ABSTRACT

Objective: Grape seed extract (GSE) containing proanthocyanidin as a root canal irrigation solution has its antibacterial effects and ability to eliminate the smear layer. In addition, proanthocyanidin acts as a cross-linking agent of collagen, which adds to dentin’s mechanical properties. This study analyzed the effect of GSE containing 2.9% proanthocyanidin on the microhardness of the dentin in the root canal.

Methods: Fifty teeth were divided into three groups and immersed in GSE solution, 3% NaOCl solution, or distilled water (controls) for 30 min for 3 consecutive days. The microhardness was measured using the Vickers method. Data were analyzed using the Kruskal–Wallis test.

Results: The GSE group presented the highest microhardness values, whereas the lowest values in the NaOCl group. No significant difference in microhardness observed between the GSE and distilled water groups.

Conclusion: The GSE solution maintains the microhardness of the root canal dentin.

Keywords: Grape seed extract, Proanthocyanidin, Microhardness, Root canal dentin.

INTRODUCTION

One of the primary purposes of endodontic treatment is hermetic obturation. A tight apical seal prevents the entry of bacteria and its products into the root canal. Low-quality root canal obturation increases the risk of microleakage, which results in endodontic treatment failure; apical microleakage is the most common cause of endodontic treatment failure. The method used to obturate the root canal influences the occurrence of the apical leakage, the formation of the smear layer, and the adhesion of the root canal filling material [1].

The chemical irrigants commonly used in endodontic treatment have certain disadvantages because they can alter the composition of the dentin’s inorganic and organic contents, mainly collagen. Type I collagen is the main constituent of the organic structure of the dentin. It acts as a scaffold and forms a framework for the mineralized material, determining the dentin’s mechanical properties [2]. Changes in the inorganic structure of the dentin and the components of the root canal due to the use of chemical irrigation solutions can increase the risk of vertical root fractures in post-endodontic teeth [3]. Alterations in the organic and inorganic components of the dentin seen in scanning electron microscope images as cracks in the intertubular dentin after irrigation with EDTA (17%) and NaOCl (2.5%) [4]. Besides, the damaged dentin collagen interferes with the formation of a hybrid layer that can affect the adhesion of the root canal sealant to the dentin [5]. One of the mechanical properties which decreased after the use of the root canal irrigation solution was microhardness – an indication of the decrease in the condition of the collagen. The damaged condition of the collagen in the root canal can reduce the adhesion of root canal sealer to the dentin, leading to microleakage and root canal failure. As a result, studies on the use of natural materials as antibacterial root canal irrigation solutions that did not reduce dentin’s mechanical properties have been conducted [5,6].

Prevention of damage to dentin’s collagen by the formation of collagen bonds is more stable and resistant to biodegradation. The application of a collagen cross-linking agent can maintain collagen stability. One of the natural collagen cross-linking agents that have recently studied is a component of grape seed extract (GSE) called proanthocyanidins [7,8]. GSE solutions have been shown to have antibacterial properties and can eliminate the smear layer [9,10]. Furthermore, GSE has shown to have a positive effect on some of the mechanical properties of dentin, such as flexural strength and microhardness [6].

However, GSE’s effect on the microhardness of the dentin in the root canal has not evaluated so far. This study aimed to analyze the effect of GSE (containing 2.9% of proanthocyanidin) on the microhardness of the root canal dentin.

MATERIALS AND METHODS

Specimens

Until the experiment starts, 50 human single root premolars extracted for orthodontic purposes stored in saline solution. The apical portions of the teeth prepared with a diamond disk and chisel under water spray. The apical third of the tooth was sectioned into 5 mm dentin disks in a mesiodistal direction, parallel to the longitudinal axis of the tooth. All the samples rinsed with saline solution, and each dentin disk embedded in an acrylic resin block with the inner side of the root canal exposed. All samples stored in a closed box with a saline solution to maintain dentin hydration until further use [11,12].

Groups

The root canal dentin disks randomly divided into three groups based on the irrigation solution they immersed in: Group 1 (n=30) immersed in GSE with proanthocyanidin (2.9%), Group 2 (n=30) immersed in sodium hypochlorite (NaOCl; 3%), and Group 3 (controls: n=30) immersed in distilled water. Each sample immersed in 5 mL of irrigation solution, 30 min per day for 3 consecutive days [13,14].

