International Journal of Phytopharmacology

Research Article

www.onlineijp.com

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

AN *IN-VITRO* COMPARATIVE STUDY ON THE BOND STRENGTH OF ROOT DENTIN AFTER TREATMENT WITH TWO DIFFERENT COLLAGEN CROSS LINKING AGENTS

***Yadav Chakravarthy and **Arun Vijayan**

* Professor and Head of the department, Department of Conservative Dentistry and Endodontics, Vinayaka Mission's Sankarachariyar Dental College, Salem, Tamil Nadu, India
** Post Graduate Student, Department of Conservative Dentistry and Endodontics, Vinayaka Mission's Sankarachariyar Dental College, Salem, Tamil Nadu, India

ABSTRACT

The aim of the studyis to evaluate whether the two different collagen- cross linking agents (Alpha-tocopherol and Punicalagin) may help to reverse the reduced bond strength of root dentin after treatment with sodium hypochlorite. Group 1: root canals were irrigated with 5.25% NaOCl followed by 5% Alpha-tocopherol for 10 minutes. Group 2: root canals were irrigated with 5.25% NaOCl followed by 5% punicalagin for 10 minutes. Group 3: root canals were irrigated using 5.25% NaOCl + EDTA solution (positive control). All the canals were then obturated with Gutta Percha Points(Dentsply) with Resin cement (AH26, Dentsply) as root canal sealer and allowed it to be cured undisturbed for 5minutes, stored for one day in water, and then cross sectioned into three slabs of 2-mm thickness that were prepared and tested for microtensile bond strength.The results demonstrated that 5.25% NaOCl caused significant reduction ($P<0.05$) in the bond strength, but this can be reversed by 5% Alpha-tocopherol significantly more than the 5% Punicalagin.

Key words: Collagen-cross linking agents; Alpha-tocopherol; Punicalagin; Dentin bond strength.

Corresponding Author: **Arun Vijayan** Email: arunvijayan83@outlook.com

INTRODUCTION

Success of root canal treatment depends on cleaning, shaping and disinfection of the root canal that is achieved by chemomechanical preparation to eliminate bacteria and their by-products, and to prevent recontamination of the root canal space. Root canal irrigants used during biomechanical preparation may alter the dentin structure and affects its interaction with the root canal filling material. Earlier studies have shown that during cleaning and shaping, root canals irrigated with 5.25% sodium hypochlorite (NaOCl) cause significant reduction in the bond strength of resin sealers and cements (Weston CH *et al*., 2007; da Cuncha LF *et al.*, 2010). These chemical substances have a negative effect on the dentin properties, which does not appear to improve over the period of time. Whereas, this reduced bond strength to NaOCl- treated dentin could be restored by the application of collagen cross linking agents on the dentin surface (Lai SCN *et al*., 2001).

Dentin is composed of two phases: an inorganic, and an organic phase of predominantly Type 1 collagen. Quality of the bond strength and its durability is greatly influenced by the structural integrity and mechanical properties of collagen fibrils, mechanical properties of collagen can be increased by forming intra and intermolecular and intermicrofibrillar cross links (Neha N *et al.*, 2017). Improvement of the mechanical properties of collagen can be achieved by the use of different collagen cross-linking agents. Dentin surface pretreatment by these agents prior to the bonding procedures can aid in

Access this article online

DOI: http://onlineijp.com/

DOI: http://dx.doi.org/10.21276/ijp.2019.10.3.3

Quick Response code

Received:25.02.19 **Revised**:02.03.19 **Accepted**:13.03.19

increasing the bond strength values. Several naturally occurring (alpha tocopherol, proantho-cyanidin and others) as well as synthetic (glutaraldehyde, carbodimides and others) collagen cross linking agents may induce exogenous collagen cross-links and also improve the mechanical properties (Castellan CS *et al*, 2010).

Type 1 collagen in dentin is essential for many structural roles such as scaffold for mineralization and viscoelasticity by forming a rigid, strong, space-filling biomaterial. Alpha tocopherol and Punicalagin stabilize and increase the cross-linkage of type 1 collagen fibrils by the hydroxylation of proline which is an essential step of the collagen biosynthesis. Intermolecular crosslinking is the prerequisite for stability, tensile strength, and viscoelasticity of the collagen fibrils (Bedran-Russo AM *et al.*, 2007). The strengthening of collagen fibrils by crosslinking agents to increase the mechanical properties and decrease enzymatic degradation is a significant step in adhesive dentistry. Thus, the purpose of this invitro comparative study was to evaluate the bond strength of root dentin, using two different collagen cross linking agents (Alpha tocopherol and Punicalagin) after treatment with sodium hypochlorite.

