

Efficacy of anti-oxidants when used for distinctive time to re-establish bond strength

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Abstract

Introduction: The objective was to evaluate the effect of three antioxidants Sodium thiosulphate, Sodium ascorbate and Rosemarinic acid with different application times in reversing the bond strength of dentine compromised by sodium hypochlorite (NaOCl) and EDTA.

Material and Method: Crowns of bovine incisors were cut to expose the dentine pulp chamber from which 84 (buccal and lingual surface) samples were selected. The specimens were polished and randomly distributed into 6 groups. Each group has n=7 i.e 6 X 7= 42 samples. Group 1:0.9% sodium chloride for 30mins (negative control), Group 2: 5.25% NaOCl for 30mins, 17% EDTA for 3mins and 5.25% NaOCl for 1min (positive control). Groups (3, 4, 5 and 6) were treated in the same manner as positive control. Group 3 samples were neutralized by immersion in an inert solution (saline) for 10mins. The samples of Groups 4, 5, 6 were neutralized with the antioxidant reagents mentioned above. Group 4, 5, 6 were sub-grouped for 3mins, 5mins and 10mins i.e 4a, 4b, 4c 5a, 5b, 5c and 6a, 6b, 6c. The specimens were dried and etched with total adhesive system according to manufactures instructions. Followed by 3 mm build-up of composite resin incrementally to bonded dentine and light cured for 20 seconds. The micro tensile bond strength was determined using universal testing machine and was statistically analysed with independent sample t-Test with 95% confidence limits.

Results: At 3 mins and 5mins rosemary extract performed better than sodium thiosulphate and sodium ascorbate (with P values: 0.000,0.000 and 0.000,0.040). Rosemary extract when used for 3mins and 5 mins showed a significantly better performance than at 10mins (with P values 0.000 and 0.001). At 3 mins and 5mins rosemary did not show any significant difference.

Keywords: Sodium Thiosulphate, Sodium Ascorbate, Rosemarinic acid, Sodium hypochlorite.

Introduction

One of the main objectives of endodontic therapy is to decrease the microbial load and to facilitate periapical healing & prevent reinfection. Bacteria and their products are regarded as the major cause of pulp and peri-radicular diseases.^{1,2} The complex anatomy of root canal spaces and use of various instrumentation techniques alone do not help in achieving the objective of endodontic therapy. This leads to the emphasis on chemical means of cleaning and disinfection of the root canal system.

Among the chemical irrigants sodium hypochlorite is widely and routinely used for endodontic therapy due to its anti-bacterial and organic dissolution properties³ in a concentration ranging from 0.5%-5.25%⁴ but the adverse effect on the resin-dentine bond strength have been previously investigated and confirmed.

An antioxidant is a molecule that inhibits the oxidation of other molecules and terminates the chain reaction such as Beta-carotene, Vitamin E, Thiols or Ascorbic acid (vitamin C). Hence the compromised bond strength could be restored by the application of an antioxidant solution before adhesive procedure, resulting in neutralization and reversal of the oxidizing effect of NaOCl of dentine surface.^{5,6}

Sodium thiosulphate an anti-oxidant has been used in medicine and as a neutralizing agent of NaOCl in microbiological analysis, showing potential to be used

as a reducing agent for NaOCl –treated dentine without damaging biological tissues.⁷⁻⁹

Ascorbic acid and its sodium salts are well known antioxidants that are capable of reducing variety of oxidative compounds especially free radicals,^{10,11} in other words it promotes polymerization reaction of free radicals of the adhesive agent without premature termination and reverses the disrupted bonding to NaOCl-treated dentine.⁵

Rosemarinic acid (RA) extracted from rosemary a polyphenol; P-toluene sulfinic acid sodium salt has shown to accelerate the polymerization of composite resin. It has a strong free radical scavenging activity and is a MMP inhibitor.⁶

These antioxidants could reverse the compromising effect of NaOCl on bond strength of enamel and dentine by restoring the altered redox potential of the oxidized bonding substrate.^{10,11,6,12,17}

This study aims to determine the effectiveness of anti-oxidants a). Sodium thiosulphate (ST), (b). Sodium ascorbate (SA) and (c). Rosemary extracts (RE) to restore bond strengths when used for different time intervals.

The null hypothesis tested was that neither the application time nor the type of antioxidant/reducing agent has any effect on reversing compromised bonding to NaOCl treated dentine.

