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Comparative Evaluation of Different Antioxidants on Reversal of Microtensile Bond Strength of Composite Resin in Endodontically Treated Tooth Surface

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ABSTRACT

Introduction: The use of Sodium Hypochlorite (NaOCl) as an endodontic irrigant changes the properties of dentine and lowers the bond strength of resin cements to the dentine, but it can be reversed by the use of antioxidants.

Aim: To evaluate the effect of antioxidants for restoring adhesion potential/reversal of microtensile bond strength of dentin in the pulp chamber treated with NaOCl and Ethylenediaminetetracetic Acid (EDTA).

Materials and Methods: This is a laboratory based experimental in-vitro study in which 40 freshly extracted human incisors were selected and cut to expose the pulp chamber below the dentine. The specimens were distributed among five groups: Group I samples were irrigated with 5.25% NaOCl for 30 minutes followed by 17% EDTA for 3 minutes and final rinse was done with 5.25% NaOCl (Positive control). Group II samples were treated with distilled water only (Negative control). In Group III, samples were treated same as in group I followed by 10% Sodium Ascorbate (SA) for 10 minutes. Samples of group IV were also treated as in group I followed by application of 5% solution of Alpha tocopherol for 10 minutes. Group V samples were treated same as in group I followed by a rinse with 5% solution of Sodium Thiosulphate ($Na_2S_2O_3$) for 10 minutes. Composite resin build up was performed and interface was tested by using Universal testing machine for Microtensile bond strength. The resulting data was analysed by one-way Analysis of Variance (ANOVA) and Mann-Whitney U test ($p<0.05$).

Results: The results demonstrated that irrigation with 5.25% NaOCl and 17% EDTA produce significant ($p<0.05$) reduction in resin dentin bond strength but this could be reversed by application of 5% $Na_2S_2O_3$. Highest bond strength was observed in group V (22.38±0.84) and lowest for group I (15.38±1.22).

Conclusion: Dentin bond strength was significantly reduced when bonding was performed immediately after use of NaOCl. Use of antioxidants after final irrigation with NaOCl can be recommended for better bonding of composite resin.

Keywords: Root canal, Sodium hypochlorite, Sodium thiosulfate

INTRODUCTION

The goal of successful endodontic therapy is complete debridement of the root canal to remove inflamed and necrosed pulp tissue and achievement of apical as well as coronal hermetic seal [1]. Furthermore, immediately after the endodontic treatment coronal leakage is prevented by using permanent post-endodontics restoration, out of which restorative resin is most commonly used [2-4]. Endodontically treated tooth restored with composite resin showed better fracture resistance when compared to amalgam because of its ability to form a hybrid layer with dentine [5-7]. NaOCl is frequently used as a root canal irrigant because of its ability to dissolve the organic matter along with its antimicrobial property [8]. Nevertheless, this irrigant may affect the interaction of dentine to the adhesive restorative materials.

The bond strength of restorative resin decreases after using NaOCl due to release of residual oxygen which interferes with polymerisation of resin monomer that ultimately affect the dentine permeability, micro hardness and fracture resistance [9]. To minimise these adverse effects antioxidants can be applied before final restoration to neutralise and repeal of oxidising effect of NaOCl on dentine surface [10,11]. In dentistry Sodium Ascorbate (SA) is a commonly used antioxidant. SA improves the bond strength by neutralising and reversing the oxidising effect of NaOCl treated dentin [12].

Alpha tocopherol (vitamin E) an alternative of SA which is neutral, biocompatible and lipid soluble antioxidant. The ability of alpha tocopherol allowes polymerisation of the free radical and reverses the negative effect of NaOCl and improves resin bonding in endodontically treated teeth [13].

Recently, sodium thiosulfate has been used as an antioxidant to attain neutralisation of free radicals produced from NaOCl and also to restore the bond strength of composite resin to dentine [14]. It has some capability to neutralise the oxidising effect of free radicals on the applied surface by redox reaction thus, assisting in complete polymerisation of composite resin. Furthermore, it can react with oxidants to neutralise unpaired electrons and form a stable product. Sodium thiosulfate has been used in many microbiological and clinical studies to neutralise NaOCl indicating its biocompatibility. Thereby making it interesting to use it as an antioxidant to recover the bond strength lost because of endodontic irrigating materials [15].

There is evidence of effects of ascorbic acid solution on NaOCl and H_2O_2 treated dentin [16]. Limited research has been done to analyse the effect on bond strength to sodium thiosulfate treated root canal dentine [17]. Hence, the present study was conducted.