MATERIALS AND METHODS

Preparation of solutions

Two solutions were prepared for this study. For the first solution, 5 g of strawberry extract in the form of powder(Srivari natural herbs, Bengaluru, India) was collected and dissolved in 100 ml of sterile distilled water to make 5% alpha tocopherol solution. For the second solution,5 g of pomegranate powder (Srivari natural herbs, Bengaluru, India) was dissolved in 100 ml of distilled water to make 5% of punicalagin.

Preparation of samples

A total of 30 extracted human single-rooted teeth were used in this study. They were extracted for periodontal and orthodontic reasons. The teeth were cleaned of calculus and debris and stored in saline. The crowns were decoronated at the cementoenamel junction such that standard lengths of 18 mm of roots were obtained. The 30 teeth were then randomly distributed into three groups of 10 teeth each. The instrumentation protocol was same for all the groups. Canal space was enlarged using Gates- Glidden drills (Mani, Tochigi, Japan) of sizes #3, #4, and #5 in a slow-speed contra-angle hand piece and hand instrumentation using K-files (Mani, Inc, Tochigi, Japan) up to #70. The irrigation protocol during instrumentation varied as follows: From Group 1 to 3, the irrigation was performed with 5.25% NaOCl (Prevest Denpro Limited, Jammu, India) followed by Ethylene diamino tetra acetic acid (EDTA) (GlydeTM, Dentsply Maillefer, Ballaigues, Switzerland) and 2% chlorhexidine (CHX) (Asep-RC, Steadman Pharmaceuticals Pvt Ltd, Thiruporur, Tamil Nadu) during the preparation of the root canal, as is done clinically. The total time of exposure to NaOCl, EDTA, and CHX was estimated to be 15 to 20 minutes per tooth. In Group 1, after irrigation with 5.25% NaOCl , the root canals were rinsed with 10 ml of water. The canals were then rinsed with 10 ml of 5% alpha tocopherol for 10 minutes. For Group 2, after irrigation with 5.25% NaOCl , the root canals were rinsed with 10 ml of water. The canals were then rinsed with 10 ml of 5% punicalagin for 10 minutes. After the irrigation procedure, no further treatment was done for Group 3 (positive control). After the irrigation protocol for each group, all the canals in groups 1 to 3 were rinsed with 10 ml of water and the canals were dried with paper points.

After that canals were obturated with Gutta Percha Points (Dentsply) with Resin cement (AH26, Dentsply) as root canal sealer and allowed it to be cured undisturbed for 5minutes. Application of root canal sealer was performed using lentulo spirals.The filled specimens were then stored in 25°C water for 24 hours to prevent dehydration. Then, the specimens were removed from the water, dried, and sectioned into coronal, middle, and apical third of 6 mm each by using a diamond disc**.** The central 2-mm thick sections were made from each coronal, middle, and apical third section. The buccal and lingual dentin portion of each slab was then cut away to permit tensile testing of the root canal–resin interface. The specimens were then glued to a custom-made jig with cyanoacrylate resin. Universal testing machine was used to ensure that pure tensile force was applied to the bonded interface during testing in a universal tester operated at 1 mm/min. Each specimen was stressed in tension until failure occurred on either side of the resin bonded canal. The load at failure was divided by the surface area (approximately 1.8 mm^2) to obtain the failure stress that was expressed in MPa.

Statistical analysis

Mean and standard deviation were estimated for each study group. Normality of the data was tested in each group by using Kolmogorov–Smirnov test. Mean values were compared between different study groups by using One way ANOVA followed by Tukey-HSD procedure.

Statistical significance was defined as $p<0.05$.

