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Comparative Evaluation of Different Dentin Deproteinizing Agents on Nanoleakage - An In-Vitro Study

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Abstract

Background and objective: Bonding to dentin has always remained a challenge in restorative dentistry the aim of this study was to evaluate the amount of nanoleakage following deproniteinization of dentin with current deproteinizing agents such as 10% Ascorbic acid and 90% Trichloroacetic acid.

Materials and Method: A total of 40 extracted human premolars were used in this study. Standardized Class V cavities were prepared on the buccal surface. The teeth were randomly divided into 4 groups (n= 10) based on the different agents used for deproteinization. Group 1: Normal saline was used as a control. Group 2: 10% Ascorbic acid Group 3: 3% NaOCl Group 4: 90% Trichloroacetic acid. The teeth were dried and dentin bonding agent (universal bond) was applied and light cured. Restorative procedure was performed by inserting composite resin in three horizontal increments. Each increment was individually light cured for 20s to ensure complete polymerization. Then for dye penetration the specimens were stored in 50% alcoholic solution of 1% wt Rhodamine B dye for 24 hours. Teeth were sectioned parallel to long axis of tooth using low speed diamond disc under copious water supply. Sectioned specimens were polished using 600 grit silicon carbide paper. For nanoleakage assessment the restorative interface region was examined using Confocal Laser Scanning microscope.

Results: Trichloroacetic acid showed the least nanoleakage.

Conclusion: 90% Trichloroacetic acid has least nanoleakage. However both 10% ascorbic acid and 3%

Sodium Hypochlorite had significantly lower nanoleakage than Saline.

Clinical Significance: Deproteinization of dentin with newer biocompatible agents such as 90% Trichloroacetic acid and 10% Ascorbic acid decreased the amount of nanoleakage when compared with routinely used agents such as Sodium hypochlorite.

Keyword: Deproteinization, Confocal laser scanning microscopy, Nanoleakage

Introduction

One of the challenges of restorative dentistry research is to develop adhesive restorative materials that provide an effective bond to dental tissues and consequently offer successful restorative treatment[1]. This is difficult to achieve, as the bonding process is different for both enamel and dentin, because dentin is more humid, more dynamic and more organic than enamel[2].

The notion of dentin adhesion presently used involves the nanomechanical retention which is due to polymerization of hydrophilic monomers around acid exposed collagen fibres. The entanglement of these monomers within the exposed collagen fibres gives rise to the so called Hybrid layer, which is also referred to as Resin infiltrated Dentin layer. The presence of hybrid layer is pivotal for attainment of a leakage free interface between the cavity walls and resin composite. After acid etching, the demineralized collagen matrix is present in a denatured state, beneath which lies the residual hydroxyapatite crystals. Monomer infiltration throughout the thickness of demineralised dentin is important for successful adhesion. However the low surface free energy of demineralised collagen could possibly minimize diffusion of hydrophilic monomer through the existing nanospaces. Also the presence of water around the collagen fibres can impede proper bonding leading to the phenomenon of nanoleakage.[3]This zone of partially demineralized dentin with microcavities may be considered as a weak point in the attachment. Similar studies in enamel showed no evidence for the formation of respective porosities or penetration paths[4].

The term nanoleakagewas introduced by Sano, et al., in 1995, to describe a specific type of leakage, which exists even in the absence of marginal gaps. This leakage occurs laterally, through submicron porosities (estimated to beabout 20 to 100 nm in width) at the base of the hybrid layer,which have not been filled with adhesive resin or whichhave been left poorly polymerized. This demineralized, but not fully hybridized dentin layer can be considered a weak point in the adhesion mechanism that could allow dentinal and oral fluid to slowly permeate the interface, and this is believed to degrade the adhesive resin. [5] Therefore there was a need to modify the organic content of enamel in teeth. Deproteinization is one such method.[6]

Scientific research is currently being carried out on alternative methods of dentin deproteinization, which is characterized by changing at the surface through removing the collagen exposed by a conditioning acid (Sato et al., 2005). According to Mountouris et al. (2004), dentin deproteinization with sodium hypochlorite (NaOCl) produces an alternative morphologicaland chemical modification of the tissues, that is relevant to the bonding substrate.[7]

