Evaluation of nanoleakage following deproteinization of dentin – a confocal laser scanning microscopic study

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Abstract

Aim: This study evaluated the amount of nanoleakage following deproteinization of dentin with newer deproteinizing agents such as Ascorbic acid and Zinc Hydroxide.

Materials and Method: A total of 40 extracted human premolars were used in this study. Standardized Class V cavities were prepared on the buccal. The teeth were randomly divided into four groups (n= 10) based on the different agents used for deproteinization.

Group 1: Normal saline was used as a control.
Group 2: 3% NaOCl
Group 3: 10% Ascorbic acid
Group 4: 5% Zinc hydroxide

All the teeth were dried and dentin bonding agent was applied and light cured.

The restorative procedure was performed by inserting composite resin in three horizontal increments. Each increment was individually light cured for 20s from all the surfaces to ensure complete polymerization. For dye penetration the specimens were stored in 50% alcoholic solution of 1% wt Rhodamine B fluorescent dye for 24 hours. The teeth were 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 adhesive region was examined using Confocal Laser Scanning microscope.

Results: In group 3, when 10% ascorbic acid was used after acid conditioning of dentin, the confocal laser scanning microscopic images showed least amount of dye penetration thus indicating least nanoleakage. Least mean nanoleakage score was seen in Group 3.

Conclusion: Ascorbic Acid has least nanoleakage which was not significant from NaOCl. However both ascorbic acid and NaOCl had significantly lower nanoleakage than Zinc Hydroxide and Saline. Zinc hydroxide has also significantly lower nanoleakage than saline.

Clinical Significance: Deproteinization of dentin with newer biocompatible agents such as Ascorbic acid decreased the amount of nanoleakage when compared with routinely used agents such as sodium hypochlorite.

Keyword: Deproteinization, Nanoleakage, Confocal laser scanning microscopy, Composite

Introduction

The notion of dentin adhesion presently used involves the nanomechanical retention which is due to polymerization of hydrophilic monomers around acid exposed collagen fibres.(1) 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.(2)

After acid etching, the demineralized collagen matrix is present in a denatured state, beneath which lies the residual hydroxyapatite crystals.(3) 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.(2) The phenomenon of nanoleakage was first advocated by Sano et al in 1994, and they described it as a microscopic leakage occurring inside the structure of resin infiltrated dentin layer or the Hybrid layer.(4)

Therefore the removal of demineralised collagen matrix with deproteinizing agents has been advocated. Various studies have proposed the use of sodium hypochlorite, a non-specific deproteinizing agent in various concentrations for complete removal of demineralised collagen, as an adjunctive procedure after etch and rinse technique to ameliorate the adhesion between dentin and composite resin.(5,6) This increases the bond strength between the dentin and composite resin and also decreases or abolishes nanoleakage.(7)

Usage of NaOCl has certain drawbacks such as the formation of fragility zone and also cytotoxicity. It also has intolerable taste and odour.(8) Thus the aim of the present study is to evaluate the nanoleakage after treating dentin with deproteinizing agents mentioned above using Confocal Laser Scanning Microscope.

Materials and Method

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 used in this study. 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 grain 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 teeth were randomly divided into four groups (n= 10) based on the different agents used for deproteinization.

Group 1: 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. 3% NaOCl 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. 10% Ascorbic acid 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. 5% Zinc hydroxide was applied for a period of 20s followed by rinsing with water for 20s.

All the teeth were dried and dentin bonding agent was applied onto the cavity with an applicator tip for 10s 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 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. The root apices were sealed using composite and the entire tooth except the bonded interface and 1mm on the tooth surfaces next to the interface were coated with two layers of nail varnish.

For dye penetration the specimens were stored in 50% alcoholic solution of 1% wt RhodamineB fluorescent dye for 24 hours. The teeth were 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 adhesive 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.

