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Effect of Different Anti-Oxidants on the Bond Strength of Resin Cement to Root Canal Dentin Treated With Sodium Hypochlorite and Ethylenediaminetetraacetic Acid – An In Vitro Study

[1]Dr. Laxmikant Late, [2]Dr. Sadashiv Daokar, [3]Dr. Priya Kulkarni, [4]Dr. Rohit Tambake, [5]Dr. Prachi Mapari, [6]Dr. Snehashree Pophli.

[1]M.D.S, Department of Conservative Dentistry and Endodontics, C.S.M.S.S Dental College and Hospital, Aurangabad, Maharashtra, India.

[2]Prof. and HOD, Department of Conservative Dentistry and Endodontics, C.S.M.S.S Dental College and Hospital, Aurangabad, Maharashtra, India.

[3]B.D.S, C.S.M.S.S Dental College and Hospital, Aurangabad, Maharashtra, India.

[4]M.D.S, Conservative Dentistry and Endodontics, C.S.M.S.S Dental College and Hospital, Aurangabad, Maharashtra, India.

[5]M.D.S, Conservative Dentistry and Endodontics, C.S.M.S.S Dental College and Hospital, Aurangabad, Maharashtra, India.

[6] M.D.S, Department of Conservative Dentistry and Endodontics, C.S.M.S.S Dental College and Hospital, Aurangabad, Maharashtra, India.

Corresponding Author: Dr. Laxmikant Arun Late, Department of Conservative Dentistry and Endodontics, C.S.M.S.S Dental College and Hospital, Aurangabad, Maharashtra, India.

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Abstract

AIM: To evaluate the effect of two different anti-oxidants on the bond strength of resin cement to root canal dentin treated with sodium hypochlorite and Ethylenediaminetetraacetic acid.

Materials and Methods: Forty freshly extracted permanent single rooted teeth were selected for the study. Teeth were sectioned at cemento-enamel junction and then instrumented. Based on the irrigation protocol, four groups were formed as: group I: (n-10) Root canals were irrigated with saline. [Negative control], group II: (n-10) Root canals were irrigated with 5% NaOCl and 17% EDTA for 10 minutes. [Positive control], group III: (n-10) Root canals were irrigated with 5%NaOCl and 17% EDTA, followed by 5% pine bark solution for 10 minutes, group IV: (n-10) Root canals were irrigated with 5% NaOCl and 17% EDTA, followed 10% lycopene solution for 10 minutes. Canals were coated with self-etch dentin bonding agent and filled with dual cure resin cement. 2mm thick coronal part of the specimens were sectioned, and prepared for tensile testing of root canal-resin interface.

Results: The results demonstrated that 5% NaOCl caused significant reduction (P<0.05) in the resin-dentin bond strength, but this can be reversed by using natural anti-oxidants significantly.

Conclusion: Anti-oxidants used restored the lost bond strength of resin cement to root canal dentin treated with

sodium hypochlorite and ethylenediaminetetraacetic acid. Pine bark showed the maximum bond strength among all the groups.

Keywords: Dentin bond strength; pine bark; proanthocyanidin; lycopene; resin cement; micro-tensile test.

Introduction

The fundamental goals of endodontic therapy are to achieve successful cleaning and shaping of root canals and an adequate apical and lateral seal. An ideal root canal sealer should adhere to both dentin and the core filling material. Different types of sealers are used in endodontic treatment like zinc oxide eugenol, glass ionomer, calcium hydroxide and resin based sealers etc. Resin based sealers have been proved to be more effective in sealing root canal systems with improved bond to radicular dentin as well as core material than any other sealer. [1] Advances in adhesive systems have lead to the use of resin cements as intracanal secondary seal and for endodontic post cementation. [2,3] The development of the microtensile testing method permits the measurement of resin bond strengths to radicular dentin. [4,5]

The bond between resin and dentin is achieved by micromechanical interlocking and this interlocking is improved by removal of smear layer and exposure of collagen. [6] To achieve this, various chemical irrigants are used, like sodium hypochlorite [NaOCl] and ethylenediaminetetraacetic acid [EDTA]. These irrigants are most commonly used in endodontic therapy, as they are effective in removing micro-organisms, debris, smear layer and makes instrumentation more efficient. NaOCl and EDTA are known to have negative influence on bond strength of resin cements to dentin. [7-11] Both, concentrations as well as time of contact of NaOCl have influence on collagen degradation of dentin, which makes endodontically treated teeth susceptible to fracture. [12] Both the irrigants, alter the dentin composition and also affect the interaction with resin based restorative material. This loss of bond strength could be detrimental to the entire root canal therapy. So, to prevent this loss of bond strength, it could be reversed with the use of anti-oxidants. Various anti-oxidants, like ascorbic acid, tannic acid & gallic acid are known to increase mechanical properties of dentin and increase the resin dentin bond, by enhancing polymerization and deeper penetration of sealer, which in turn leads to better seal of root canal space. [7-11] Naturally occurring anti-oxidants like, Lycopene, is known to have anti-oxidising properties. [13] Pine bark contains, oligomeric proanthocyanidine compounds, (OPCs) which are powerful anti-oxidants. [14] Hence, aim of this study is to evaluate the effect of Lycopene & Pine bark on the bond strength of resin cement to root canal dentin previously treated with sodium hypochlorite and ethylenediaminetetraacetic acid (EDTA).