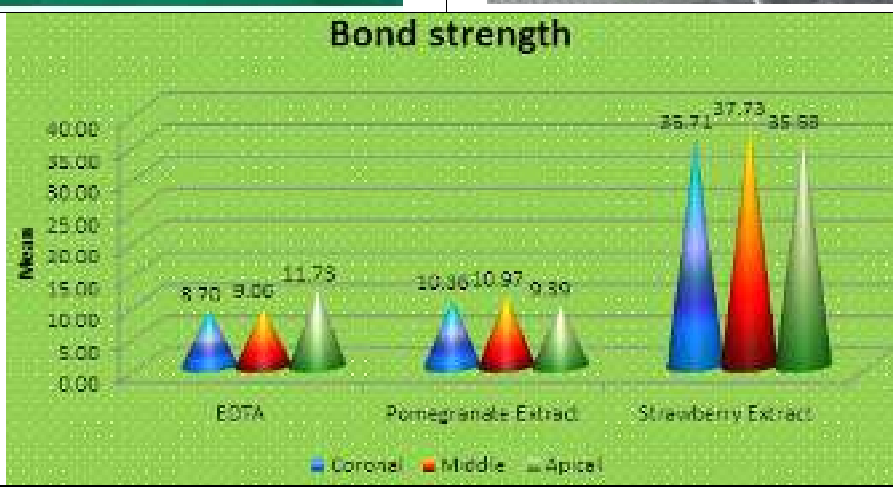
RESULTS

The effects of the various treatments on the bond strength of root canal dentin are shown in the Table 1. The bond strength is the maximum in Group I (37.73 ± 0.821), followed by Group II (10.97 ± 0.635) and Group III(9.06 ± 0.362) exhibits the least bond strength among all the other groups. Within sub-groups, middle third reveals the maximum bond strength, followed by coronal and apical third. Group I (alpha tocopherol) exhibits statistically higher value than all the other groups in coronal, middle, and apical third with respect to bond strength.

Table 1. Comparison of mean values among different study groups

	Group	Mean+/-SD	Significant groups
Coronal	I	35.71 +/- 1.703	I vs. II, III
	II	10.36 +/- 0.547	II vs. I,III
	III	8.70 +/- 0.222	III vs. I,II
Middle	I	37.73 +/- 0.821	I vs. II, III
	II	10.97 +/- 0.635	II vs. I,III
	III	9.06 +/- 0.362	III vs. I,II
Apical	I	35.59 +/- 1.578	I vs. II, III
	II	9.39 +/- 0.348	II vs. I,III
	III	11.73 +/- 0.655	III vs. I,II

P <0.0001 (Sig.), *One Way ANOVA was used to calculate the *P* value; Tukey- HSD procedure was employed to identify the significant groups at 5% level.



DISCUSSION

Endodontic irrigants like sodium hypochlorite and hydrogen peroxide are potent biological oxidants, which causes the oxidation of some of the dentin matrix components, particularly collagen (Moreira DM *et al*., 2009). Sodium hypochlorite (NaOCl) is still a gold standard as a chemical irrigant for endoontic therapy because of its antimicrobial activity and ability to dissolve organic matter, but it has certain disadvantages like, disrupts the organic matrix of dentin leaving the dentin surface more porous and reducing its bond strength (Sim TPC *et al* , 2001). Ethylene-diamine-tetra-acetic acid (EDTA), a lubricant though it acts on both the organic and inorganic portion of the dentin, causes dentin demineralization and leads to the erosion of dentin, eventually causing an impairment of implications with the hybrid layer (Santos JN *et al*., 2006). Thus, the current irrigation protocol regime, reduces the physical properties of dentin like the micro-hardness, flexural strength, elastic modulus, etc. which does not appear to improve over a period of time (Ana Carolina Pimentel Correa *et al*., 2016). This calls for the need of collagen cross-linking agents which could reverse the compromising effect of the chemical agents on the dentin bonding substrate.

Among all types of collagen, type I collagen is the predominant genetic product and it is an essential molecule to provide tissues and organs with tensile strength, form, and cohesiveness. Type I collagen is present in a tissue as fibrils that are stabilized by covalent intermolecular cross linking (Yamauchi M *et al*., 2002). To further stabilize collagen fibrils in biological tissues, various cross linkers have been used to induce additional intra and intermolecular and intermicrofibrillar cross links. Collagen in biological tissue is strengthened by the formation of native cross links which provides the fibrillar resistance against enzymatic degradation as well as greater tensile properties. Natural cross linkers such asalpha tocopherol and punicalagin can increase the number of inter- and intramolecularcross links (Bedran-Russo AK *et al*., 2007). The present study was undertaken to assess the feasibility of using crosslinking agents to increase the mechanical properties of dentin.