Measurement

The microhardness measurement uses the Vickers method with 500 g per 15 s indentations [14]. Three indentation points measured in each...
RESULTS AND DISCUSSION

Root canal dentin microhardness value

The highest median microhardness observed in Group 1 (GSE group) followed by Group 3 (distilled water) and Group 2 (NaOCl; Table 1). Likewise, the highest average value of dentin root canal microhardness was Group 1 followed by Group 3 and Group 2. The highest microhardness value measured in the 2.9% GSE group was 32.21 ± 15.14 HV, and the lowest value in the 3% NaOCl group was 21.24 ± 9.23 HV. No significant difference in microhardness value noted between Group 1 and Group 3 (controls).

Irrigation solution influence to dentin’s microhardness

Chemomechanical preparation is one of the most critical factors that determine the success of root canal treatment. The 3% NaOCl irrigation solution commonly used for endodontic treatment has antibacterial activities and dissolves the necrotic tissue in the root canal [15]. However, it alters the composition of the organic and inorganic structures of the tooth [13,14]. Moreover, it is toxic to the tissues due to its chemical nature [16]. Therefore, various studies evaluating the potential of natural materials for use as root canal irrigation solutions have been conducted [5,6,9,10,17,18].

GSE is one such natural ingredient, which contains a high proportion of proanthocyanidin. GSE solution has shown to eliminate the root canal smear layer and act against Enterococcus faecalis [9,10]. Another advantage of GSE is the ability to increase collagen cross-linking, which can improve the quality of the organic and inorganic tooth structures, thus increasing dentin’s mechanical properties [5,6,17].

Dentin microhardness, one of the dentin’s mechanical properties, is influenced by the amount of collagen matrix and mineral content in the dentin. Collagen acts as a scaffold for mineralized material in the dentin, resulting in the association between the amount of dentin collagen matrix and minerals contained in dentin. As the amount of collagen matrix formed on the dentin increases, the mineral component also increases. The increase in dentin microhardness indicated by an increase in the number of calcified matrices per mm². Therefore, microhardness measurements have commonly used to demonstrate the loss or increase of minerals in hard tooth tissues, including dentin [19,20].

Changes may occur in the organic and inorganic components of the dentin in post-endodontically treated root canals. One such change is the decrease in microhardness, commonly caused by the irritation solutions used for endodontic treatment [13]. The decrease in microhardness provides evidence of the reduction in the dentin’s inorganic components and an overview of the dentin collagen matrix’s condition. These changes result in the formation of cracks in the intertubular dentin, which will reduce the resistance of the root canal wall and increase the risk of fractures in the endodontically treated teeth [4].

Damage to the dentinal collagen after the use of the NaOCl irrigation solution disrupts the formation of a hybrid layer due to the formation of an oxygen-rich layer on the dentin; this eliminates the organic matrix (collagen) and exposes the dentin surface, thus affecting the seal against the dentin [5]. The adhesion of the root canal sealer affects the quality of the root canal obturation. It determines the success of the treatment [1].

Proanthocyanidin in GSE as cross-linking agent

GSE contains proanthocyanidin, which is known to have antibacterial properties, can eliminate smear layers, and improve dentin’s mechanical properties. Proanthocyanidins can induce the cross-linking of collagen molecules in the dentin by increasing the number of inter- and intra-molecular collagen cross-links, thus strengthening the dentin collagen matrix and increasing the stability of the collagen by preventing enzymatic degradation, which in turn will improve the mechanical properties of dentin [5,17].

Proanthocyanidin molecules consist of multiple free phenyl hydroxyl groups that contain free hydroxyl groups, which can form bridge-type hydrogen bonds with the amide groups of the collagen matrix and mineral molecules, and collagen molecular amides. The formation of hydrogen bonds creates the stability of proanthocyanidin-collagen interactions. Furthermore, because of its position between the collagen molecules, proanthocyanidin can form ionic and covalent bonds with collagen fibrils [21]. During the process of forming hydrogen bonds, proanthocyanidin molecules can replace water molecules that bind to collagen in the extra fibrils [21]. This results in cross-linking and the formation of more stable collagen, thus enabling the maintenance of the mineral component and microhardness of the root canal dentin.

The GSE (Vitis vinifera) used in this study originates from Turkey and has been used to prepare solutions for healthy drinks. The majority of the local grape varieties cultivated in Indonesia belong to this type [22]. The climate conditions in the lowlands with the hot weather and loose sandy soil in Indonesia, especially in Bali, Kediri, and Probolinggo, are suitable for grape cultivation. The weather does not affect the quality of the wine originating from Indonesia, as well as the quality of the wine from Europe and Central Asia [23,24].