Materials and Method

Forty freshly extracted human permanent single rooted teeth were selected for the study. **Inclusion criteria**: Single rooted teeth with single canal, Teeth with fully formed apex. **Exclusion criteria:** Carious teeth & root caries, Teeth with root fracture & immature apex. Teeth collected were cleaned and sterilized according to occupational safety and health hazards (OSHA norms) and stored in saline until further use. Teeth were then sectioned at cemento-enamel junction using a diamond disc at low speed. Patency was confirmed by inserting size 10 k file. (Mani, Tochigi, Japan)

Preparation of Anti-Oxidant Solutions

Two solutions were prepared, First, 5g of pine bark extract powder (Navchetana Kendra, health care private limited, delhi, india) dissolved in 100 ml of distilled water

to make 5% pine bark solution. Second solution, 10g of tomato seed extract powder (Navchetana Kendra, health care private limited, delhi, india) dissolved in 100 ml of distilled water to make 10% lycopene solution.

Root Canal Preparation

Forty teeth were then randomly distributed into four groups containing 10 teeth in each group based on the irrigation protocol. Canal orifice were enlarged using gates glidden drills (Mani, Tochigi, Japan) in a slow speed contra-angle hand piece. Root canals were then instrumented and enlarged with endodontic K files upto size 70 K files. All instrumentations were performed with copious irrigation based on irrigation protocol.

Based on the irrigation protocol, four groups were formed as:

Group I: (n-10) Root canals were irrigated with normal saline for 10 minutes. [Negative control]

Group II: (n-10) Root canals were irrigated with 5% NaOCl (Vishal dentocare pvt. Ltd. Gujrat, india) followed by 17% EDTA (Dent wash, prime dental products pvt. ltd.) for 10 minutes. [Positive control]

Group III: (n-10) Root canals were irrigated with 5%NaOCl and 17% EDTA for 10 minutes, then rinsed with 10ml of water and followed by rinse with 10 ml of 5% pine bark solution for 10 minutes

Group IV: (n-10) Root canals were irrigated with 5% NaOCl and 17% EDTA for 10 minutes, then rinsed with 10ml of water and followed by rinse with 10 ml of 10% lycopene solution for 10 minutes.

All the canals in group I to IV were then rinsed with 10ml of water and then dried with paper points.

Etching of canals: Canals were etched with 37% phosphoric acid, rinsed and dried. **Bonding:** Canals were coated with self-etch dentin bonding agent (3M ESPE Adper Easy bond, Germany) according to manufacturer's instructions and cured. **Sealing of canals:** canals were then filled with dual cure resin cement (3M ESPE RelyX Ultimate, Germany) with the help of lentulo-spirals. (Mani, japan) **Curing:** The resin cement was then light cured and kept undisturbed for 5 minutes. Filled specimens were stored in water for 24 hours to prevent it from dehydration.

Sectioning: Specimens were then sectioned into coronal, middle and apical third by using a diamond disc, 2mm thick sections were made from coronal third section with the help of diamond disc. Then mesial and distal dentin portion of all slabs (sections) were cut away. The specimens were then glued to a custom-made jig with cynoacrylate resin and secured in universal testing machine (TUF-C-1000 SERVO) to permit for tensile testing of the root canal-resin interface. Each specimen was then stressed in tension at 1 mm/min until failure occurred on either side of the resin bonded canal. The load at failure was divided by the surface area to obtain the failure stress that was expressed in MPa.

Statistical Analysis

Mean and standard deviation were estimated for each study group. Mean and standard deviation were estimated 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 values for each group were tabulated, and the mean and standard deviation were calculated for each of the groups [Table 1]. The bond strength is maximum in Group III Pine bark (32.96 ± 5.75), followed by Group IV Lycopene (25.32 ± 3.09) and Group I Saline (24.24 ± 4.44). Group II NaOCl (12.15 ± 4.21) exhibits the least bond strength among all the other groups.

Table - 1

GROUPS	N	MEAN (MPa)	SD
GROUP I	10	24.24	4.4431
GROUP II	10	12.15	4.2194
GROUP III	10	32.96	5.7554
GROUP IV	10	25.32	3.0951

The *f*-ratio value is 36.90749. The *p*-value is < .0001. The result is significant at $p < .05$.