In this study, 5% alphatocopherol showed the maximum bond strength among all the groups (group 1[37.73 ± 0.821]). There was a significant difference (P<0.01) in the bond strength between 5% alpha tocopherol (group I [37.73 ± 0.821]) and control (group III [9.06 ± 0.362]) and also between 5% alpha tocopherol (group 1 [37.73 ± 0.821]) and 5% punicalagin (group 2 [10.97 ± 0.635]). The probable reason may be that the antioxidant ability of alpha tocopherol is 20 times more than punicalagin and also it can be quickly and completely absorbed (Baker NA *et al*., 1975). Collagen cross linking and stabilization property of alphatocopherol is also an important factor for the increased bond strength.

CONCLUSION

Within the limitations of this *in vitro* study, both the collagen cross lining agents tested, Alpha tocopherol and Punicalagin, prove to overcome the bond strength of dentine compromised due to the endodontic irrigants such as sodium hypochlorite used during root canal therapy. Hence exhibiting a great potential to be clinically used and promises better results.

ACKNOWLEDGEMENTS AND FUNDING

The authors thank Alpha-Omega Hi-Tech Bio Research Center, Salem for its assistance in this research. This project was supported and funded by the authors.

CONFLICT OF INTEREST

No interest

REFERENCES

Ana CPC, Doglas C, Jose flavio affonso de A, Brenda paula figueiredo de almeida G, Alexandre AZ, Caio CRF et al. Sodium thiosulfate for recovery of bond strength to dentin treated with sodium hypochlorite. *JOE*, 42(2), 2016.

Baker NA, Eleazer PD, Averbach RE, Seltzer S. Scanning electron microscopic study of the efficacy of various irrigatingsolutions. *J Endod*, 1975, 127e35.

Bedran-Russo AK, Pereira PN, Duarte WR, Drummond JL, Yamauchi M. Application of crosslinkers to dentin collagen enhances the ultimate tensile strength. *J Biomed Mater Res B Appl Biomater*, 80, 2007, 268-272.

Bedran-Russo AK, Pereira PN, Duarte WR, Drummond JL, Yamauchi M. Application of crosslinkers to dentin collagen enhances the ultimate tensile strength. *J Biomed Mater Res B Appl Biomater*, 80, 2007, 268-272.

Castellan CS, Pereira PN, Viana G, Chen SN, Pauli GF, Bedran-Russo AK. Solubility study of phytochemical cross-linking agents on dentin stiffness. *J Dent*, 38, 2010, 431-436.

da Cunha LF, Furuse AY, Mondelli RF, Mondelli J. Compromised bond strength after root dentin deproteinization reversed with ascorbic acid. *J Endod*, 36, 2010, 130-134.

Han B, Jaurequi J, Tang BW, Nimni ME. Proanthocyanidin: A natural cross linking reagent for stabilizing collagen matrices. *J Biomed Mater Res A*, 65, 2003, 118 –124.

Lai SCN, Mak YF, Cheung GSP, Osorio R, Toledano M, Carvalho RM *et al*., Reversal of compromised bonding to oxidized etched dentine. *Journal of Dental Research*, 80, 2001, 1919–1924.

Moreira DM, Almeida JF, Ferraz CC *et al*., Structural analysis of bovine root dentin after use of different endodontics auxiliary chemical substances. *J Endod*, 35, 2009, 1023–1027.

Neha N, Lotika B, Kosha J**.** Effect of Various Collagen Cross Linkers on the Bond Strength of Dentin Bonding Agents to the Dentin**.** *International journal of contemporary medical research*, 4(10), 2017, 2190-2194.

Santos JN, de Oliveira Carrilho MR, de Goes MF, et al. Effect of chemical irrigants on the bond strength of a self-etching adhesive to pulp chamber dentin. *J Endod*, 32, 2006, 1088–1090.

Sim TPC, Knowles JC, Ng Y-L, et al. Effect of sodium hypochlorite on mechanical properties of dentine and tooth surface strain. *Int Endod J*, 34, 2001, 120–132.

Weston CH, Ito S, Wadgaonkar B, Pashley DH. Effects of time and concentration of sodium ascorbate on reversal of NaOCl – induced reduction in bond strengths. *J Endod*, 33, 2007, 879-881.

Yamauchi M. Collagen biochemistry: An overview. In: Phillips GO. Advances in Tissue Banking, World Scientific, New Jersey, 6, 2002, 455–500.