Materials and Methods

Specimen Preparation: Eighty-four extracted incisors, stored in 0.2% thymol was used in this study.¹⁰ The crowns were sectioned buccally on the middle third to expose the dentine area 3-5mm of the incisal portion were removed horizontally with a double-sided diamond disc under running water. The dentin area was penetrated by the disc longitudinally, a 10mm buccal section was made from the incisal edge perpendicular to

the long axis of the tooth. Then the pulp tissue was carefully extracted with a spoon excavator.¹³ The resulting slabs of the intracoronal dentin were flattened and polished with 180-grit and 600-grit SiC papers under running water for 30 seconds to standardize the smear layer of the dentin surface.

The specimens were randomly distributed into 6 groups according to the chemical irrigant used as in Table 1.

Material information for endodontic irrigation:

Endodontic Material	Concentration
Sodium hypochlorite (Hypochlorite SPEIKO)	5.25% NaOCl
Sodium Ascorbate 500mg (eq. to ascorbic acid 400mg) manufactured by Abbott healthcare pvt. Ltd.	10% Sodium Ascorbate/ Neutral Ascorbic acid
Sodium thio-sulphate	5% Sodium thiosulphate
Rosemarinic acid	6% Rosemarinic acid

Bonding Procedure: All specimens were dried with absorbent papers before the bonding procedures, etched with 37% phosphoric acid (N- Etch, Ivoclar Vivadent, India) for 20 second and a total etching adhesive system (Kerr OptiBond S, United States) was applied to the surface of the pulp chamber dentine. Three layers with 1mm of increment of resin composite (Kerr Herculite Precise Kit, United States) was added to the bonded dentine¹⁴ and each was light cured for 20 seconds. After the restoration, the blocks were stored in distilled water at 37⁰ C for 24hrs.

Micro- Tensile Bond Strength: 24 hrs later, the blocks from each group were removed from water, dried and fixed to an acrylic plate to permit creation of serial cross-sections by using a diamond saw (Isomet; Buehler Lake Bluff, IL) operating at 300 rpm. Rectangular slabs (4cmX 3cm X 4cm) were made from the central crown segment to obtain a linear resin/dentine interface. Each slab was submitted to tensile load individually in a universal testing machine¹⁴ (UTM- EZ-Test; Shimadzu Co, Kyoto, Japan) at a crosshead speed of 1mm/min until fracture.

The micro tensile bond strength was then determined and analyzed using independent sample t-Test with confidence limits for inter and intra group comparison by the Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, Medical 9.0.1, Systat 12.0 and R environment ver.2.11.1.

Results

The micro tensile bond strength values for means and standard deviations are shown in the Table 2, 3 and 4. Statistical analysis revealed that there were significant differences between all the groups while Group 2 (NaOCl + EDTA) showed least bond strength showed to pulp chamber dentine ($P \leq 0.05$).

3 and 5 mins application of rosemary extract significantly increased the micro tensile bond strength to NaOCl treated dentine compared to sodium thiosulphate and sodium ascorbate groups. Rosemary extract showed a significant better performance at 3 and 5 mins than at 10 mins. Sodium ascorbate performed better than sodium thiosulphate at 3 and 5 mins. At 10mins there was no significant difference among the experimental groups.

Discussion

In this study, NaOCl was used as an irrigant in all the groups. NaOCl is widely used irrigant during endodontic treatment due to its capacity to dissolve organic tissues and its antibacterial effects.^{6,15} This study found out that NaOCl decreases the bond strength between the dentine and composite resins. This results in accordance with many previous studies.^{15,16} This is due to the reactive residual free-radicals released by the oxidizing effect of NaOCl, which hinder the polymerization of dental adhesive system.

Some researchers have shown that application of reducing agents/anti-oxidants before the adhesive procedures can interact with NaOCl by-products and neutralize the oxidizing effect of NaOCl- treated dentine surface.^{17,5} Recently, the use of anti-oxidants / reducing agents has been increased before the adhesive procedures to increase the bond to dentine and to decrease micro-leakage.^{6,17-20} In addition, reducing agents are now-a-days frequently used as polymerization facilitating²¹ agents and cross-linkers. The three main antioxidant mechanisms are chelation to metals, quenching free radicals and chain breaking of the free radicals.²² Antioxidants are synthetically produced but some are derived from natural sources and their natural extracts.

In this study, the application of rosemary extract to NaOCl treated dentine significantly increased bond

strength with application times of 3mins and 5mins. Rosemarinic acid which is extracted from rosemary is a polyphenol and the best antioxidant activity of 72 species/herbs.^{23,24} The antioxidant activity of rosemarinic acid is due to the ability of catechol to form an intermolecular hydrogen bond between the free hydrogen of its hydroxyl and phenoxyl radicals, thus improving the radical stability.^{25,26}

Sodium ascorbate improves the bond strength via redox reaction on the treated substrate. It allows free radical polymerization of the adhesive to continue without premature termination and reverses the compromised bonding of the treated dentine.⁵ But the beneficial effect of sodium ascorbate mostly occurs within 1 min and also the reducing action of sodium ascorbate does not change with more concentration.

