

Original Research

A Comparative Evaluation of the effect of two different antioxidants on the bond strength of composite resin to pulp chamber dentin treated with sodium hypochlorite and Ethylenediaminetetraacetic Acid: An In Vitro Study

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ABSTRACT:

OBJECTIVE- The aim of this in vitro study is to evaluate and compare the effect of two different antioxidants – 6.5% proanthocyanidin and 10% ascorbic acid on the bond strength of composite resin to pulp chamber dentin treated with sodium hypochlorite and ethylenediamine tetraacetic acid. **MATERIALS AND METHODS-** Thirty extracted human mandibular molar teeth were sectioned in a mesiodistal direction to expose the pulp chamber to obtain a total of sixty samples. The enrolled specimens were divided into 3 groups: Control Group(n=20), treated with 3% sodium hypochlorite (NaOCl) for 5 mins followed by treatment with 17% ethylenediamine tetraacetic acid (EDTA) for 5 mins ; Group A (n=20) ,after pretreatment with NaOCl and EDTA , the specimens were immersed in a solution of 6.5% Proanthocyanidin (PA) for 10 mins ; Group B(n=20) , following pretreatment with NaOCl and EDTA , specimens were further treated with 10% ascorbic acid solution for 10 mins. After drying the specimens, a self-etch adhesive, Prime and Bond [™] (Dentsply Caulk) was applied on pup chamber dentin followed by composite restoration (Ceram X [™] Sphere Tec [™] One Universal, Dentsply Sirona). Each specimen was then tested under a universal testing machine at dentin/resin interface to determine shear bond strength. **RESULTS-** The group in which 6.5% PA was used showed significantly higher bond strength than the 10% ascorbic acid group followed by the control group. (p <0.05). **CONCLUSION-** Within the limits of this in vitro study it can be concluded that immediate application of proanthocyanidin and ascorbic acid can restore the compromised bond strength of composite resin to NaOCl/EDTA treated dentin. Proanthocyanidin can improve the bond strength significantly more than ascorbic acid.

Key words: Sodium Hypochlorite, Ethylenediamine Tetraacetic Acid, Proanthocyanidin

Received: 02/05/2020

Modified: 20/05/2020

Accepted : 15/06/2020

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This article may be cited as: Tandon R, Bhushan J, Rawla A. A Comparative Evaluation of the effect of two different antioxidants on the bond strength of composite resin to pulp chamber dentin treated with sodium hypochlorite and Ethylenediaminetetraacetic Acid: An In Vitro Study. J Adv Med Dent Scie Res 2020;8(7):16-21.

INTRODUCTION

Successful root canal treatment relies on efficient cleaning, shaping and compact filling of the root canal under aseptic conditions. In addition to a good apical seal, a coronal seal is also mandatory in order to prevent micro leakage.¹ Apical periodontal health depends considerably more on the coronal restoration than on the technical quality of the endodontic treatment.² Therefore, the need for an immediate and proper restoration after endodontic treatment is of utmost importance.

Restoration using direct composite resin is a viable treatment option post endodontic therapy.³ The resin-dentin interdiffusion zone, called the “hybrid layer,” plays a key role in the micromechanical retention of the restoration.⁴

Chemicals used during root canal preparation may alter the composition and therefore the interaction of the dentin surface with restorative materials.⁵ Sodium hypochlorite (NaOCl), an efficient organic solvent and antimicrobial agent is used widely as an irrigant for endodontic therapy.⁶ Dentin is degenerated by NaOCl due to dissolution of dentinal collagen.⁷ Also, NaOCl releases oxygen, and this oxygen could cause inhibition of the adhesive system penetration and polymerization, thus decreasing the bond strength values.^{8,9}

Ethylenediamine tetraacetic acid (EDTA), a chelating agent on the other hand can expedite the destructive hypochlorite effect and facilitate hydroxyl ion diffusion through dentinal tubules.¹⁰

The strengthening of collagen fibrils by crosslinking agents may be of clinical importance in improving dentin bond strength.¹¹ Some studies have shown that application of an antioxidant after NaOCl treatment can restore compromised bond strengths.^{8,12,13,14}

In the present study, we compared the potential effects of proanthocyanidin (PA) and ascorbic acid on the recovery of shear bond strength to pulp chamber dentin treated with NaOCl and EDTA. The null hypothesis was that neither 6.5% proanthocyanidin nor 10% ascorbic acid solution would be effective in reversing the bond strength to dentin compromised by NaOCl and EDTA.

MATERIALS AND METHODS

Preparation of Solutions

Two solutions were prepared for this study:

- 1) 6.5 g of grape seed extract (NuSci, ebay) in the form of powder dissolved in 100 mL of distilled water to make 6.5% proanthocyanidin solution and,
- 2) 10 g of ascorbic acid powder (VitaeGen Life Sciences, Amazon) were dissolved in 100 mL of distilled water to make 10% ascorbic acid solution.

