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In Vitro Comparative Evaluation of Reducing Agents for Recovery of Bond Strength to Dentin Treated with Sodium Hypochlorite

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Abstract: ***The aim of the study is to evaluate the anti- oxidant efficacy of sodium thiosulphate & sodium ascorbate on the recovery of shear bond strength to pulp chamber dentin treated with NaOCI & EDTA. Methodology: 40 dentin samples were divided in 4 groups (n=10); Positive control received no treatment; negative control received only irrigation treatment, while the other two experimental groups were treated with their respective anti-oxidant following the irrigation. All the samples were then cured with composite and subjected to SBS test under universal testing machine. Statistical analysis: ANOVA & Post hoc tukey were used with P <0.5. Results: Both the anti- oxidant groups showed greater mean SBS value than the negative control, comparable to positive control. Sodium ascorbate group showed the highest value with significant difference with the negative control group. Conclusion: Both the anti-oxidants used were able to restore the SBS of pulp chamber dentin altered due to endodontic irrigants. Hence, possesses a great potential in its clinical implication.***

Keywords: *Anti-oxidant, shear bond strength, sodium hypochlorite, sodium thiosulfate, sodium ascorbate, ascorbic acid*

1. Introduction

The successful outcome of endodontically treated teeth relies not only on the apical seal but also on the coronal seal. Coronal leakage is one of the negative contributors to the prognosis of endodontic treatment.[18] Thus, immediate sealing of endodontically treated teeth is very essential to prevent coronal leakage. Recently, adhesive restorative materials are preferred for its obvious advantages over the conventional ones, such as bonding to dentin by the hybrid layer and reduced marginal leakage. Also, teeth restored with resin composite are better resistant to fracture than those restored with amalgam because their physical-mechanical properties are closer to those of dentin.[1]

Root canal irrigants used during biomechanical preparation may alter the dentin structure and affect its interaction with composite resin. Sodium hypochlorite (NaOCl) is a gold standard in endodontic irrigation, and so it is widely used as a chemical irrigant for endodontic therapy along with other irrigants like hydrogen peroxide & EDTA. These chemical substances have a negative effect on the dentin properties, which does not appear to improve over the period of time.[3`4] Whereas, this reduced bond strength to NaOCl-treated dentin could be restored by the application of an antioxidant on the dentin surface.[7] Ascorbic acid and its sodium salts (eg. Sodium ascorbate) are widely studied anti-oxidants that are capable of reducing oxidative products.[2] Sodium thiosulfate is another anti-oxidant that has been used in medicine and as a neutralizing agent of NaOCl in microbiological analysis.[1] Previous studies reported these materials exhibiting potential to be used as reducing agents for NaOCl-treated dentin without damaging biological tissues.

This in vitro study aimed to evaluate the antioxidant efficacy of sodium ascorbate & sodium thiosulphate on the recovery of the shear bond strength to pulp chamber dentin treated with NaOCl and EDTA. In this study, the effect of 10% sodium ascorbate & 5% sodium thiosulphate solution on dentin bond strength after treatment with root canal irrigants were tested. The null hypothesis was no difference would be seen in sodium ascorbate & sodium thiosulphate in reversing the bond strength to dentin compromised by NaOCl and EDTA.

2. Materials and Methods

Specimen Preparation

Forty extracted human anterior teeth were stored in 0.1% thymol before use. The dentin area of the buccal middle third was chosen as a sample. The crown portion was cut down horizontally exposing the incisal portion with a double-sided diamond disc under running water (Image-1). Then the disc penetrated the tooth centrally and longitudinally (Image-2). The depth of penetration was determined by placing the mandrel in the tooth. The last cut was made onto the buccal section perpendicular to the long axis of the tooth, 10 mm from the incisal edge till it reaches the cut previously made (Image-3,4). Any pulp tissue remnant was excavated using a spoon excavator. The specimens of intracoronary dentin were then treated to standardize the smear layer with 600-grit SiC papers under running water for 30 seconds (Image-5). The specimens were randomly divided into 4 groups (n=10).

