

The Effect of Grape Seed Extraction Irrigation Solution towards Cleanliness the Smear Layer On Apical Third of the Root Canal Wall

Anggraini Margono^{1*}, Afriani Nov Angellina², Endang Suprastiwi¹

1. Lecturer of Departement of Conservative Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

2. Resident of Departement of Conservative Dentistry, Faculty of Dentistry, Universitas Indonesia, Jakarta, Indonesia.

Abstract

Grape seed Extract (GSE) contains 74-78% proantosianidin that serves as an accelerator of crosslinking collagen. The aim of this study was to analyze the ability of GSE as root canal irrigation solution in cleaning smear layer on the apical third area.

Fifty extracted incisors teeth were divided into 5 groups. As an irrigation solution; group 1 were using distilled water (aquadest), group 2: 3.25% GSE, group 3: 6.5% GSE, group 4: 13% GSE and group 5: 17% EDTA. The cleanliness of smear layer were evaluated by SEM and then scored. The data were analyzed by using Kolmogorov-Smirnov by SPSS 17.

The highest score is on the 13% GSE group (40%) followed by 17% EDTA and 3.25% GSE (30%), while in group of aquadest was 0%. Score 2 showed that aquadest was the lowest level of cleanliness around 80%, followed by 13% GSE group, 3.25% GSE and 6.5% GSE. Whereas in 17% EDTA group there was no score 2. The whole group of GSE compared with aquadest and EDTA showed no significant differences amongst them

Grape seed extract solution has a potency for cleaning the smear layer on apical third area of the root canal.

Experimental article (J Int Dent Med Res 2017; 10(2): pp. 244-247)

Keywords: Grape seed extract, irrigation solution, smear layer.

Received date: 28 September 2016

Accept date: 29 October 2016

Introduction

Chemomechanical preparation is a combination of chemical and mechanical preparation that will clean the smear layer in the root canal. Traditionally, much of the attention placed on irrigation in endodontics has focused on smear layer removal. Smear layer consist of inorganic/organic particles such as debris from pulp tissue, odontoblasts, bacteria and blood cells which can be source of infection, prevent the irrigation solution, and filler to penetrate into the dentin tubules. The effect of sodium hypochlorite and decalcifying solutions on dentin structure and dentin strength has become an important topic, which is related to the possible harmful effects of irrigation such as dentine

erosion and even vertical root fracture. Root canal irrigation solution serves as a lubricant, and has a broad spectrum antimicrobial properties.^{1,2}

Type of root canal irrigation solution that has been used include : NaOCl, EDTA, MTAD, citric acid, chlorhexidine, potassium iodide and hydrogen peroxyde.^{3,4} Irrigation solution is a synthetic substance that is toxic and irritating. In order to improve the safety biology in root canal treatment, we need a biological irrigation solution.

Grape seed extract (GSE) has a polyphenol content which comprises of 74-78% proanthocyanidin (PA).^{5,6}

Proanthocyanidin is a weak acid that can serve as a natural crosslinking proline-rich protein that is collagen.⁷ Al-ammari (2009) stated that PA concentration of 6.5% can increase the crosslinking collagen.⁸ Because of that chemical properties it can be assumed that GSE has the ability as an irrigation solution in cleaning the smear layer. The aim of this study was to analyze the ability of a grape seed extract solution (GSE) to clean the smear layer on apical third of the root canal.

*Corresponding author:

Anggraini Margono
Departement of Conservative Dentistry, Faculty of Dentistry,
Universitas Indonesia
Jln. Salemba Raya No 4, Jakarta 13410, Indonesia
E-mail: margonodewi@yahoo.com

Materials and methods

Grape seed extract (GSE) is made by using maceration method with solvent solution of 70% ethanol, pH 5.84. GSE was diluted with distilled water to get concentration of 3.25%, 6.5% and 13%. Fifty single teeth fresh extraction with closed apices were clean and soak in saline solution. Two longitudinal groove on the lingual and buccal root surface were made to facilitate vertical cuts. Apical foramen patency was standardized by using K-file #15 (Dentsply Maillefer, Switzerland) and working length was measured to 0.5mm from at the apical ends. Root canals were prepared by using NiTi instruments Revo-S (Micro Mega, France), with 4 files such as SC1, SC2, SU and AS 30. Samples were randomly divided into five groups with the number of samples in each group of 10 teeth. The instruments used for irrigation is a safe-ended tip syringe 2.5 ml with a needle size 30G (Dentsply Maillefer, Switzerland). Group 1 using irrigation with aquadest, group 2 with 3.25% GSE, group 3 with 6.5% GSE, group 4 with 13% GSE, group 5 with 17% EDTA (MD-Cleanser, Meta Biomed, Korea) . The root was split into two parts and cut at the apical third.