Specimen Preparation

Thirty freshly extracted human mandibular molar teeth were collected for the study, cleaned of debris and stored in distilled water until use. Any teeth with carious lesion or surface cracks were excluded from the study. They were then sectioned in a mesiodistal direction using a low speed circular diamond disc to expose the pulp chamber and a total of sixty sectioned samples were obtained. The contents of pulp chamber were removed with a spoon excavator. Further the buccal/ lingual wall of each half of the crown occlusal to the cemento-enamel junction was removed with a diamond disc until the remaining dentin thickness (RDT) was approximately 1.5mm as measured with a metal caliper (Safeseed[®] Electronic Digital Vernier Caliper Ruler). Specimens were kept in distilled water prior and after examination. The enrolled specimens were divided into 3 groups of 20 specimens each.

CONTROL GROUP (n=20) - Initially, specimens were immersed in a solution of 3% NaOCl solution (Hyposol, Prevest Denpro Limited) for 5 mins (Fig 1A), then rinsed with saline. Next specimens were immersed in a solution of 17% EDTA (Prevest Dento Limited) for 5 mins (Fig 1B), followed by saline.

GROUP A (n=20) - After pretreatment with NaOCl and EDTA (Fig 2A & 2B), the specimens were immersed in a solution of 6.5% PA solution for 10 mins (Fig 2C)

GROUP B (n=20) - After pretreatment with NaOCl and EDTA (Fig 3A & 3B), the specimens were immersed in 10% ascorbic acid solution for 10 mins (Fig 3C)

Bonding Protocol

Before bonding procedure, specimens were blot dried using cotton pellets. A self-etch adhesive system, Prime and Bond[™] (Dentsply Caulk) was coated on the surface of pulp chamber dentin using a micro applicator tip (Dentsply) according to manufacturer's instructions and cured for 20 secs. After the application of the bonding agent, each specimen received a nano-ceramic composite resin (Ceram X[™] Sphere Tec[™] One Universal, Dentsply Sirona), placed incrementally. Each bonded specimen was then cured using light-curing unit (Elipar Highlight, 3M ESPE) for 20 secs. After the bonding procedure, the samples were stored in distilled water until testing.

Shear Bond Strength Testing

Shear bond strength of the composite resin to the tooth surface was determined using a universal testing machine (BISS, Bangalore Integrated System Solutions, India) at a crosshead speed of 1 mm/min. The mounted

specimen with the test load has been shown in Fig 4. Load was applied until restoration failure. The results were tabulated and statistically analyzed. Statistical analysis was performed with the help of SPSS software v20. Data was analyzed using one way analysis of variance (one way ANOVA). Level of statistical significance was defined at $p < 0.05$

RESULTS

The shear bond strength means and standard deviations are shown in Table 1. Statistical Analysis revealed that control group with NaOCl + EDTA showed significantly lower bond strength to pulp chamber dentin ($P < 0.05$) as compared to Group A and Group B. The specimens that were immersed in a solution of 6.5% PA (Group A) yielded significantly higher shear bond strength values than the ones immersed in 10% ascorbic acid solution (Group B) ($p < 0.05$)

Table 1 : Shear Bond Strengths(MPa) And Standard Deviations

GROUPS	SAMPLE SIZE	MEAN (MPa)	STANDARD DEVIATION
CONTROL(NaOCl+EDTA)	20	22.05	4.35
GROUP A (NaOCl+EDTA+6.5% PA)	20	29.84	5.11
GROUP B (NaOCl+ EDTA+ 10% ascorbic acid)	20	26.85	4.74
Significant groups- Control vs Group A vs Group B ($p < 0.05$)			



Fig 1A

Fig 1A- TOOTH SAMPLES IMMERSSED IN SODIUM HYPOCHLORITE

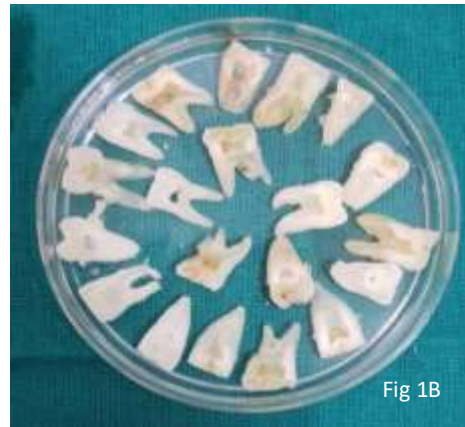


Fig 1B

Fig 1 B- TOOTH SAMPLES IMMERSSED IN ETHYLENEDIAMINE TETRAACETIC ACID



Fig 2A



Fig 2B



Fig 2A: TOOTH SAMPLES IMMERSSED IN SODIUM HYPOCHLORITE
Fig 2B: TOOTH SAMPLES IMMERSSED IN ETHYLENEDIAMINE TETTRAACETIC ACID
Fig 2C: TOOTH SAMPLES IMMERSSED IN PROANTHOCYANIDIN SOLUTION