A null hypothesis was formulated stating that there is no difference in the bond strength to dentin treated with root canal irrigants and use of SA, alpha tocopherol and $Na_2S_2O_3$ would not affect the bond strength to dentin after root canal irrigation with NaOCl.

MATERIALS AND METHODS

This in-vitro study was done in the Department of Conservative Dentistry and Endodontics, King George's Medical University Lucknow, Uttar Pradesh, India from October 2018 to March 2019 and bond strength was tested at Central Institute of Plastics Engineering and Technology (CIPET) Lucknow. After taking the ethical approval from the Institutional Ethical Committee

(Ref.no. 77th ECMII-B IMR faculty/p16) study was carried out on extracted human teeth.

Sample size calculation: The sample size was calculated by an online statistical calculator Dhand NK and Khatkar MS (2014) (http://statulator.com/SampleSize/ss2P.html) for comparing two independent mean and the power of the study was 80% [18].

Forty extracted human maxillary incisors were taken for this study that were extracted for periodontal and prosthodontic reasons and were stored in 0.2% thymol and used within 6 months. The crowns of teeth were separated from the root at cemento-enamel junction using a diamond disc. The crowns were cut into two halves mesio-distally. The pulp tissue was removed and dentin surface was flattened with 180-grit and 600-grit silicon Carbide (3M Imperial) paper under running water for 30 seconds to standardise the smear layer of the dentin surface.

Samples were then divided into five groups according to the antioxidant used by simple randomisation method. (n=8). In negative control group no treatment with NaOCl (Vishal Dento care Pvt., Ltd.,) or EDTA (Meta Biomed) was done, rest all samples were treated with 5 ml of 5.25% of NaOCl for 30 minutes according to the protocol described by Correa ACP et al., and then immersed in the 17% EDTA for three minutes following which final rinse was performed with 5% NaOCl for one minute [15]. In Positive control group composite resin was bonded to NaOCl and EDTA treated tooth surface immediately without using any antioxidant. For antioxidant treatment $Na_2S_2O_3$ (Thermo fisher scientific India Pvt., Powai, Mumbai) group specimens were immersed in the 5 mL of 5% solution of $Na_2S_2O_3$. For α tocopherol (sigma- Aldrich, Bangalore, India) group, α tocopherol group was dissolved in the ethanol to prepare 5% of solution of alpha tocopherol and SA (Rolex chemical industries, Mumbai, India) was dissolved in the water to produce the 5% of the solution of SA. Samples were immersed in solution for 10 minutes according to groups divided [Table/Fig-1].

Groups	Irrigation protocol	Antioxidant procedure
I	5.25% NaOCl for 30 minutes + 17% EDTA for 3 minutes + 5.25% NaOCl for 1 minute	No antioxidant
II	No irrigant	No antioxidant
III	Same as in Group I	5 mL of 5% solution of SA
IV	Same as in Group I	5 mL of 5% solution of tocopherol
V	Same as in Group I	5 mL of 5% solution of $Na_2S_2O_3$

[Table/Fig-1]: Experimental groups according to irrigation protocol and antioxidant used.

Group I (Positive control): Dentin surface was irrigated with 5 mL of 5.25% NaOCl for 30 minutes and then immersed in 17% EDTA for three mintues then final rinse done with 5.25% NaOCl for one minute.

Group II (Negative control): No irrigation and no antioxidant was used.

Group III: After treatment as in group 1, freshly prepared 5 mL of 5% SA (Rolex chemical industries, Mumbai, India) was applied to the dentin surface for 10 minute [16].

Group IV: After treatment as in group I, 5 mL of 5% solution of Alpha Tocopherol (Sigm-aldrich, Bangalore, India) was applied to the dentin surface for 10 minutes.

Group V: After treatment as in group I, 5 mL of 5% solution of $Na_2S_2O_3$ (Thermo fisher scientific India Pvt., Powai, Mumbai) was applied to the dentin surface for 10 minute.

Bonding procedure: After pre-treatment with antioxidants, surfaces of all the specimens were dried with absorbent papers, then treated sections from each group were mounted on the acrylic block with dentine surface facing upwards [Table/Fig-2]. Tooth Surface was etched with 37% of phosphoric acid (Dentsply Caulk, Int. Milford DE, USA) for 20 seconds and bonding agent (Te-Econom Bond, Ivoclar Vivadent) was applied on the surface for 10 seconds and light cured for 20 seconds. Ryle's tube of 18 gauge is cut to produce segments of 3 mm length and blocks of composite resins (Te-Econom plus Ivoclar Vivadent) were prepared over dentin surface and each one was light cured for 20 seconds with curing light (Ivoclar Vivadent Bluephase N). The blocks were stored in distilled water at 37°C for 24 hours before testing [13].