Sodium thiosulphate neutralizes the oxidizing agent, through the redox reaction and leads to optimal

polymerization of the resin composite. But 3.5ml and more quantity of Sodium thiosulphate are required to neutralize 5% sodium hypochlorite²⁷. This neutralization reaction also results in formation of yellow precipitates of sodium sulphate and sodium chlorides²⁸ and reducing the effect of sodium thiosulphate.

The antioxidant activity of the reducing agent and NaOCl treated dentine depends on their total antioxidant capacity value.

Polyphenols like Rosemarinic acid were shown to have higher total antioxidant capacity than sodium ascorbate and sodium thiosulphate. Also Rosemarinic acid has a MMP-inhibitor effect. MMP-activation leads to degradation of extra cellular matrix.⁶ So the use of MMP inhibitor promises to increase the durability of resin-dentin bonds.

Table 1: Sample Division

Negative Control (NaCl) (n=7)	Positive Control (NaOCl+17% EDTA) (n=7)	Saline (NaOCl+17% EDTA+NaCl) (n=7)
group 1 (n=7)	group 2 (n=7)	group 3 (n=7)

Experimental Groups

Sodium Thiosulphate group 4 (n=21)	Sodium Ascorbate group 5 (n=21)	Rosemary Extract group 6 (n=21)
Subgroup 4 a (n=7) 3mins	Subgroup 5 a (n=7) 3mins	Subgroup 6 a (n=7) 3mins
Subgroup 4 b (n=7) 5mins	Subgroup 5 b (n=7) 5mins	Subgroup 6 b (n=7) 5mins
Subgroup 4 c (n=7) 10mins	Subgroup 5 c (n=7) 10mins	Subgroup 6 c (n=7) 10mins

Table 2: Comparison of the mean values between the groups

Negative control	Positive control	saline	Sodium thiosulphate			Sodium Ascorbate			Rosemary extract		
			3mins	5 mins	10 mins.	3 mins	5 mins	10 mins	3 mins	5 mins	10 mins
3.89	0.88	2.77	0.45	0.47	0.47	1.16	3.63	1.80	5.16	6.30	1.64

Table 3: Inter Group

Comparison	Groups	Mean	S.d.	P-value
4a vs 5a	4a	0.45	0.08	0.007*
	5a	1.16	0.56	
4b vs 5b	4b	0.47	0.04	0.000*
	5b	3.63	1.26	
4c vs 5c	4c	0.45	0.08	0.000*
	5c	1.8	0.121	
5a vs 6a	5a	1.16	0.56	0.000*
	6a	5.16	1.08	
5b vs 6b	5b	3.63	1.26	0.040*
	6b	6.30	2.87	
5c vs 6c	5c	1.80	0.12	0.449**
	6c	1.64	0.52	
6a vs 4a	6a	5.16	1.08	0.000*
	4a	0.45	0.08	
6b vs 4b	6b	6.3	2.87	0.000*
	4b	0.47	0.04	
6c vs 4c	6c	1.64	0.52	0.000*
	4c	0.45	0.08	

* Significant @ 95% confidence level.

** Insignificant @ 95% confidence level.

Table 4: Intra Group

Comparison	Groups	Mean	S.D.	P-Value
4a vs 4b	4a	0.45	0.08	0.656**
	4b	0.47	0.04	
4b vs 4c	4b	0.47	0.04	0.656**
	5b	0.45	0.08	
4c vs 4a	4c	0.45	0.89	1.000**
	4a	0.45	0.89	
5a vs 5b	5a	1.16	0.56	0.001*
	5b	3.63	1.26	
5b vs 5c	5b	3.63	1.26	0.002*
	5c	1.80	0.12	
5c vs 5a	5c	1.80	0.12	0.013*
	5a	1.16	0.56	
6a vs 6b	6a	5.16	1.08	0.347**
	6b	6.3	2.87	
6b vs 6c	6b	6.3	2.87	0.001*
	6c	1.64	0.52	
6c vs 6a	6c	1.64	0.52	0.000*
	6a	5.16	1.08	

* Significant @ 95% confidence level.

** Insignificant @ 95% confidence level.

Conclusion

Within the limitations of this invitro study, amongst the three antioxidants, use of 6% Rosemarinic acid for 3 and 5 min did significantly improved the resin cement bond strength to NaOCl treated dentine. Sodium thiosulphate exhibited the least reversal effect. Further research is required to determine the long term stability of the adhesive materials to dentine after the use of reducing agents.

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