The disadvantages generated by using 10% Sodium hypochlorite to deproteinize acid etched dentin formed a fragility zone and the cytotoxicity of the Sodium hypochlorite; which are aggravated by the depth of the dentin, and the intolerable taste and odor have stimulated a new and different treatment philosophy to deproteinize dentin[8]

Hence, an attempt was made in this study to overcome the drawbacks created by the earlier existing 10% sodium

hypochlorite in relation to nanoleakage using deproteinizing agents such as 10% ascorbic acid and 90% trichloroacetic acid. An observation was made under Confocal Laser Scanning Microscope.

Materials and Methods

Tooth Preparation

A total of 40 extracted human premolars free of dental caries, restorations, cracks or any obvious defects were cleaned and stored in 0.5% thymol aqueous solution until use. Standardized Class V cavities were prepared on the buccal aspect having dimensions of 3mm high × 3mm wide × 2mm depth, using a high speed handpiece with a medium grit diamond bur No.848 under water coolant. The outline of the cavity was drawn on the surface of the tooth. The measurements were standardized by using a matrix band with a precut hole of 3 x 3 mm which was fixed on the tooth. The gingival floor of the cavity was placed within the cemento-enamel junction. The prepared cavity was completed using a round bur No. 2 in a low speed handpiece. The enamel margins were not beveled. The prepared class V cavities were randomly divided into four groups (n= 10) based on the different agents used for deproteinization.

Group 1 (negative control): Teeth were etched with 37% phosphoric acid for 15s, rinsed with water for 20s and blot dried. Normal saline was used as a control.

Group 2: Teeth were etched with 37% phosphoric acid for 15s, rinsed with water for 20s and blot dried. 10% Ascorbic acid was applied for a period of 20s followed by rinsing with water for 20s.

Group 3: Teeth were etched with 37% phosphoric acid for 15s, rinsed with water for 20s and blot dried. 3% NaOCl was applied for a period of 20s followed by rinsing with water for 20s.

Group 4: Teeth were etched with 37% phosphoric acid for 15s, rinsed with water for 20s and blot dried. 90% Trichloroacetic acid was applied for a period of 20s followed by rinsing with water for 20s.

All the cavities were dried and dentin bonding agent was applied onto the cavity with an applicator tip and were left for a period of 10s, the excess was removed using an air stream, followed by light curing for 20s. The restorative procedure was performed by inserting composite in three horizontal increments. Each increment of 1mm was individually light cured for 20s from all the surfaces to ensure complete polymerization. Before placing the final increment a transparent matrix band was used to contour the restoration. The restoration surfaces were polished using a sand paper disc.

Preparation for Dye Penetration Test

The root apices were sealed using light cured composite and the entire tooth except the restorative surface and 1mm around the restoration were coated with two layers of nail varnish.

Confocal Laser Scanning Microscopic Analysis

The prepared tooth specimens were stored in 50% alcoholic solution of 1% wt Rhodamine B dye for 24 hours, then sectioned parallel to the long axis of the tooth using low speed diamond disc under copious water supply. The sectioned specimens were then polished using 600 grit silicon carbide paper. The dentin and restorative interface region was examined using Confocal Laser Scanning microscope. The obtained images were analyzed using Zeiss Zen 2 software. The distance of dye penetration from the interface into the dentin was measured.

Stastical Analysis

Data obtained was statistically analyzed using One-Way analysis of variance for mean comparison among groups and post-hoc Tukey HSD was used to compare the nanoleakage between groups at a significance level of 0.01. The statistical analysis was performed using SPSS

version 12.0.1 for windows. The sample size has been estimated using the G power software v. 3.1.9.2

Result

Table 1 represents the mean dye penetration length measured in millimetre between the four study groups. This was done using one-way ANOVA test. The mean dye penetration for Group 1 was 1139.32±184.70, Group 2 was 594.81±7.91, Group 3 was 136.58±28.59 and Group 4 was 68.43±4.28. This difference in the mean dye penetration between 04 groups was statistically significant at P<0.001. [***Refer Graph no. 1***]

Table 1: Comparison of Mean Dye Penetration

Comparison of mean dye penetration length (in mm) between 04 study groups using one-way ANOVA test