The level of cleanliness in apical third analyzed by using SEM (Scanning Electro Microscope) with 1000x magnification and evaluated with scoring system, double-blind manner by a trained operator. Score 0 was measured if more than 75% showed a clean area of the smear layer, score 1 indicates 50 to 75% of the surface is clean, and score 2 if more than 50% in the apical third surface covered by smear layer.⁹ Data were analyzed statistically by the Kolmogorov-Smirnov test using SPSS17 and the level of significance $p < 0.05$.

Results

Table 1 showed the highest score is on the 13% GSE group (40%) followed by 17% EDTA and 3.25% GSE (30%), while in group of aquadest was 0%. Score 2 showed that aquadest was the lowest level of cleanliness around 80%, followed by 13% GSE group, 3.25% GSE and 6.5% GSE. Whereas in 17% EDTA group there was no score 2. Conclusion: GSE solution was capable of cleaning the smear layer on the apical third area.

Group	0		1		2		Total
	N	%	N	%	N	%	
Aquadest	0	0	2	20	8	80	10
3.25% GSE	3	30	4	40	3	30	10
6.5% GSE	1	10	7	70	2	20	10
13% GSE	4	40	2	20	4	40	10
17% EDTA	3	30	7	70	0	0	10
Total	11	22	22	44	17	34	50

Table 1. Percentage of apical third cleanliness of the root canal.

Score 0: If > 75 % net of the smear layer.

Score 1: If 50-75% net of the smear layer.

Score 2: If > 50% are covered by smear layer.

Group	Aquadest	3.25%GSE	6.5%GSE	13%GSE	17%EDTA
Aquadest		0.164	0.055	0.400	0.003*
3.25%GSE	0.164		0.988	1.00	0.759
6.5%GSE	0.055	0.988		0.759	0.988
13%GSE	0.400	1.00	0.759		0.400
17%EDTA	0.003*	0.759	0.988	0.400	

Table 2. Comparison of significance between groups. *The predictive value of $p < 0.05$.

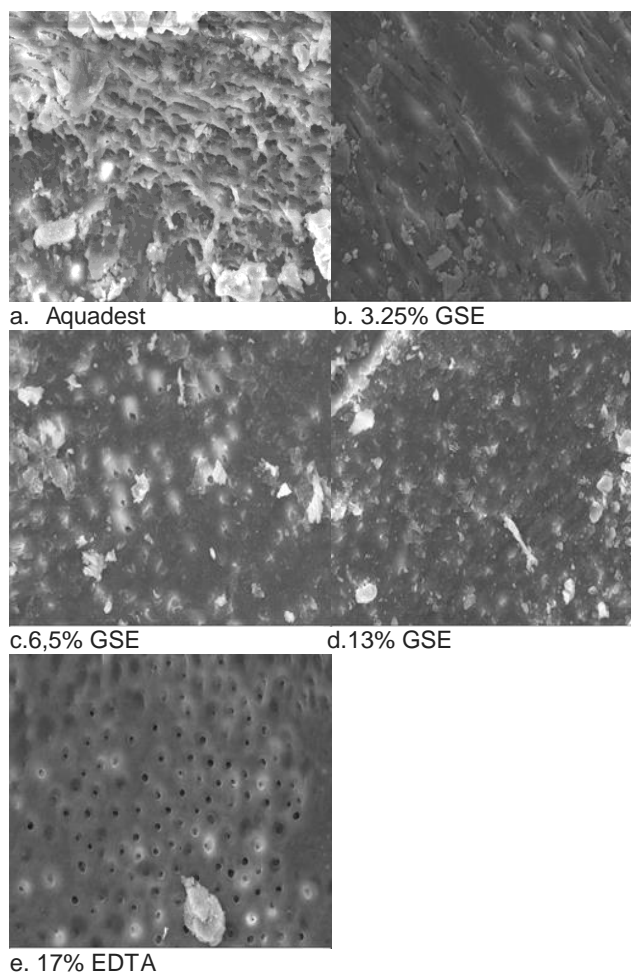


Figure 1. SEM picture group of a. aquadest, b. 3.25%, c. GSE Group 6.5% GSE Group, d.13% GSE Group, and e.17% EDTA Group.

Table 2 when compared amongst the groups there were significant differences between groups of aquadest with 17% EDTA ($p=0.003$). Whereas amongst groups of 3.25%, 6.5% and 13% GSE, the results did not differ significantly. The whole group of GSE compared with distilled water and EDTA showed no significant difference. Conclusion: GSE has strength cleaning ability that exists between aquadest and 17% EDTA.

Figure 1, described the result of SEM of each groups showed the level of smear layer cleanliness Aquadest group (score 2), 3.25% GSE group (score of 0), 6.5% GSE (score of 0), 13% GSE (score of 0) and 17% EDTA (score of 0). Conclusion: aquadest group showed an inability to clean the smear layer.