[Table/Fig-2]: Composite resin build-up on dentin surface.

Bond strength measurement: Microtensile bond strength was measured by subjecting the specimens to the universal testing machine (Instron 3340, Instron Co., Canton, MA, USA) operated at the crosshead speed of 1 mm/minute and 5.00 N (Newton) load was applied until failure had occurred. Experiment for the measurement of Bond strength was done by author under supervision of Scientist of CIPET. It was the machine based reading so no issues of inter-examiner reliability occurred.

STATISTICAL ANALYSIS

The data was evaluated using Statistical Package for Social Sciences (SPSS) Software Version (19.0). Under the normal distribution, the data was analysed by one-way ANOVA (p=0.05). Comparison of Microtensile bond strength between the groups was explained by Mann-Whitney U test.

RESULTS

Statistical analysis revealed that there was a significant difference between the groups ($p<0.05$) thus null hypothesis was rejected. The mean Microtensile bond strength of different groups are summarised in [Table/Fig-3]. The positive control (Group I) exhibited the lowest mean bond strength value (15.38±1.22 MPa), closely followed by the group IV (17.90±1.54MPa) and highest mean value by the $Na_2S_2O_3$ Group V (22.38±0.84).

Groups	Mean±SD (Mpa)
I	15.38±1.22
II	21.49±1.48
III	19.36±1.39
IV	17.90±1.54
V	22.38±0.84

[Table/Fig-3]: Mean and standard deviation of micro-tensile bond strength of different groups (MPa).
SD: Standard deviation

The Mann-Whitney U test revealed that p value in Groups I vs II, I vs III, I vs IV and I vs V was statistically significant at $p < 0.05$. However, it was not significant between Groups III vs IV and Group II vs V [Table/Fig-4,5]. Intergroup comparison of mean microtensile bond strength between Group III, IV and V is shown in [Table/Fig-5].

Groups	Z	p-value
I vs II	3.31	0.00094
I vs III	3.31	0.00094
I vs IV	2.47	0.01352
I vs V	3.31	0.00094

[Table/Fig-4]: Comparison of Microtensile bond strength in different groups using Mann-Whitney U Test.
(p<0.05 is statistically significant, p<0.001 is statistically highly significant)

Groups	Z	p-value
II vs V	1.103	0.27134
III vs IV	1.418	0.1556
III vs V	3.20	0.00138
IV vs V	3.31	0.00094

[Table/Fig-5]: Intergroup comparison of Microtensile bond strength in different groups using Mann-Whitney U Test.
(p<0.05 is statistically significant, p<0.001 is statistically highly significant) NS=Not significant

DISCUSSION

This study is one of the few studies that evaluate the resin bond strength to dentin and pulp chamber after the use of common endodontic irrigants, and it is the first to assess the comparison of three different antioxidant like sodium thiosulfate, SA, and alpha tocopherol to restore the lost dentin bond strength. The methodology was adjusted to compare the effect of different antioxidants on the area of the tooth surface which is fully affected by chemical substance during endodontic treatment [19].

Previous researches [20-24] as in [Table/Fig-6] have shown the detrimental effect of different root canal irrigants on the bond strength of postendodontic restorative resin cement to the radicular/coronal dentin. In a study done by Erdemir A et al., they found that 3% hydrogen peroxide significantly lowered the bond strength [25].

of the treated substrate and facilitating the complete polymerisation of adhesive material [28]. On the other hand it can react with antioxidants to neutralise unpaired electrons and form a stable product [29]. Then, it becomes important to use this antioxidant substance to recover the bond strength lost due to the use of endodontic irrigants. $Na_2S_2O_3$ can be used up to 6 months when stored in a refrigerator [30].

SA is capable of reducing a variety of oxidative compounds, particularly free radicals [16]. It is used just immediately after preparation because of its short shelf life. The antioxidant ability of SA can help to neutralise and reverse the oxidising property of NaOCl. In the present study, SA allows free-radical polymerisation of resins to proceed without premature termination hence reverse the action of NaOCl [10].

In case of bleached enamel, Suneetha R et al., studied 10% SA solution as an antioxidant for restoring the shear bond strength immediately after bleaching [31]. Subramanian R et al., observed that after application of 10% SA just after beaching could neutralize the residual oxygen and restore the bond strength [32]. In another study it was also observed that in scanning electron microscopic images ascorbic acid causes excessive etching of bleached enamel surface [12].