Groups	N	Mean	SD	Min	Max	P-Value
Group 1	5	1139.32	184.70	932.7	1352.4	
Group 2	5	594.81	7.91	588.9	608.2	<0.001*
Group 3	5	136.58	28.59	103.0	161.5	
Group 4	5	68.43	4.28	63.8	73.3	

Table 2: Multiple Comparisons of Mean Differences Between The Groups

Multiple comparison of mean differences between 04 study groups using Tukey's HSD post hoc Analysis

Groups	G1 Vs G2	G1 Vs G3	G1 Vs G4	G2 Vs G3	G2 Vs G4	G3 Vs G4
P-Value	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.66

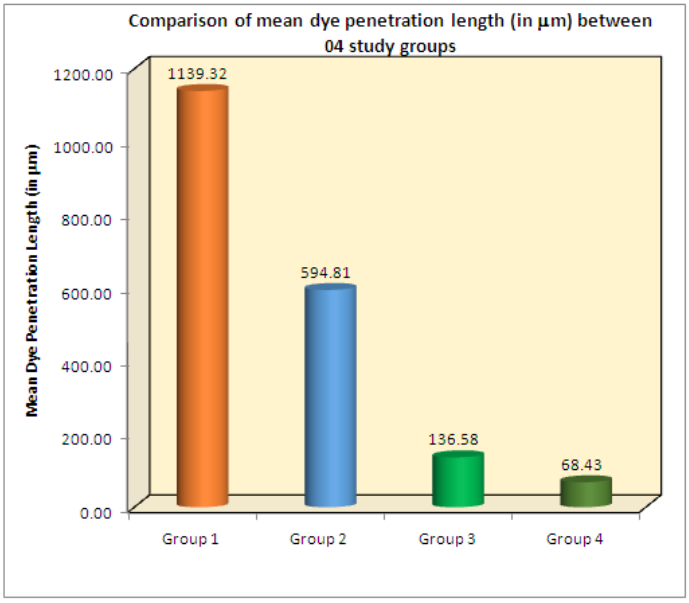
Table 3: Statistical Significance between the Groups

* - Statistically Significant

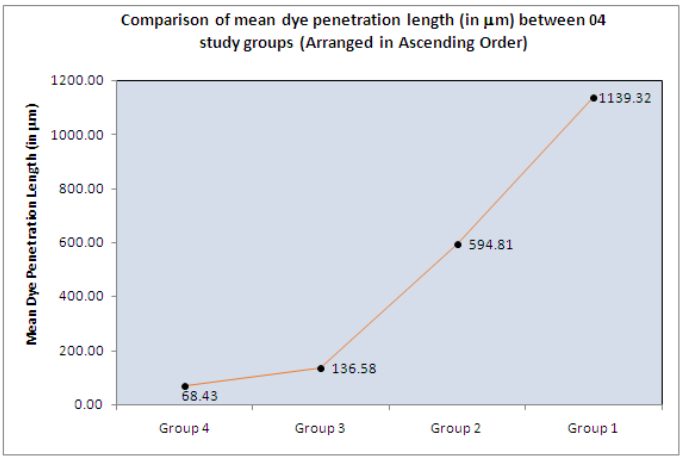
Note: G1 - Group 1, G2 - Group 2, G3 - Group 3 & G4 - Group 4

Graphical Representation of Mean Dye Penetration

Graph 1:



Graph 2:



Confocal Laser Scanning Images of Dye Penetration of Various Groups.

Fig.1a and 1b: saline.

Fig. 2a and 2b:10% Ascorbic Acid.