Discussion

Materials tested in this study was grape seed extract that contain most proanthocyanidin (74-78%).^{3,9} Mandibular incisors were used to obtain uniformity of the samples.¹⁰ Teeth samples were stored in saline after extracted to maintain humidity and conditioning biological situation in the oral cavity.

Root canal were prepared with crown down techniques using Revo-S to facilitate more irrigation solution volume to get more optimum penetration.¹¹ EDTA 17% were used as a comparison because it is a standard liquid irrigation for cleaning smear layer. The volume of irrigation solution used in this study was 2.5 ml, whereas volume between 0.75-3 ml did not show any significant differences.

Irrigation needle diameter was adjusted to the size of the apex preparation and according to Sedgley et al. (2005), states that the use of irrigation needle should be up to a depth of 1 mm from the working length.¹² Then the irrigation needle size 30 with outer diameter 0.32 mm, corresponding medical standardization dimensional stainless steel needle, (specification ISO 9626: 1991 / Amd 1: 2001). The effectiveness of irrigation materials could be delivered 1-2 mm of working length without hindrance in the root canal that has been prepared to increase the effectiveness in dissolving the smear layer.¹³ Part of the apical third was analyzed with scanning electron microscope (SEM) by taking two points differences then the average value taken. SEM

analysis conducted by two examiners with double blind method manner.¹⁴ Using the reference ring imaginary column to facilitate the perception there reducing the bias in the objective assessment between two examiners.¹⁵

In Table 1 showed the highest score of 0 in the 13% GSE group (40%) followed by 17% EDTA group and GSE 3:25% (30%). 17% EDTA group does not have a score of 2. This proves that EDTA 17% has the most excellent cleaning ability. EDTA 17% is a polyprotic acid containing 4 carboxylic acid groups and 2 amine groups. EDTA acts on calcified tissues by replacing sodium ions which combine with dentine to provide solubility of salts in calcium ion. EDTA has a normal pH (7.3), and within normal concentrations, EDTA dissolves 10.6-100 grams of calcium. So that EDTA chelation is effective in the process of dentin and dispose smear layer.^{16,17,18,19}

Grape seed extract contains 74-78% proanthocyanidin which serves as a crosslinking collagen. So it assumed that, smear layer on dentin can be removed by the PA. However, the results in this study showed that the cleanliness level of the apical third smearlayer in the root canal walls at GSE group is not as good as 17% EDTA. This may be due to its natural characteristic that contains other ingredients than the PA, so it is not equivalent to EDTA which is a synthetic material that has been proven to clean the smear layer. Grape seed extract used in this study is a natural ingredient that can increase safety biology. However, GSE is not toxic and not irritating compared to 17% EDTA.^{5,20,21,22}

Table 2 showed there was a significant difference between the groups of distilled water with EDTA 17% ($p=0.003$), while amongst the third group GSE concentration showed no significant differences. The third group GSE concentration when compared with distilled water also showed no significant differences. This proves that the three concentrations of GSE have the cleaning ability that exists between distilled water and 17% EDTA. In Table 2 there were no significant differences between 3.25% GSE, 6.5% GSE and 13% GSE in the level of smear layer cleanliness on the apical third of the root canal walls. This proves that the three concentrations of GSE have the strength cleaning ability that exists between aquadest and 17% EDTA.

It also seen in Table 1 the three concentrations of GSE which has score of 0 at most (40%) is the GSE with a concentration of 13% and at least on the GSE with a concentration of 6.5%. Concentration of 6.5% has been selected according to the research by lampaglia and Al-Ammar (2009), who use such concentration as crosslinking kolagen.^{6,24} The changes of GSE concentration to 3.25% and 13% did not affect the level of cleanliness in the third apex of the root canal walls. The opposite occurs in a synthetic material that if there is change in concentration, it leads to increasing the effectiveness of the material.

Conclusions

In this study it can be concluded that the solution of grape seed extract can clean the smear layer on the apical third area, even though the result was not as good as EDTA 17%. The concentration of grape seed extract does not affect the cleaning ability of smear layer on the apical third of the root canal.

Acknowledgements

We thank to the Universitas Indonesia for the support.

Declaration of Interest

The authors report no conflict of interest.