Group A – Negative control
Group B – Positive control
Group C – Sodium thiosulphate
Group D – Sodium ascorbate

Image : 1

Image : 2

Image : 3

Image : 4

Image : 5

Irrigation Regime

The Negative control group was immersed in 0.9% sodium chloride (NaCI) for 10 minutes without any treatment with NaOCI/EDTA, whereas the Positive control & two experimental groups were immersed in 5.25% NaOCl for 30 minutes (solution was replenished every 10 minutes), 17% EDTA for 3 minutes followed by a final washing with NaOCl for 1 minute. Further, the two experimental groups received the anti-oxidant treatment with immersion in 5 mL 5% Na2S2O3 & 10% Ca_6 H $_7$ NaO_6 solution for 10 minutes according to the respective groups.

	Group A Negative Control	Group B Positive Control	Group c Sodium thiosulphate	Group d Sodium ascorbate
Samples (N)	10	10	10	10
Irrigation Regime	0.9% NaCl for 10 min	5.25% NaOCl for 30 min + 17% EDTA for 3 min + 5.25% NaOCl for 1 min		
Anti-Oxidant Protocol	NO	NO	5% $Na_2S_2O_3$ for 10 min	10% Ca_6 H $_7$ NaO_6 for 10 min

Adhesive Procedure

Immediately after the irrigation protocol, the adhesive procedure was carried out. Specimens were dried with absorbent papers and etched with 37% phosphoric acid for 20 seconds. A total-etch/ Self-etch adhesive system (Single bond Universal; 3M ESPE, St Paul, MN) was applied to the pulp chamber dentin surface according to the manufacturer's instructions. 2 mm layer of a resin composite (Filtek Z250, 3 M/ESPE) was incrementally cured for 20 sec to the bonded dentin, using a LED curing unit operating at 1200 mW/cm² (Image-8). After the adhesive procedures, the sample blocks were stored in distilled water at 37˚C for 24 hours.

Shear Bond Strength

After 24 hours of water storage, the sample blocks from each group were dried and fixed to an acrylic cylindrical slab to allow for shear bond test under universal testing machine (ACME Engineers, India; Model No- Unitest 10). Each slab was attached individually to a testing apparatus and then submitted to a shear load in a universal testing machine at a crosshead speed of 1 mm/min until failure (Image-9). The load applied was measured in Newton (N) & the shear bond strength values in Megapascals (MPa).

Statistical Analysis

The shear bond strength was determined and analyzed by One-way analysis of variance. The statistical significance level was set at $P < .05$.

Image-6
(Sodium thiosulfate)

Image-7
(Sodium ascorbate)

Image-8

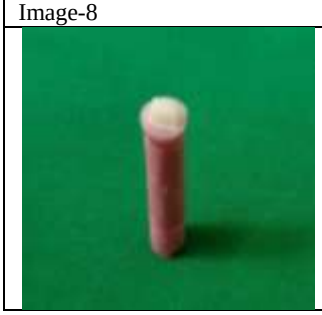

Image-9

3. Results

The mean and standard deviations of shear bond strength for groups are as listed below in the table. The highest & lowest mean shear bond strength was seen in sodium ascorbate group & positive control, respectively. No statistical significant difference was seen between the tested anti-oxidants. Also, No statistical significant difference was seen between the negative control & the experimental groups. But the difference was significant between the positive control & sodium ascorbate group.

The *f*-ratio value is 3.262. The *p*-value is 0.032. The result is significant at $p < 0.05$.

Group	N	Mean (Mpa)	Sd	Max. Sd	Min. Sd
Group A	10	24.68	7.601	32.281	17.079
Group B*	10	18.29	6.105	24.395	12.185
Group C	10	22.55	5.361	27.911	17.189
Group D*	10	26.06	4.170	30.230	21.890

* Superscript groups shows significant difference between them.