Discussion

Successful endodontic therapy depends on three-dimensional obturation as well as sufficient chemomechanical preparation of root canal system. Mechanical instrumentation of root canals leaves a smear layer on the canal dentinal walls. Alternating use of ethylenediaminetetraacetic acid (EDTA) and 5% NaOCl has been proved to be efficient in removing smear layer. [6,15] The excellent sealing ability of contemporary adhesive resins makes them useful both as secondary intracoronal diffusion barriers and for cementing endodontic posts. [2,3]

Debonding is still considered one of the important reason for the failure of intraradicular fiber posts. This type of failure is related to the degradation of exposed collagen that was incompletely covered by the adhesive. [10] Chemical substances used as irrigation during instrumentation might affect the dentin substrate. One of the disadvantages of this irrigant is that it interferes with the polymerization of bonding resin including root canal sealers because of residual oxygen left in the dentinal tubules after irrigation. Sodium hypochlorite breaks down to sodium chloride and oxygen. Oxygen from such chemicals causes strong inhibition of the interfacial polymerization of resin bonding materials, free radicals inhibit the polymerized reaction, resulting in premature chain termination and incomplete polymerization. [8,9,10] The generation of oxygen bubbles at the resin-dentin interface may also interfere with resin infiltration into the tubules and intertubular dentin. This decreases the bond strength of resin obturating materials with root canal dentin and also the retention of the intraradicular fibre post. [2,3]

Ascorbic acid and its salts such as sodium ascorbate both are potent anti-oxidants and can neutralize the adverse effects of oxidative solutions *via* redox reactions. This antioxidant with its radical scavenging activity has the ability to remove residual oxygen and reverse the effect of sodium hypochlorite on root dentin, thus leading to better sealer penetration into the dentinal tubules and is thought to aid the polymerization of the dentin methylmethacrylate /polymethylmethacrylate resin. [7-11] In this study two antioxidants used are, the prominent carotenoid in serum is the antioxidant red pigment called lycopene. Lycopene has the unique feature of getting bound to chemical species that react to oxygen, thus being the most efficient biological antioxidizing agent. [13] Second is naturally occurring antioxidants such as pine bark, contain oligomeric proanthocyanidin complexes (OPC). Proanthocyanidins (PAs) have free radical scavenging activity. PAs found in pine bark are high molecular weight polymers. As a naturally occurring plant metabolite, PAs have been proven to be a safe antioxidant in various clinical applications and dietary supplements. [14]

The present study observed the effect of cross-linking agents after 10 min of treatment, which is much more clinically feasible, so a less time-consuming treatment can

be possible and achievable with the increased agent concentration. In this study, regional bond strengths of resin cement to root canal dentin walls and the effect of NaOCl were evaluated. To simplify the laboratory procedures, the root canals were filled with resin cements. We do not advocate the use of resin cement as a root canal filling material, rather, we elected to use the resin cement without endodontic posts to simplify the experimental design and to permit the evaluation of resin-dentin bonds without the complication of resin post bonds. In this study, we used the microtensile bond strength test because the main advantage of this test is that failures mainly occur in the adhesive, because the dispersion of forces improves over small surfaces. When adhesive failure occurs, it is easier to evaluate the real bond strength of an adhesive. [16]

Study confirms that treatment with **5% NaOCl** significantly decreases bond strength compared with Saline. According to the results of this study, there is a statistically significant increase in the bond strength by pine bark and lycopene, compared with 5% NaOCl which indicates that they reverses the lost bond strength. There was a significant difference ($P<0.05$) in the bond strength between 5% Pine bark (group-III) and saline (group-I) and also 5% Pine bark (group-III) and 10% lycopene (group-IV). It was also found that there was no significant difference ($P>0.05$) in the bond strength between 10% lycopene (group-IV) and saline (group-I), which implies that lycopene reverses the bond strength comparable with the normal.

In this study, 5% **Pine bark** (group-III) showed the maximum bond strength among all the groups. The probable reason may be that the antioxidant ability of Pine bark is more than lycopene, and also it can be quickly and completely absorbed. NaOCl treatment removes exposed organic matrix type-1 collagen or soft tissue from the dentin and leaves a mineralized surface less receptive to bonding with this resin cement. PA is a natural collagen cross linker that can increase collagen synthesis and decrease the degradation of collagen. Collagen is strengthened by the formation of native cross links which provides the fibrillar resistance against enzymatic degradation as well as greater tensile properties. PAs enhance the mechanical properties of dentin matrix which increase the properties of dentin-resin bonds. [17]

Conclusion

Within the limitations of this *in vitro* study it was observed that NaOCl irrigation compromised the bond strength between the adhesive and dentin. Pine bark contain PAs improves the bond strength significantly more than lycopene due to its high antioxidant and collagen crosslinking ability. More research would be required to confirm the concentration and the time of irrigation of antioxidants used.

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