Vitamin E is the term used for a group of tocopherols and tocotrienols, of which α tocopherol has the highest biological activity. Vitamin E functions as a chain-breaking antioxidant that prevents propagation of free radical reactions [33]. Vitamin E (alpha-tocopherol), same as Ascorbic acid restored the bond strength by removing the free radicles molecular oxygen. It has recently been suggested for improving composite bonding with coronal dentine after endodontic treatment [33].

Results of this study showed that group V and group II (Negative Control) have higher bond strength followed by group III and

Author and Year	Test modality	Antioxidants agent used	Application time	Concentration of NaOCl/ EDTA used	Results
Lai SC et al., 2001[9]	Microtensile bond strength	10% sodium ascorbate solution	10 minutes	5% NaOCl	Effective
Soeno K et al., 2004 [19]	Tensile bond strength	10% ascorbic acid	15-60 seconds	10% NaOCl	Effective
Weston CH et al., 2007 [11]	Tensile bond strength	10% ascorbic acid	10 minutes	5.25% NaOCl	Effective
da Cunha LF et al., 2010 [21]	Push out bond strength	10% ascorbic Acid	10 minutes	5% NaOCl	Effective
Manimaran VS et al., 2011[24]	Microtensile bond strength	10% sodium ascorbate	10 minutes	5.25% NaOCl	Effective
Prasansuttiporn T et al., 2011 [27]	Microtensile bond Strength	10% sodium ascorbate Solution/ Rosmarinic acid solution	5-10 minutes	6% NaOCl	Effective
Khoroushi M and Kachuei M [20]	Tensile bond strength	10% sodium ascorbate Hydrogel	2 minutes	2.5% NaOCl	Effective
Stevens CD 2014 [22]	Shear Bond Strength	10% sodium ascorbate	1 minutes	6% NaOCl	Effective
Gonulol N et al., 2015 [16]	Microtensile Bond strength	10% sodium ascorbate solution	10 minutes	5.25% NaOCl	Effective
Ebrahimi-Chaharom ME et al., 2015 [23]	Shear bond strength	10% sodium ascorbate	10 minutes	5.25% NaOCl	Effective
Correa ACP et al., 2016 [15]	Microtensile bond strength	0.5% or 5% sodium thiosulfate	1, 5 or 10 minutes	5.25% NaOCl	Effective
Bharti R and Chandra A (Current Study)	Microtensile bond strength	5% solution of sodium ascorbate, sodium thiosulfate and Alpha tocopherol	10 minutes	5.25% NaOCl	Effective

[Table/Fig-6]: Description of in-vitro studies using different antioxidants/reducing agents to revert the decrease in bond strength after use of NaOCl/EDTA in root canal wall dentin [9,11,15,16,19-24,27].

Use of NaOCl on the surface of coronal dentine causes changes in the mechanical properties of dentin, such as micro hardness, elastic modulus, and fracture resistance that might produce a "new" dentin surface and this may be due to damage caused to the organic matrix, collagen, and finally leaving mineralised coronal dentine surface [26]. Decreased bond strength is the result of the oxidising action of NaOCl. It may be possible for the compromised bond strength to be reversed by a reduction of the oxidised surfaces with an antioxidant such as $Na_2S_2O_3$, SA, and alpha tocopherol [27].

$Na_2S_2O_3$ has been used in many microbiology studies to neutralise NaOCl and it is a potent antioxidant. It is possible that it has the potential to neutralise the oxidising agents through redox reaction

Group IV. SA also reversed the bond strength in this study which is supportive to the previous study [34]. Alfa tocopherol was used as a gel in this study. It also resulted in the bond strength reversal but less than in group III (SA). This may be due to the nonaqueous nature of this antioxidant.

Limitation(s)

This study did not evaluate the bond strength before the use of anti-oxidants. As this is an invitro study, clinical evaluation is necessary to determine the efficacy of different antioxidants in restoring the microtensile bond strength. Sodium ascorbate has a short shelf-life so it can not be stored for a long time, it should be prepared freshly whenever it is to be used for better results.

CONCLUSION(S)

The reversal effects of $Na_2S_2O_3$, ascorbic acid and alpha tocopherol on the compromised bond strength in NaOCl/EDTA-treated dentin using the total etching adhesive system were found to be satisfactory. Nevertheless, the use of 5% $Na_2S_2O_3$ for 10 minutes showed the best result compared to ascorbic acid and alpha tocopherol. These findings are clinically important/significant because they suggest SA, alpha tocopherol and $Na_2S_2O_3$ can be used for reversal of bond strength of post-endodontic restorative resin to root canal treated dentin surface. Considering the available literature and the limitations of each antioxidants under consideration, none of them completely reverse the bond strength to its original. So, development of new antioxidants will help in safe and effective use before the postendodontic restoration.

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