References

1. Haapasalo M, Qian W, Shen Y. Irrigation: beyond the smear layer. *Endodontic Topics*. 2013;27(1):35-53.
2. Torabinejad M, Walton WE. *Endodontics : Principles and Practice*. 4th ed. Missouri: Saunders Elsevier. 2009.
3. Bergenholtz G. *Textbook of Endodontology*. 2th ed. United Kingdom: Wiley-Blackwell. 2010.
4. Ehrlich H, Koutsoukos PG, Demadis KD, Pokrovsky OS. Principles of demineralization: modern strategies for the isolation of organic frameworks. Part I. Common definitions and history. *Micron*. 2008;39(8):1062-91.
5. Shrestha S, Torneck CD, Kishen A. Dentin Conditioning with Bioactive Molecule Releasing Nanoparticle System Enhances Adherence, Viability, and Differentiation of Stem Cells from Apical Papilla. *J Endod*. 2016;42(5):717-23.
6. Manimaran VS, Srinivasulu S, Ravesh AV, Ebenezar, Mahalaxmi S, Srinivasan S. Application of a proanthocyanidin agent to improve the bond strength of root dentin treated with sodium hypochlorite. *J Conserv Dent*. 2011;14(3):306-08.
7. Perumalla AVS, Hettiarachchy NS. Green Tea and Grape Seed Extracts Potential Applications in Food Safety and Quality. *Food Res Inter*. 2011;44:827-39.
8. Furiga A, Lonvaud-Funel A, Dorignac G, Badet C. In vitro anti-bacterial and anti-adherence effects of natural polyphenolic compounds on oral bacteria. *J Appl Microbiol*. 2008;105(5):1470-6.
9. Ioana VS, Vasile L. Recovery of Total Polyphenolic Compounds from Different Grape Skins Varieties, Under Enzymatic Treatment. *Analele Universitatii din Oradea, Fascicula: Ecotoxicologie, Zootehnie si Tehnologii de Industrie Alimentara*. 2010:1283-93.
10. Kokani F, Kamberi B, Dragusha E, Mrasori S, Haliti F. The cleaning efficiency of the root canal after different instrumentation technique and irrigation protocol: A SEM analysis. *Open J Stomatol*. 2012;2:69-76.
11. Chunningham WT, Martin H. A Scanning Electronic Microscope Evaluation of Root Canal Debridement with The Endodontic Synergistic System. *J Oral Surg*. 2002;53(2):527-31.
12. Sedgley CM, Nagel AC, Hall D, Applegate B. Influence of irrigants into instrumented root canals using real time imaging in vitro. *Int Endod J*. 2005;38:97-104.
13. Green B, Yao X, Ganguly A, et al. Grape Seed Proanthocyanidins Increase Collagen Biodegradation Resistance in the Dentin Adhesive Interface when Included in an Adhesive. *J Dent*. 2010;38(11):908-15.
14. Zand V, Ghaziani P, Rahimi S, Shahi S. A Comparative SEM Investigation of The Smear Layer following Preparation of Root Canals Using Nickel Titanium Rotary and Hand Instruments. *J Oral Sci*. 2007;49(1):47-52.
15. Ring J, Murray P, Kenneth N, Namerrow, Moldauer BFG. Removing Root Canal Obturation Materials: a Comparison of Rotary File Systems and Retreatment Agents. *J Am Dent Asso*. 2007;140(2):680-8.
16. Čujić N, Šavikin K, Janković T, Pljevljakušić D, Zdunić G, Ibrić S. Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique. *Food Chem*. 2016;194:135-42.
17. Lima Mdos S, da Conceição Prudêncio Dutra M, Toaldo IM, Corrêa LC, Pereira GE, de Oliveira D, Bordignon-Luiz MT, Ninow JL. Phenolic compounds, organic acids and antioxidant activity of grape juices produced in industrial scale by different processes of maceration. *Food Chem*. 2015;188:384-92.
18. Tosić G, Miladinović M, Kovačević M, Stojanović M. Choice of root canal irrigants by Serbian dental practitioners. *Vojnosanit Pregl*. 2016;73(1):47-52.
19. Chen G, Chao Chang Y. Effects of liquid-and paste type EDTA on smear layer removal during rotary root canal instrumentation. *J Dent Sci*. 2011;6(1):41-7.
20. Dakshita J, Ashish A. Recent concepts on root canal chelation. *Endo*. 2011;5(3):175-94.
21. Cecchin D, Farina AP, Souza MA, Albarello LL, Schneider AP, Vidal CMP, Russo AKB. Evaluation of antimicrobial effectiveness and dentin mechanical properties after use of chemical and natural auxiliary irrigants. *J Dent*. 2015;43(6):695-702.
22. Vidhya S, Srinivasulu S, Sujatha M, Mahalaxmi S. Effect of grape seed extract on the bond strength of bleached enamel. *Oper Dent*. 2011;36(4):433-8.
23. Liu Y, Wang Y. Proanthocyanidins's efficacy in stabilizing dentin collagen against enzymatic degradation: MALDI-TOF and FTIR analyses. *J Dent*. 2013;41(6):535-42.
24. Al-Ammar A, Drummond JL, Bedran-Russo AKB. The use of collagen cross-linking agents to enhance dentin bond strength. *J Biomed Mater Res B Appl Biomater*. 2009;91(1):419-24.