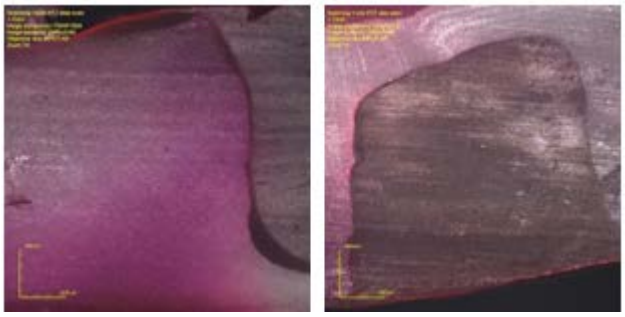


Fig. 3a and 3b: 3%sodium hypochlorite

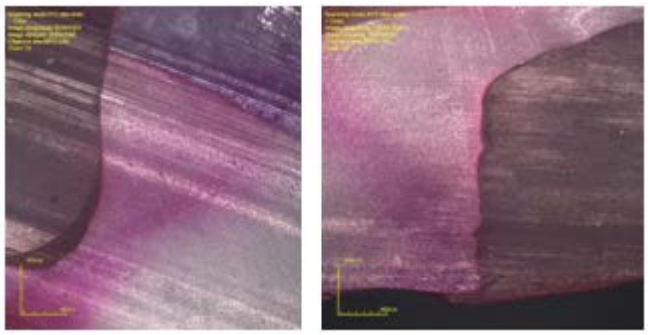


Fig. 4a and 4b: 90% trichloroacetic acid.

Discussion

In aesthetic procedures, resin cements are indicated as the materials of choice for performing clinical cementing work, particularly because they provide good aesthetics and mechanical strength, low solubility, and bond to enamel and/or dentin.9

The bond of resin materials to the tooth is important for retention and durability of the restoration, thus they are the key to clinical success. Hence, the use of conventional bonding techniques could result in early adhesive failures due to the susceptibility of the collagen fiber network. In this connection, some researchers have pointed out the solubility of dentinal collagen as one of the possible strategies for optimizing bonding.

After the etch and rinse procedure, there is exposure of demineralised dentin comprising of collagen fibres underneath which lies hydroxyapatite crystals. The collagen has a low surface energy compared with hydroxyapatite with high surface energy. After acid etching the surface energy is decreased by the exposure of collagen fibres.10

The removal of collagen fibers from previously etched surfaces with the use of deproteinizing agent has been shown to be a way of minimizing the technique-sensitivity of hybridization, and in some situations, depending on the bonding agent, promote favourable improvement in adhesive effectiveness.11

The deproteinized dentin has higher hardness, wettability and permeability, modulus of elasticity than demineralised dentin. In accordance with Pashley et al the tubule diameter is about 2µm wider in deep dentin compared to superficial dentin, thus enhancing the wettability. Deproteinized surfaces when compared with only demineralised substrate showed dentinal tubules with widened openings, exposure of several smaller orifices in the intertubular dentin which are lateral ramifications.12

Prati et al introduced the concept of Reverse Hybrid layer. In conventional hybrid layer, the mineral phase of dentin is removed by acid etching and replaced by resin infiltration around the exposed collagen fibres. In reverse hybrid layer, after acid etching and exposure of collagen fibres, application of NaOCl is done to remove exposed collagen fibres and also solubilise the fibres down into the underlying mineralised matrix to create submicron porosities within the mineral phase.13

Compromised bond strengths were observed for some single-bottle adhesives when dentin was treated with sodium hypochlorite either before or after acid-etching

(Perdigão et al., 2000; Lai et al., 2001). This drop in bond strength was attributed to the oxidizing instead of the deproteinizing effect of sodium hypochlorite, since the compromised bond strength may be reversed by the application of a reducing agent such as sodium ascorbate to the oxidized dentin (Lai et al., 2001) .Removal of demineralized collagen layer by sodium hypochlorite eliminated nanoleakage formation (Pioch et al., 2001a). 14

Hence in the present study nanoleakage was compared between 10% Ascorbic acid, 3% Sodium Hypochlorite and 90% Trichloroacetic acid as deproteinization agents.