4. Discussion

The objective of the study was to check the efficacy of anti-oxidant to rejuvenate the bond strength, lost due to endodontic irrigants. The results achieved support the hypothesis. It could be attributed to the redox mechanism of anti-oxidants. Also could be, as Weston et al hypothesized the mechanism to be an instantaneous process.[17]

Dentin surface of buccal middle third chosen as specimen as that part of coronal dentin is mostly in contact with the endodontic irrigants with all surface changes occurring. Also the bond strength of the intracoronal dentin is detrimental for the prognosis of post endodontic restoration. The negative control group (NaCI) was added, to determine if the neutralization achieved by Sodium thiosulphate & ascorbate is due to chemical or physical reaction with dentin surface. While, in other groups, replenishing the NaOCI after 10 minutes interval would have allowed for further oxidation of dentin surface leading to difference in the resultant bond strength. Liquid EDTA wash was preferred over the gel form, because it removes the smear layer more effectively than the gel form.[1]

Shear Bond Strength to dentin differs with different adhesive systems. Studies have been performed showing, total-etch adhesives system give better bond strength result than the self-etch systems.[8`11] Also, there is an effect of the composite thickness on the SBS to dentin, 2-mm thick specimen produced greater SBS than the 5-mm thick specimen.[14] Therefore, to avoid this, an incremental technique for the composite core is recommended over the bulk fill

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.[3] Sodium hypochlorite (NaOCI) is still a gold standard as a chemical irrigant for endodontic therapy because of its antimicrobial activity and ability to dissolve organic matter,[1o`12] but it has certain disadvantages like; disrupts the organic matrix of dentin leaving the dentin surface more porous,[3] they liberate oxygen which inhibit polymerization in the adhesive system,[4] also result in incomplete polymerization by premature chain termination by forming protein-derived radicals that compete with the propagating vinyl-free radicals generated by the photoactivation of resin adhesives.[2`4] Ethylene-diamine-tetra-acetic acid (EDTA), a lubricant though it acts on both the organic & inorganic portion of the dentin, causes dentin demineralization & leads to erosion of dentin, eventually causing an impairment of imbrications with the hybrid layer.[15] Thus, the current irrigation protocol regime, reduces the physical properties of dentin like the microhardness, flexural strength, elastic modulus, etc, which does not appear to improve over the period of time.[1] This calls for the need of an anti-oxidant which could reverse the compromising effect of these chemical agents on the dentin bonding substrate.

Sodium thiosulphate ($Na_2S_2O_3$) is an inorganic compound, typically available as pentahydrate, $Na_2S_2O_3 \cdot 5H_2O$. [1] While ascorbic acid and its sodium salts [eg. Sodium ascorbate (Ca_6 H $_7$ nao_6)] are organic compounds, capable of reducing oxidative compounds (free radicals). Studies have reported the use of both the antioxidants to reverse the compromised bond strength of dentin due to endodontic irrigants, by restoring the altered redox potential of the oxidized bonding substrate, prior to resin bonding.[2]

Sodium Thiosulphate is available in crystal form while ascorbate as a powder. The solution for both was derived by dissolving the appropriate amount of solute in 100 ml of distilled water by w/v ratio for desired concentration. In this study, 5% sodium thiosulphate & 10% sodium ascorbate was chosen considering their potent efficacy at the concentration used in their respective studies.[1`2] Sodium thiosulphate solution been inorganic has a better shelf life up to 6 months while that of an organic ascorbate solution is about a month. Both the anti-oxidants, sodium thiosulphate & sodium ascorbate are biocompatible at the concentrations they were used, 5% & 10% respectively. Studies have shown the anti-oxidants exhibit cell viability in a MTT assay (Methyl-Thiazolyl-Diphenyl Tetrazolium Bromide).[1`5] Anti-oxidants were applied for 10 minutes in our study and is considered to be clinically desirable. Anti-oxidants are said to be used for at least one-third of the time of application of oxidizing agents to achieve the desired result (Lai et al).[7]

5. Conclusion

Under the limitations of in vitro study, both the anti-oxidants tested, Sodium thiosulphate & Sodium ascorbate, prove to overcome the bond strength of dentine compromised due to the endodontic irrigants used during root canal therapy. Hence, exhibiting a great potential to be clinically used and thus, promises more better results.

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