Statistical 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. (SPSS Inc, Chicago, IL, USA)

Results

Table 1, shows the mean values of the nanoleakage scores and the standard deviation after treating dentin with different deproteinizing agents. Fig. 1 depicts the mean values graphically. Fig. 2 A-D are the confocal laser scanning microscopy images. In group 3, when 10% ascorbic acid was used after acid conditioning of dentin the confocal laser scanning microscopic images showed least amount of dye penetration thus indicating least nanoleakage (Fig. 2C). The same can be appreciated in Table 1 by observing the mean values for nanoleakage. Least mean score for nanoleakage was seen in Group 3 (i.e., 26.869).

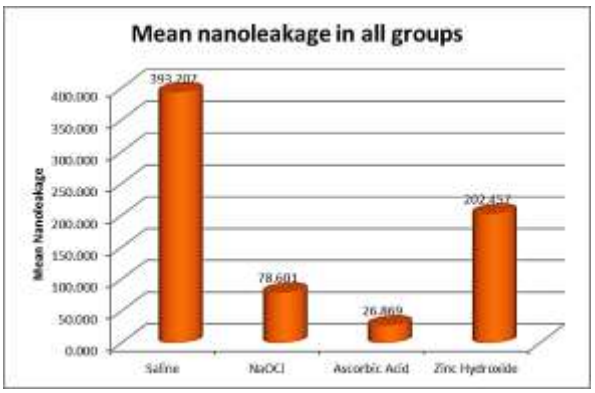

Fig. 1: Depicts the mean values graphically

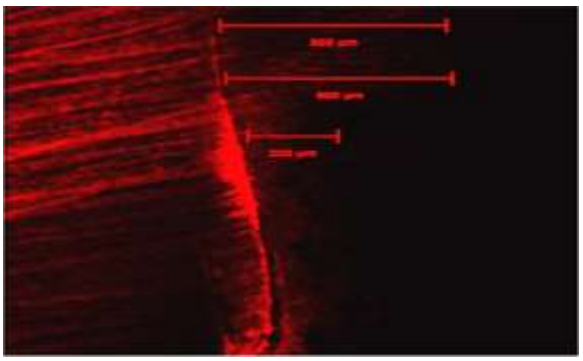

Fig. 2a

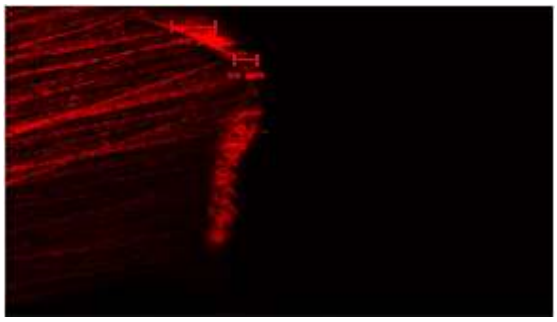

Fig. 2b

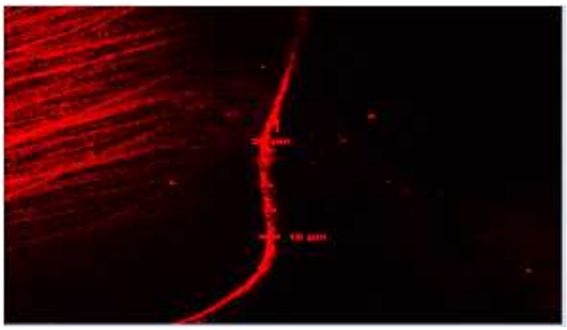

Fig. 2c

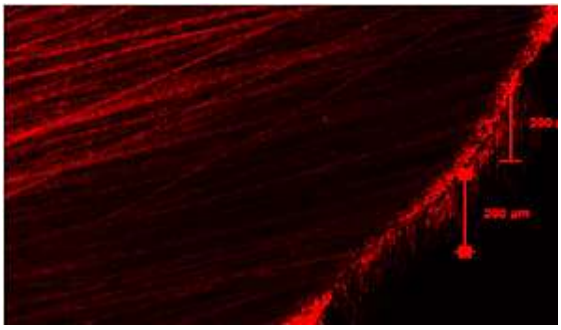

Fig. 2d

Table 1: Descriptive Analysis of Nanoleakage (µm)