Gwinnett demonstrated that the collagen-rich zone offers no direct contribution to the interfacial bond strength.The unprotected fibers may undergo hydrolysis after long term exposure to water leading to deterioration of adhesion between resin and dentin and thus to decreased bond strength. There appeared ways to promote adhesion to dentin. First is to improve the monomer impregnation into the substrate and the second is to increase the diffusibility or penetrability of the dentinal substrate itself. 15

According to Mountouris et al. (2004), dentin deproteinization with sodium hypochlorite (NaOCl) produces an alternative morphological and chemical modification of the tissues, that is relevant to the bonding substrate. NaOCl is a non-specific proteolytic agent (Yamauti et al., 2003) and its effects on the composition of dentin have been investigated in many studies (Barboza de Souza et al., 2005; Perdig~ao et al., 1999; Uno and Finger, 1995), but this solution is limited in terms of the concentration and duration of use; additionally, the fatty acids produced with the use of NaOCl can harm the resin–dentin bonding mechanism. 16

The findings of another study demonstrated that the application of 10% NaOCl, in gel or solution increased the microleakage of composite resin restorations since this alters the dentin surface, changing the hydrophilic properties that might influence the intimate attachment at the interface.16 Also in accordance with the study conducted by J. Perdiga ˜oa et al it was concluded that that the application of NaOCl induces morphological changes beyond the area corresponding to the deepest penetration of the phosphoric acid gel.17

The most commonly used deproteinization agents are tannic acid, citric acid, papain gel, bromalein enzymes, maleic acid, ascorbic acid, tricloroacetic acid, etc

In the search for an alternative substance to NaOCl, in the present study 90% tricholoroacetic acid and 10% ascorbic acid was been compared.

Ascorbic acid and its salts are anti-oxidants which are non toxic and extensively used in food industries, therefore it is unlikely it may have any deleterious effects intra orally. They are capable of reducing a variety of oxidative compounds, especially free radicals.18 According to Buettner et al, the hypothesis that the application of ascorbic acid to bleached surfaces provides adhesiveness with bond strengths equivalent to those of non- bleached surface 19. Ascorbic acid has also been used as an accelerator component in formulating intiator system, and is comparable to EDTA as a cleansing solution. It has been previously reported that an experimental conditioner, containing 10% ascorbic acid and 5% ferric chloride, was effective at improving the bonding process between META/MMA-TBB resin and dentin treated with phosphoric acid and NaOCL.20 proving the deproteinization property

90% Trichloracetic acid (TCA) is a chemical escarotic agent that has been used in medicine and dentistry for more than a century. TCA is produced by the oxidation of chloral hydrate with nitric acid and is manufactured by the chlorination of acetic acid. Its aqueous solution is highly acidic, yielding a pH of 1.0. It is predominantly used for decalcification and fixation in microscopic studies and as

a precipitating agent for proteins. It has been used as a cauterizing agent in medical procedures, especially for dermatologic and ophthalmologic purposes.20

One study conducted by (Lewinstein and Rotstein, 1992) evaluated the effect of TCA on microhardness and surface morphology of human dentine and enamel. The study reported that the duration of TCA treatment has a progressive effect on the microhardness of enamel. In addition, application of 90% TCA on enamel surface for 60 sec leaves an etched appearance and after 90 sec, the etching pattern is destroyed and the enamel surface becomes erosive. In accordance with the current study, 90% TCA showed the least nanoleakage followed by 10% AA and 3% NaOCl. This is in accordance with the study conducted by (Galun et al., 1994). who introduced 90% TCA as an effective dentin conditioner with acceptable shear and tensile bond strengths for bonded restorations.21 Also, the study conducted by Leonardo et al stated that NaOCl- treated group demonstrated significantly lower bond strength. The oxygen in NaOCl causes suoerficial oxidation and inhibits the interfacial polymerization of resin-based materials. The residual chemical solutions and their by-products likely diffuse into the dentin, affecting the polymerization of monomers at the demineralized dentin and decreased bond strength. On the other hand ascorbic acid was able to restore the bond strength of dentin. 22

Conclusion

It is concluded trichloroacetic acid showed the least nanoleakage followed by ascorbic acid and NaOCl. As it is an invitro study, it does not mimic the actual invivo conditions of oral cavity. Hence, further clinical trials are required to substantiate the results. Further studies on the application of TCA alone are required, without phosphoric acid as an etchant and as a hemostatic agent for bonded restorations on dental tissues. Other bonding agents, such as self-etch and self-adhesive systems, also need to be studied. Additional investigations of bond strength stability and clinical trials are needed to confirm the promising enamel etching effectiveness of TCA reported in the current study. Until then, it remains clinically advisable to use the conventional etching application technique.

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