SD) of the mean.

3. Results

This study evaluated the impact of GSE containing 2.9% proanthocyanidin on the flexural strength of root canal dentin and compared the result to that of 3% NaOCl solution and distilled water. The Kolmogorov-Smirnov normality test yielded $p > 0.05$, which suggested that the distribution of data from the three groups was normal. The homogeneity test ($p < 0.05$) suggested that the group data had were inhomogeneous and, therefore, a non-parametric Kruskal-Wallis test was used.

Table 1 Mean±SD and CI of the flexural strength (Mpa) of root canal dentin after immersion in EBA 2.9%, NaOCl 3% and distilled water

Treatment group	N	Mean±SD	95% Confidence Interval
EBA 2.9%	30	156.69± 50.36	137.88 - 175.49
NaOCl 3%	30	90.40±24.65	81.20 - 99.60
Aquabides	30	146.79± 52. 41	127.22 - 166.36

Table 1 shows the flexural strength of dentin after immersion. EBA (2,9%) treatment group had the highest flexural strength (156.69 MPa) of root canal dentin than the 3% NaOCl (90.40 MPa) and distilled water (146.79 MPa) groups. From the obtained data, it could be concluded that the best flexural strength was observed in root canal dentin immersed in GSE with 2.9% proantHocyanidin than that immersed in 3% NaOCl and distilled water.

Tabel 2 The impact of the treatment group on the flexural strength of root canal dentin

Experimental Group	NaOCl 3%	Distilled Water
GSE 2.9%	0.000*	0.438
NaOCl 3%		0.000*

Key: *level of significance $p < 0,05$ using post hoc Mann-Whitney test
GSE: Grape Seed Extract
NaOCl : sodium hypochlorite

Table 2 shows the impact of each treatment on the flexural strength of root canal dentin. There was no significant difference in the flexural strength of root canal dentin between the 2.9% GSE and distilled water group ($p > 0.05$); thus 2.9% GSE maintained the flexural strength of root canal dentin to the same extent as maintained by distilled water. In contrast there was no significant difference in the flexural strength of root canal dentin between the 2.9% GSE and 3% NaOCl groups [$p < 0.05$ ($p = 0.00$)]; thus the flexural strength of root canal dentin immersed in 2.9% GSE was better than that of root canal dentin immersed in 3% NaOCl. Further, there was a significant difference in the flexural strength of root canal dentin between the 3% NaOCl and distilled water groups [$p < 0.05$ ($p = 0.00$)]; thus, the flexural strength of root canal dentin immersed 3% NaOCl was lower than that of root canal dentin immersed in 3% NaOCl.

4. Discussion

This study evaluated the impact of a GSE solution (containing 2.9% proanthocyanidin) on the flexural strength root canal dentin compared with that of 3% NaOCl solution and distilled water. The background of this study referred to research results from lampaglia (2012), which suggested that proanthocyanidin or

tannic acid induces collagen crosslinks in biological tissues. Collagen crosslinks enhance the mechanical properties and lower the rate of enzymatic degradation [3].

In this study, the flexural strength of root canal dentin was maintained after immersion in the GSE solution. This was demonstrated by the absence of statistical differences between the 2.9% GSE and distilled water groups. Distilled water was used as control because of its neutrality and the lack of effects on root canal dentin. Thus, it likely represents a normal flexural strength of root canal dentin, as evidenced by the similar effects of 2.9% GSE and distilled water.

NaOCl was used as a comparison because it is the most abundantly used irrigant in root canal procedures. However, NaOCl could lower the flexural strength of dentin, which was confirmed in the present study by the lowest flexural strength observed in dentin immersed in 3% NaOCl when compared with that in dentin immersed in 2.9% GSE and distilled water (Table 1). There was also a significant difference between the 2.9% GSE and 3% NaOCl groups. The results suggest that the overzealous use of irrigants during root canal procedures likely decreases the flexural strength of dentin and makes root-canal-treated teeth more prone to fracture. There was a difference in the flexural strength of root canal dentin between GSE and sodium hypochlorite groups.

The specimens used in this study were one-rooted upper premolars extracted for orthodontic purposes. Samples were chosen from extracted teeth to obtain a uniform sample so the anatomical variance between each sample was minimal. Following extraction, teeth were immersed in saline for no longer than 1 month to maintain the humidity of the sample, primarily within the collagen inside the dentin. Samples were then shaped into a slab so that they were compatible with the universal testing machine that requires specimens to be rectangular with a flat surface, and the size used to refer to the teeth used (premolar) which if made up into a specimen, the possible size is 7-mm length, 4-mm width, and 1-mm thickness.

This study suggests that the flexural strength of root canal dentin immersed in 2.9% GSE was higher than that of sample immersed in the 3% NaOCl, likely because the active ingredient in proanthocyanidin could enhance collagen crosslinking. These results are the likely consequences of the impacts of proanthocyanidin on collagen interaction and stability as suggested by lampaglia. Moreover, because of the position of proanthocyanidin between collagen molecules, it can form an ionic and covalent bond with collagen fibers. Thus, collagen acts as a dentinal scaffold and plays an important role in the mechanical properties of dentin, one of which is flexural strength [3].

GSE used in this study was prepared from that produced in Turkey, where it is usually used as an herbal drink. This solution typically undergoes nanotechnology during the processing phase so that it is a high-quality and pure form of GSE, which can be stored for prolonged periods of time without compromising the quality. This preliminary study suggests that GSE development yields improved outcomes in patients undergoing root canal procedures [9, 10].

The GSE solution used herein contained 2.9% proanthocyanidin and a thickener (i.e., glycerin). This concentration of proanthocyanidin was lower than that used in the Iampaglia study in 2012 (20%) and was prepared using a powder mixed form. Differences in the preparation methodology, the use of other additional ingredients (i.e., glycerin), and the concentration are likely the reasons for discrepancies in results obtained from this study and those obtained from other investigations [3].

5. Conclusion

The immersion of root canal dentin slabs in GSE for 30 minutes per day of 3 d resulted in a higher flexural strength of root canal dentin than that of samples immersed in 3% NaOCL solution.

6. References

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