The local GSE is processed based on the concentration of GSE in the solution. According to Angellina (2013), the GSE solution’s concentration did not affect the ability to clean the smear layer in the root canal [9]. GSE is an irrigation solution that cleanses the smear layer not based on the GSE concentration. However, the role of GSE in the cross-linking of the collagen influenced by the concentration of proanthocyanidin. Based on several previous studies, 3.75% and 6.5% of proanthocyanidin in GSE can significantly increase collagen cross-linking [25,26]. Additional studies are required to evaluate the effects of various concentrations of proanthocyanidin in GSE on the dentin in the root canal.

Application of irrigation solution

The 3% NaOCl solution in this study based on a previous study by Ghommode et al. (2013) compared the antibacterial effect of GSE with 3% NaOCl on E. faecalis [18]. Similarly, Kalluru (2014) demonstrated the effects of several commonly used irrigation solutions on the dentin’s microhardness in the root canal [11]. Antibacterial effect and ability to dissolve pulp and necrotic tissue at a concentration of 3% is superior to those at concentrations of 0.5% and 1%. Higher concentrations of NaOCl will exert more toxic effects on the periapical tissue [27].

Until now, there has been no standard regarding the ideal duration of root canal irrigation. Based on an in vitro study by Retamozo et al. (2010), the best effect of irrigation with 5.25% NaOCl observed for 40 min. If a lower concentration of 1.3% or 2.5% used, the duration...
In this study, the role of proanthocyanidin in GSE as a cross-linking agent of collagen, which can increase dentin microhardness, did not differ significantly compared with distilled water control solution. This condition possibly caused by the content of glycerin in the GSE solution used. Glycerin as a thickener increases the GSE solution’s surface tension so that the penetration into the dentinal tubules will be lower. Therefore, the role of proanthocyanidin molecules in GSE solution with glycerin could not be optimum to increase the microhardness of dentin in the root canal. In a previous study by Liu et al. (2015), the small molecular size of proanthocyanidin reported providing the best results in triggering an increase in dentin collagen’s mechanical properties [30].

The microhardness evidenced by mineral loss or an increase in the hard tooth tissue and indirectly illustrates the extent of collagen matrix bonding in the dentin. Dentin microhardness is determined by the strength of the collagen matrix bonding to the dentin and the number of calcified matrices per mm² [19]. The collagen matrix’s amount and stability in the dentin root canal will affect the strength of the adhesion of the resin-based sealer to the dentin. Application of GSE with 5% proanthocyanidin shown to increase the adhesive strength of the resin to a root canal that irrigated with 5.25% of NaOCl [5].

Proanthocyanidin molecules consist of free hydroxyl groups that form bridge-type hydrogen bonds with the side chains of hydroxyl, carboxyl, amino groups, and collagen amides. These hydrogen bonds account for the stability of the proanthocyanidin-collagen interactions. Furthermore, due to its position between the collagen molecules, proanthocyanidin can form ionic and covalent bonds with collagen fibrils. The proanthocyanidin molecules replace the water molecules that bind to collagen in the extra fibrils to form these hydrogen bonds.

Proanthocyanidin stabilizes and enhances the cross-linking of type I collagen fibrils by proline hydroxylation, an essential stage of collagen biosynthesis. Thus, the collagen cross-links are more stable in the dentin matrix. Intermolecular cross bonds determine the stability, strength, and viscoelasticity of the collagen fibrils. Collagen fibrils reinforced by cross-linking agents such as proanthocyanidin will have increased mechanical properties and reduced enzymatic degradation, which plays essential roles in material adhesion to the dentin [2].

**CONCLUSION**

The findings of this study show that GSE can increase the dentin’s microhardness in the root canal. GSE solution can be used as an irrigation material during root canal treatment, and a solution containing 2.9% of proanthocyanidin can maintain the microhardness of the root canal dentin.

Further research is needed to evaluate the microhardness of the root canal using GSE in conjunction with various irrigation techniques and durations; also, the effect of local GSE from Indonesia containing various concentrations of proanthocyanidin needs evaluation.

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Nil.

**AUTHORS’ CONTRIBUTIONS**

All the authors have contributed equally.

**CONFLICTS OF INTEREST**

Declared none.

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