Group	n	Minimum	Maximum	Mean	SD	SEm
Saline	10	212.210	512.090	393.207[a]	103.248	32.649
NaOCl	10	50.340	113.760	78.601[b]	21.162	6.692
Ascorbic Acid	10	9.000	51.160	26.869[b]	15.419	4.876
Zinc Hydroxide	10	108.650	345.780	202.457[c]	72.684	22.985
Total	40	9.000	512.090	175.284	155.719	24.621

SD: Standard deviation; SEm: Standard error of Mean; Different alphabets in superscript show statistically significant difference ($p<0.01$)

Discussion

Bonding to dentin relies on the formation of Hybrid layer, which is formed by the polymerization of monomers into the spaces between the collagen fibres of demineralised dentin.[1] Some studies have demonstrated that the hybrid layer is of utmost importance for increasing the bond strength between dentin and composite resin,[9-14] while others have shown that it is accountable for nanoleakage, possibly because of hydrolytic degradation of exposed collagen. This ensues because of the formation of a fragile zone of poorly impregnated monomers into dentin collagen which is vulnerable to breakdown by long term contact with water.[14,15]

The stability of the hybrid layer depends on several factors such as permeability of adhesive system, non effective sealing of dentinal tubules, inadequate polymerization of adhesive system, cytotoxicity of non polymerized adhesive system to pulp and technique

sensitivity in maintaining moist dentin during application.[16]

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.[17] To increase the bonding between composite resin and dentin the weak zone in the hybrid layer, the exposed collagen fibres should be completely removed. The dentin obtained after removal of the collagen fibres in known as deproteinized dentin.

The deproteinized dentin has higher hardness, modulus of elasticity, wettability and permeability than demineralised dentin.[1] Pashley et al have shown that 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.[18] Based on these properties it can be asserted that infiltration into deeper dentin may be more efficient than in superficial dentin. Also cathepsins and metalloproteinases hamper the bond between dentin and composite resin if the collagen exposed by acid etching cannot be adequately filled.[19] Consequently bonding to deproteinized dentin has shown reduced nanoleakage and good bond strengths.

Prati et al have 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.[20]

NaOCl is a widely used non specific proteolytic agent for deproteinization of dentin. The proteolytic action of NaOCl is due to extensive fragmentation of long peptide chains and formation of N – Chloramines with terminal amine groups that further decompose to other by products.[7]Various concentrations of NaOCl have been used ranging from 0.5% to 12%. Generally it has been agreed that higher the concentration of NaOCl used, greater the bond strength.[21]

In this study, the nanoleakage was more for NaOCl group compared with 10% ascorbic acid group. This can be explained by the fact that presence of routine residual free radicals in dentin treated with NaOCl may compete with the propagating vinyl free radicals generated during lighter activation of the adhesive. This results in premature termination and incomplete polymerization.[22]

Ascorbic acid is a reducing agent and is widely used as an anti oxidant. Since ascorbic acid and its salts are non toxic, they are extensively used in food industries, therefore it is unlikely it may have any deleterious effects intra orally. In the present study least amount of nanoleakage was found when the acid etched dentin was deproteinized by using 10% ascorbic acid. This may be attributed to the pH of ascorbic acid. Ascorbic acid has a pH of 2.0,[23] at this pH it may be contemplated that it makes the deeper dentin more porous, hence greater penetration of monomer into the nanospaces around the hydroxyapatite crystals, hence decreased nanoleakage.

Zinc hydroxide is a deproteinizing agent used in the medical field for deproteinization of uric acid serum. In this present study this agent did not show any favorable result compared with the other two agents.

Conclusion

Ascorbic Acid has least nanoleakage which was not significant from NaOCl. However both ascorbic acid and NaOCl had significantly lower nanoleakage than Zinc Hydroxide and Saline. Zinc hydroxide has also significantly lower nanoleakage than saline.

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