Fig 3A- TOOTH SAMPLES IMMERSSED IN SODIUM HYPOCHLORITE
Fig 3 B- TOOTH SAMPLES IMMERSSED IN ETHYLENEDIAMINE TETTRAACETIC ACID
Fig 3C- TOOTH SAMPLES IMMERSSED IN ASCORBIC ACID SOLUTION

FIG 4 : MOUNTED TOOTH SAMPLE WITH TEST LOAD IN THE UNIVERSAL TESTING MACHINE



DIRECTION OF TEST LOAD

DISCUSSION

Resin- dentin bonding is a special form of tissue engineering that relies on a demineralized dentin collagen matrix used as a scaffold to create a hybrid layer which couples the adhesive/ resin composite to the underlying dentin.¹⁵ Bonding efficiency depends largely on structural integrity and mechanical properties of acid-demineralized collagen fibers.

To our knowledge, this study is one of the few comparing the potential of proanthocyanidin and ascorbic acid to recover the dentin bond strength lost with the use of commonly used endodontic irrigants.

Endodontic irrigants alter the structure of dentin, mainly collagen that interferes with dentin/resin bonding.¹⁶ NaOCl treatment has a negative impact on the mechanical properties of dentin i.e. its flexural strength and elastic modulus.¹⁷ Removal of collagen fibrils from the dentin surface by sodium hypochlorite may impede the formation of a consistent hybrid layer.¹⁸ Moreover the oxidizing capacity of NaOCl leads to generation of oxygen free radicals that might compete with the propagating vinyl-free radicals generated during light activation of the adhesive system, resulting in premature chain termination and incomplete polymerization.⁹

EDTA has a strong demineralizing effect, causing widening of the dentinal tubules, denaturation of collagen fibers and softening of dentin.¹⁹

On the other hand antioxidants (eg. PA and Ascorbic acid) with their free radical scavenging action can reverse the NaOCl and EDTA compromised bond strength. Grape seed extract (GSE) is a naturally occurring antioxidant. It contains oligomeric proanthocyanidin (PA) complexes (OPCs). PA or condensed tannins is composed of flavon-3-ol subunits, catechin, epicatechin and epicatechin-3-O-gallate, linked mainly through C4 - C8 (20). They possess antibacterial, antiviral, anticarcinogenic, anti-inflammatory, and anti-allergic activities. They are natural extracts, thus lack toxicity. OPCs contain numerous electron donor sites (hydroxyl sites) that allow it to bind to free radicals by giving away its hydrogen atoms. They also increase the number of inter- and intramolecular collagen cross links.²¹

In the present study we observed increased shear bond strength with 6.5% PA [group A (29.84±5.11)] as compared with 3% NaOCl [Control (22.05±4.35)]. This is in accordance with the study conducted by Benin Dikmen et al. The reducing activity of PA reverses the oxidizing effect of NaOCl, restoring the redox potential of the oxidized dentin.²²

Ascorbic acid is the most essential component in the synthesis of hydroxyproline and hydroxylysine in collagen.²³ In the present study, there is a statistically significant increase in the bond strength with 10% ascorbic acid [Group B (26.85 ±4.74)], as compared

with 3% NaOCl [Control(22.05±5.35)] which indicates that ascorbic acid reverses the lost bond strength. Its antioxidant properties convert the oxidized substrate to a reduced substrate which restores the redox potential of the dentin. This allows free radical polymerization of the adhesive to proceed without premature termination.²⁴ It is nontoxic, and its intraoral use is unlikely to cause any harmful biological effects.

In this study, 6.5% PA [group A (29.84±5.11)] showed the maximum bond strength among all the groups. There was a significant difference ($p<0.05$) in the bond strength between 6.5% PA [group A (29.84±5.11)] and 10% ascorbic acid [Group B (26.85 ±4.74)]. PA contains gallic acid that may act as a critical factor in maximizing its free radical scavenging ability. Gallic acid is only found only in grape seed extract.²⁶

Hence, the null hypothesis was rejected as both 6.5% proanthocyanidin and 10% ascorbic acid solution were seen to be effective in reversing the bond strength to dentin compromised by NaOCl and EDTA.

CONCLUSION

Within the limits of this in vitro study it can be concluded that application of proanthocyanidin and ascorbic acid can revert the bond strength compromised by the use of sodium hypochlorite and ethylenediamine tetraacetic acid. Proanthocyanidin was seen to improve the bond strength significantly more than ascorbic acid.

This new protocol could be an adjunct approach to achieve more durable dentin bonding. Yet further research is required to evaluate optimum level, biocompatibility and safety factors of these antioxidants to be used in routine endodontic practice. In vivo studies remain necessary to validate these laboratory results.

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